horticulturae

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Role of Humic Acid on Inducing Salt Tolerance of Ivy Geranium (*Pelargonium peltatum* L.) Plants

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Abstract: Saline water is used in floriculture as an alternative to freshwater in arid regions such as Saudi Arabia (SA). However, salt stress considerably accelerates serious physio-biochemical changes associated with a decline in plant establishment. Recently, humic acid (HA) foliar spraying has induced plant stress tolerance in the era of climate change; however, its precise roles in the floriculture industry within saline conditions are not yet well documented. A factorial pot experiment throughout the 2022/2023 season was conducted in the Nursery of Sustainability and Environmental Developmental Department, King Saud University, Riyadh, SA, to evaluate the potential effects of HA (0, 500, 1000 and 2000 mg/L) on growth, flowering and some physiological characteristics of Ivy geranium (*Pelargoniumpeltatum*) plants irrigated with saline water (230 "control", 2000 and 4000 mg/L NaCl). Irrigation with saline water markedly inhibited plant growth, flowering attributes, the chlorophyll index, as well as macro and micro-nutrient levels, but increased the content of iron, sodium and proline in plant shoots relative to plants irrigated with non-salinized water. However, HA mainly at 1000 mg/L significantly improved plant growth, flowering capacity, nutrient status, proline accumulation and chlorophyll index under salinized or non-salinized irrigation water. Additionally, spraying of HA concentrations (500, 1000 and 2000 mg/L) under normal or salinity conditions significantly increased shoot sodium content relative to non-treated plants under such salinity levels. Our findings highlight the significance of HA concentrations (500, 1000 and 2000 mg/L) in improving the salt tolerance of Ivy geranium. Within the scarcity of irrigation water, it is recommended to irrigate Ivy geranium with saline water up to 4000 mg/L NaCl associated with spraying HA concentrations in special 1000 mg/L.

Keywords: chlorophyll; flowering; humic acid; Ion; *Pelargonium*; saline water

Citation: Elhindi, K.M.; Almana, F.A.; Al-Yafrsi, M.A. Role of Humic Acid on Inducing Salt Tolerance of Ivy Geranium (*Pelargonium peltatum* L.) Plants. *Horticulturae* **2023**, *9*, 1012. https://doi.org/10.3390/horticulturae9091012

Academic Editors: Pedro Loeza-Lara, Gustavo Santoyo and Rafael Jiménez-Mejía

Received: 26 July 2023
Revised: 4 September 2023
Accepted: 5 September 2023
Published: 8 September 2023

1. Introduction

Ornamental and flowering plants (OFP) take an imperative place in the horticultural business as they are utilized in gardening, roads, landscaping and as cut flowers [1,2]. Growing OFP is a dynamically developing and profitable sector of plant production. The global market of OFP moves 250 to 400 billion dollars annually in the European Union, USA and Japan [3,4]. Due to the variety of temperatures, soil and flora in Saudi Arabia (SA), the OFP agribusiness has the potential to expand, resulting in a rise in the production of native and foreign species. *Pelargonium* is a genus of 400 species widely distributed worldwide. It comes in the third rank within potted OFP with USD 2.5 billion yearly production [5,6]. In addition to the bioremediation capacity, they may also be grown in harsh environments, such as saline and calcareous soil [7]. Recently, the study of exploiting saline water on Ivy geranium (*P. peltatum* (L.) L'Hér. ex Aiton) plant development and flowering has been very scarce and needs more investigation.

Water consumptionis the main constraint to OFP production (each one kg plant dry mass needs 100–350 kg of water) [8]. So, the utilization of marginal water resources in irrigation ranging between 1000–6000 mg/L NaCl [9], such as recycled or salinized water,

has been encouraged by the growth in population and agricultural production along with the decline of high-quality water sources, particularly in arid areas like SA [10,11]. Irrigation with saline water has a drastic impact on soil–water–plant relations, i.e., intermittently deteriorating soil resources, strictly hampering the regular physio-biochemical pathways, alongside plant retardation, and initiating salty soil [12–14]. Crop development, flowering, productivity and primary carbon metabolism are all adversely impacted by high salt levels due to osmotic effects, nutritional imbalances and oxidative stress [14–16]. Additionally, salinity induces ion toxicity and nutritional imbalance due to the excessive uptake of sodium [11,12]. This makes it urgent to improve salt tolerance through spraying with attenuating substances like ions, plant growth substances and biostimulants [16–21].

Humic acid (HA) represents the prime component of organic humus [22,23], and its structure is still a matter of discussion [24,25]. HA is assumed to be complex aromatic macromolecules with amino acids, amino sugars, peptides and aliphatic complexes elaborate in connections among the aromatic groups. The hypothetical structure for HA contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units, and COOH groups variously located on aromatic rings [26]. HA application directly or indirectly improved OFP establishment due to its positive role in accelerating several plant metabolic pathways and mitigating the drastic impact of environmental stresses; meanwhile, its precise mechanisms in lessening stress injury have not been well documented [27]. Amir and Hani [28] indicate that foliar spraying with HA increased biological yield, chlorophyll and carotenoid content, as well as essential oil yield of *Dracocephalum moldavica* L. plants, and the highest positive effect was observed under 400 mg/L HA. Nofal et al. [29] found that the spraying of HA significantly increased *Erantheumum pilchellum* plant height, leaf number per plant, fresh and dry weight, flower diameter and chlorophyll content. Hammam et al. [30] revealed that spraying geranium plants with HA significantly increased plant height, shoot fresh and dry weight, proline concentration and water use efficiency. Previous research has suggested that the beneficial effects of HA can be attributed to the activation of several metabolic enzymes, improving plant water status, maintaining ion and redox homeostasis and promoting secondary metabolite assimilation [31]. HA also helps plants absorb nutrients, and it is particularly crucial for the movement and availability of micronutrients [32]. Recently, Ennab et al. [33] recorded that the use of HA as soil addition and/or foliar spraying increased significantly macro and micro-nutrient contents as well as chlorophyll and proline concentration associated with improving plant growth trials.

Little is understood about the potential role of HA foliar spraying in alleviating salt injury in Ivy geranium plants. Subsequently, the existing study's goal was to assess the influence of HA concentration on growth, flowering and some other physiological attributes of salt-affected Ivy geranium plants. In addition, the opportunity to investigate the possible application of saline water to Ivy geranium plants is expected to open new avenues for the development of the ornamental plant industry in SA.

2. Materials and Methods

2.1. Experimental Layout

The two factorial pot trials were carried out in an automated greenhouse of the Nursery of Sustainability and Environmental Developmental Department, King Saud University, Riyadh, SA from 15 December 2022 to 25 April 2023 for assessing the effect of irrigation with saline water (230 'tap water control', 2000 and 4000 mg/L NaCl), HA foliar application (0, 500, 1000 and 2000 mg/L) and their interactions (3 saline water levels x 4 HA concentration) on Ivy geranium plant growth, flowering and some other physiological trials. The experiment had 12 treatments with 6 replicates (pots, one plant per pot). The garden soil was sandy in texture (88.22% silt, 8.78% clay and 3% sand), with pH 7.64, EC 1.47 dSm^{-1}, bulk density 1.40 g/cm^3, cation exchange capacity 35.94 meq/100 g soil, organic matter 1.82%, available N 57.20 mg/kg soil, available P 7.93 mg/kg soil and available potassium 120.16 mg/kg soil.

Terminal cuttings of Ivy geranium were taken from the F1 seed mother plants (Kim variety, Blocompic, Holland, MI, USA) on 15 December 2022 and then their bases were dipped in rooting hormone (Rhizopona, 0.8%, Schutz Company, Briogeton, NJ, USA) and consequently planted in 10 cm plastic pots containing peat moss and perlite for rooting. The 35 days of homogenous rooted cuttings were planted in 25 cm plastic pots with 5 kg garden soil. The seedlings were endorsed to establish for 14 days under irrigation with a nutrient solution (Sangral NPK 20:20:20, SQM Europe, Antwerp, NV, Belgium) before the initiation of saline water irrigation treatments. Three levels of saline water (230 'tap water control', 2000 and 4000 mg/L NaCl) were used for irrigation every 3 days throughout the experimental time at 80% of soil field capacity. HA levels (0, 500, 1000 and 2000 mg/L) were sprayed 5 times at intervals of 15 days starting on 6 February 2023.

Six plants (90 days from planting) from each treatment (every 2 plants represent one replicate) were used for recorded morphological and flowering attributes as well as some physiological characteristics.

2.2. Vegetative Growth

Vegetative growth attributes were determined, including stem length (cm), stem diameter (cm), number of leaves/plant, as well as shoot fresh and dry weights/plant (g). Additionally, leaf area per plant (cm^2) was estimated using leaf area meter LI-3000 COR (Walz Co., Forest Grove, OR, USA).

2.3. Flowering Attributes

The numbers of inflorescences $plant^{-1}$, inflorescence diameter (cm), inflorescence stock length (cm), as well as inflorescences fresh and dry weights (g), were also recorded at 90 days from planting.

2.4. Physiological Growth Characteristics

Leaf greenness (SPAD) or chlorophyll content index was estimated with a SPAD-502 Plus chlorophyll meter (Konica Minolta, Tokyo, Japan). Nine measurements were occupied per leaf and averaged to provide a single record per leaf.

Proline concentration in the plant shoot was estimated following the protocol of Bates et al. [34] using a ninhydrin reagent. An aliquot of fresh leaf tissues was extracted by aqueous sulfosalicylic acid. The extract was combined with acid ninhydrin reagent for 1 h in a boiling water bath. The chromophore was collected by toluene; the optical density was measured at 520 nm; and proline concentration (µg/g fresh weight 'FW') was determined based on the calibration curve by proline.

Nitrogen (N) and phosphorus (P) contents were extracted from shoot dry weight with 5 mL of H_2SO_4 at 100 °C for 2 h; an aliquot of $H_2SO_4/HClO_3$ mix was dispensed dropwise; subsequently, the digestible was chilled for 15 min at Laboratory Temperature (22–24 °C) following Association of Official Analytical Chemists (A.O.A.C.) protocol [35]. N content was measured with the micro-Kjeldahl outline. The technique of Cooper [36] was used for the estimation of P with a phosphate standard curve. Meanwhile, potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), iron (Fe), zinc (Zn) and sodium (Na) were extracted by acid digestion (70% nitric acid and 30% hydrochloric acid) in a Milestone MLA 1200 Mega microwave digestion device, then assessed using iCAPTM 7000 Plus Series ICP-OES (Thermo ScientificTM, Waltham, MA, USA), following A.O.A.C. scheme [35].

2.5. Statistical Analysis

Data was exposed to the two-way analysis of variance (ANOVA) using the CoHort Software, version number 6.303 statistical package (CoHort software, 2006; Birmingham, UK) to evaluate the role of humic acid in mitigating salt injury. When significant ($p \leq 0.05$), a comparison of means (Tukey test) was implemented. The data existing are mean with standard error (SE). The statistical significance was considered as * $p \leq 0.05$, ** $p \leq 0.01$; and *** $p \leq 0.001$.

3. Results

3.1. Growth Attributes

Ivy geranium plant growth significantly ($p \leq 0.05$) decreased by increasing salinity levels up to 4000 mg/L in irrigation water relative to irrigation with tape water (230 mg/L NaCl)(Figure 1a, Table 1). Irrigation with 2000 mg/L NaCl strongly reduced stem length, leaf area per plant and shoot dry weight (50–57%) and had less effect on stem diameter, leaf number plant and shoot fresh weight (15–29%). When treated with 4000 mg/L NaCl dramatically decreased stem length, leaf number/plant, leaf area and shoot dry weight (61–71%) and had less effect on stem diameter and shoot fresh weight (35–40%), respectively, over non-salinized control plants (Figure 1a, Table 1).

Application of HA concentrations significantly ($p \leq 0.05$) increased all growth attributes of Ivy geranium plants over untreated plants. The uppermost values of vegetative trials were obtained and recorded once spraying with 1000 mg/L HA, following 500 mg/L HA, and finally by 2000 mg/L HA. HA at 1000 mg/L significantly boosted ($p \leq 0.05$) stem length, stem diameter, leaves number/plant, leaf area, shoot fresh and dry weights by 98.6, 57.2, 97.4, 81.7, 43.2and 81.6%, respectively, over 0 mg/L HA treated plants.

Application of HA concentration gave an encouraging impact and growth improvements at all levels of salt stress and consequently performed as growth stimulants. Accordingly, it is possibly mentioned that HA levels could be lessened by the detrimental impacts of salty water (2000 and 4000 mg/L NaCl), which boosted entire growth trials of Ivy geranium plants under salinity. HA at 1000 mg/L attained the greatest tolerance against severe salinity (4000 mg/L) and enhanced plant growth attributes (Figure 1b, Table 1)

Table 1. Effect of humic acid (HA), salinity and their interactions on Ivy geranium plant growth attributes at 90 days from planting.

Treatments	Stem Length (cm)	Stem Diameter (mm)	Leaf Number plant^{-1}	Leaf Area (cm^2)	Shoot FW (g)	Shoot Dry Weight (g)
NaCl salinity (mg/L)						
230 tap water (S0)	32.2 ± 2.8a	29.1 ± 1.1a	55.2 ± 4.3a	655.2 ± 51.4a	195.0 ± 8.2a	92.1 ± 6.8a
2000 (S1)	15.8 ± 0.9b	20.7 ± 1.1b	45.4 ± 3.0b	322.0 ± 26.0b	166.0 ± 8.0b	39.4 ± 2.7b
4000 (S2)	12.4 ± 0.9b	18.8 ± 1.1c	21.5 ± 1.7c	253.4 ± 17.4b	116.8 ± 3.5c	26.5 ± 1.8c
ANOVA *p* values	***	***	***		***	***
HA (mg/L)						
0 (H0)	13.5 ± 1.7b	17.8 ± 1.6d	25.6 ± 3.0c	290.8 ± 43.6c	130.0 ± 10.1d	38.5 ± 7.4c
500 (H1)	22.9 ± 3.8a	24.1 ± 1.6b	47.7 ± 6.4a	441.2 ± 75.9ab	169.3 ± 12.3b	58.6 ± 11.5b
1000 (H2)	26.8 ± 4.2a	28.1 ± 1.5a	50.6 ± 5.8a	528.5 ± 93.0a	186.2 ± 14.9a	70.0 ± 13.5a
2000 (H3)	17.4 ± 2.4b	21.4 ± 1.6c	38.7 ± 4.9b	380.2 ± 44.3bc	151.5 ± 9.4c	43.5± 7.7c
ANOVA *p* values	***	***	***	***	***	***
Interaction effects						
S0H0	20.0 ± 0.5d	24.0 ± 0.5de	34.0 ± 0.5d	451.7 ± 18.8cd	167.3 ± 5.8d	67.6 ± 1.4c
S0H1	38.4 ± 0.2b	30.3 ± 0.8b	68.3 ± 0.8a	719.3 ± 15.3b	205.0 ± 4.0b	104.0 ± 4.0b
S0H2	43.6 ± 0.7a	34.0 ± 0.5a	68.6 ± 0.0a	893.8 ± 39.0a	233.0 ± 4.5a	123.0 ± 0.5a
S0H3	27.0 ± 0.6c	28.0 ± 0.5bc	50.0 ± 0.5bc	556.0 ± 3.53bc	174.6 ± 6.8d	74.0 ± 0.5c
S1H0	12.0 ± 0.5fg	16.3 ± 0.3hi	29.0 ± 0.5de	266.4 ± 0.8ef	123.6± 0.8e	30.0 ± 0.5fg
S1H1	16.6 ± 0.7e	22.0 ± 0.5ef	51.0 ± 1.1bc	318.6 ± 103.3d–f	180.6 ± 1.2cd	42.0 ± 0.5e
S1H2	20.2 ± 0.4d	26.3 ± 0.8cd	54.3 ± 2.4b	393.0 ± 2.9c–e	194.6 ± 2.6bc	53.0 ± 1.1d
S1H3	14.3 ± 0.6ef	18.3 ± 0.3gh	47.3 ± 0.8c	310.2 ± 8.2d–f	165.0 ± 0.5d	32.6 ± 0.3f
S2H0	8.5 ± 0.5h	13.3 ± 0.3i	14.0 ± 0.5g	154.4 ± 0.8f	99.0 ± 0.5f	18.0 ± 0.5h
S2H1	13.9 ± 0.5e–g	20.0 ± 0.5fg	24.0 ± 0.5ef	285.7 ± 0.5d–f	122.3 ± 0.8e	30.0 ± 0.5fg
S2H2	16.5 ± 0.5e	24.0 ± 0.5de	29.0 ± 0.5de	298.8 ± 0.5d–f	131.0 ± 0.5e	34.0 ± 0.5f
S2H3	11.0 ± 0.5gh	18.0 ± 0.5gh	19.0 ± 0.5fg	274.6 ± 0.5ef	115.0 ± 1.0ef	24.0 ± 0.5gh
ANOVA *p* values	***	***	***	***	***	***

Significance levels are denoted by *** $p \leq 0.001$. Mean values ± standard error in a column for each characteristic, with dissimilar letters are significantly different (Tukey test at $p \leq 0.05$).

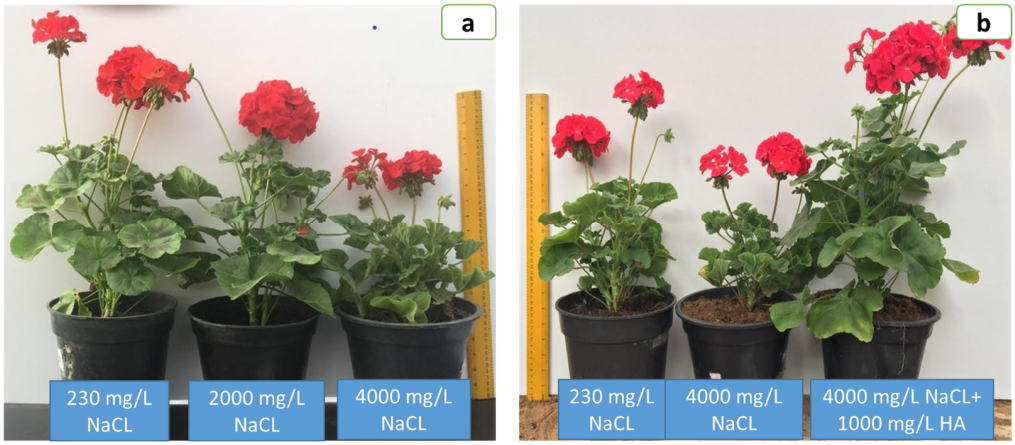

Figure 1. Effect of saline water (**a**) and role of 1000 mg/L humic acid (HA) on mitigation of the drastic effect of 4000 mg/L NaCl (**b**) on Ivy geranium plant growth attributes at full flowering (90 days from planting).

3.2. Flowering Attributes

Salinity levels up to 4000 mg/L significantly ($p \leq 0.05$) decreased all flowering characteristics, over control plants. The lowermost values were achieved under 4000 mg/L which decreased inflorescence number/plant, inflorescence stalk length, inflorescence diameter and inflorescence fresh and dry weights by 49.4, 67.0, 62.8, 60.9 and 74.4%, respectively, over the control Ivy geranium plant (Table 2).

Table 2. Effect of humic acid (HA), salinity and their interactions on Ivy geranium plant flowering attributes at 90 days from planting.

Treatments	Inflorescence No/Plant	Inflorescence Stalk Length (cm)	Inflorescence Diameter (mm)	Inflorescence Fresh Weight (g)	Inflorescence Dry Weight (g)
NaCl salinity (mg/L)					
230 tap water (S0)	68.0 ± 2.8a	23.5 ± 1.9a	50.0 ± 2.4a	57.5 ± 6.4a	27.5±2.9a
2000 (S1)	43.0 ± 1.6b	10.0 ± 0.7b	36.1 ± 2.2b	33.7 ± 1.8b	8.3 ± 0.5b
4000 (S2)	34.4 ± 2.2c	7.7 ± 0.7b	18.5 ± 1.4c	22.5 ± 1.8c	7.0 ± 0.7b
ANOVA *p* values	***	***	***	***	***
HA (mg/L)					
0 (H0)	37.4 ± 4.2d	9.8 ± 1.8c	25.1 ± 3.9d	23.7 ± 2.4c	8.9 ± 1.6c
500 (H1)	52.0 ± 5.7b	15.2 ± 2.7a	37.7 ± 4.8b	40.7 ± 6.0b	15.4 ± 3.6b
1000 (H2)	56.7 ± 4.9a	18.8 ± 3.3b	43.4 ± 5.3a	54.0 ± 8.8a	21.1 ± 5.1a
2000 (H3)	47.8 ± 5.4c	11.0 ± 1.9c	33.3 ± 4.0c	33.1 ± 3.9bc	11.6 ± 2.8bc
ANOVA *p* values	***	***	***	***	***
Interaction effects					
S0H0	52.6 ± 1.2c	17.3 ± 0.3c	38.6 ± 0.3d	30.6 ± 0.3de	15.3 ± 0.3d
S0H1	74.3 ± 0.3ab	26.0 ± 1.0b	53.3 ± 0.8b	64.0 ± 1.5b	30.0 ± 1.1b
S0H2	76.3 ± 0.8a	32.3 ± 0.6a	61.0 ± 0.5a	88.0 ± 5.1a	41.6 ± 1.7a
S0H3	69.0 ± 0.5b	18.3 ± 1.8c	47.0 ± 0.5c	47.3 ± 1.4c	23.0 ± 0.5c
S1H0	35.2 ± 2.1g	7.0 ± 0.5f	25.3 ± 0.3f	26.0 ± 1.1d-f	6.8 ± 0.1gh
S1H1	45.0 ± 0.5de	11.0 ± 0.5de	40.0 ± 0.5d	34.0 ± 0.5d	8.6 ± 0.3e-g
S1H2	50.0 ± 0.5cd	13.3 ± 0.3d	45.3 ± 0.3c	43.0 ± 0.5c	11.0 ± 0.5e
S1H3	42.0 ± 0.5ef	8.8 ± 0.6ef	34.0 ± 0.5e	32.0 ± 0.5de	7.0 ± 0.5f–h
S2H0	24.4 ± 2.1h	5.3 ± 0.3f	11.3 ± 0.3h	14.6 ± 0.3g	4.8 ± 0.1h
S2H1	36.6 ± 2.8fg	8.6 ± 0.3ef	20.0 ± 0.5g	24.3 ± 1.6ef	7.6 ± 0.6e–h
S2H2	44.0 ± 0.5de	11.0 ± 0.5de	24.0 ± 0.5f	31.0 ± 0.0de	10.6 ± 0.3ef
S2H3	32.6 ± 0.3g	6.0 ± 0.5f	19.0 ± 1.1g	20.0 ± 0.5fg	5.0 ± 0.5gh
ANOVA *p* values	***	***	***	***	***

Significance levels are denoted by *** $p \leq 0.001$. Mean values ± standard error in a column for each characteristic, with dissimilar letters are significantly different (Tukey test at $p \leq 0.05$).

Alternatively, the application of HA boosted flowering attributes. Spraying with 1000 mg/L HA provided the maximum values of flowering characteristics, the increment was 51.7, 90.9, 72.9, 127.2 and 134.9% for inflorescence number/plant, inflorescence stalk length, inflorescence diameter, inflorescence fresh and dry weights, respectively, as compared with untreated plants (Table 2).

The interactive impact of saline water and HA on flowering attributes indicates that the highest inflorescence number plant^{-1}, inflorescence stalk length, inflorescence diameter and inflorescence fresh and dry weights were obtained from the treatment of tap water plus 1000 mg/L HA over all treatments. Within severe salinity (4000 mg/L) utilization of 1000 mg/L HA increased inflorescence number/plant, inflorescence stalk length, inflorescence diameter, inflorescence fresh and dry weights by 7%, respectively, over non-treated plants that irrigated with 4000 mg/L NaCl (Table 2).

3.3. Chlorophyll

The value of chlorophyll index was individually affected ($p \leq 0.05$) by saline water and/or HA spray (Table 3). The maximum chlorophyll level (SPAD) was achieved from Ivygeranium irrigated with tape water. Increasing salinity levels induced a significant decline in SPAD value. This reduction reached 37% for geranium irrigated with 4000 mg/L NaCl relative to plants irrigated with tap water.

Table 3. Effect of humic acid (HA), salinity and their interactions on Ivy geranium plant chlorophyll (mg/g FW) and proline (µg/g FW) concentration at 90 days from planting.

Treatments	Chlorophyll	Proline
NaCl salinity (mg/L)		
230 tap water (S0)	22.0 ± 1.9a	147.4 ± 5.9c
2000 (S1)	15.5 ± 0.7b	228.0 ± 3.7b
4000 (S2)	13.9 ± 0.6b	246.7 ± 3.3a
ANOVA *p* values	***	***
HA (mg/L)		
0 (H0)	12.5 ± 0.5c	188.4 ± 17.7d
500 (H1)	18.1 ± 1.5b	213.7 ± 15.1b
1000 (H2)	22.6 ± 2.2a	226.1 ± 13.5a
2000 (H3)	15.4 ± 0.8bc	201.3 ± 14.5c
ANOVA *p* values	***	***
Interaction effects		
S0H0	14.0 ± 0.5ef	118.7 ± 0.5j
S0H1	24.0 ± 0.5b	154.0 ± 0.9h
S0H2	31.6 ± 0.8a	172.9 ± 0.6g
S0H3	18.6 ± 0.3c	144.2 ± 0.5i
S1H0	12.6 ± 0.3fg	212.3 ± 0.6f
S1H1	16.0 ± 0.0de	234.5 ± 0.6d
S1H2	19.3 ± 0.3c	244.3 ± 0.6c
S1H3	14.3 ± 0.3ef	221.0 ± 0.4e
S2H0	11.0 ± 0.5g	234.3 ± 0.4d
S2H1	14.3 ± 0.3ef	252.7 ± 0.7b
S2H2	17.0 ± 0.5cd	261.1 ± 0.7a
S2H3	13.3 ± 0.3fg	238.8 ± 0.1cd
ANOVA *p* values	***	***

Significance levels are denoted by *** $p \leq 0.001$. Mean values ± standard error in a column for each characteristic with dissimilar letters are significantly different (Tukey test at $p \leq 0.05$).

Conversely, HA spraying caused a substantial rise in chlorophyll index in ivy geranium plants over nontreated plants (0 mg/L HA). The greatest values of chlorophyll were recorded once plants were sprayed with 1000 mg/L, followed by 500 mg/L and, finally, 2000 mg/L relative to untreated plants (Table 3).

Likewise, the data in Table 3 reveal that HA as a biostimulant mitigated the harmful impacts of saline water on the chlorophyll index in Ivy geranium plants. HA at 1000 mg/L was utmost effective in this respect and increased chlorophyll by 52.7 and 54.5% as compared with untreated plants (0 mg/L HA) that were irrigated with 2000 and 4000 mg/L NaCl, respectively.

3.4. Proline

Considerable concentrations of free proline were observed under salinity and HA compared with control plants (Table 3). Irrigation with saline water up to 4000 mg/L induced the hyperaccumulation of proline in Ivy geranium plant shoots. Severe salinity produced 67.3% greater proline concentration compared to non-salinized plants (Table 3).

Proline accumulation was raised by the supplementation of HA concentrations as compared to untreated plants. The more effective concentration of HA was 1000 mg/L compared to untreated plants (Table 3).

Regarding interactive effects, spraying HA substantially induced proline accretion in Ivy geranium under saline and non-saline conditions over plants with no HA addition (Table 3). The ultimate concentration of proline was recognized with 1000 mg/L HA spraying together with severe salinity, which boosted proline by 120%.

3.5. Ion Content

Ion contents (macro and micro-nutrient) were significantly ($p \leq 0.05$) affected by salinity, HA and their combinations (Tables 4 and 5). Data recorded that salinity stress progressively decreased all ions except Fe and Na, which is raised with increasing salinity stress. The lowest content of N (54.8%), P (50.7%), K (48.2%), Ca (45.1), Mg (45.9), Mn (36.8%), Cu (33.5%) and Zn (35.6%) was recorded under 4000 mg/L NaCl over nonsalinized plants. Meanwhile, 4000 mg/L NaCl significantly ($p \leq 0.05$) increased Fe and Na by 44.3 and 61.3% relative to non-salinized treatment.

Table 4. Effect of humic acid (HA), salinity, and their interactions on Ivy geranium macro-nutrient concentration (mg/total dry weight) at 90 days from planting.

Treatments	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
NaCl salinity (mg/L)					
230 tap water (S0)	372.2 ± 12.1a	81.7 ± 3.0a	502.1 ± 6.8a	48.7 ± 3.0a	47.6 ± 3.0a
2000 (S1)	264.1 ± 4.8b	76.7 ± 2.9b	398.0 ± 15.8b	33.1 ± 2.1b	32.1 ± 2.1b
4000 (S2)	168.0 ± 9.2c	40.2 ± 1.7c	259.7 ± 8.5c	26.7 ± 2.4c	25.7 ± 2.5c
ANOVA *p* values	***	***	***	***	***
HA (mg/L)					
0 (H0)	243.9 ± 32.5c	55.7 ± 5.4c	340.5 ± 38.0c	23.1 ± 3.3c	22.0 ± 3.3c
500 (H1)	289.7 ± 32.2a	73.0 ± 6.9a	410.8 ± 35.5a	38.9 ± 3.1b	42.3 ± 5.0a
1000 (H2)	285.1 ± 33.5ab	75.9 ± 7.3a	424.6 ± 36.4a	45.6 ± 4.5a	40.5 ± 2.5a
2000 (H3)	253.7 ± 22.9bc	60.3 ± 6.4b	370.6 ± 31.8b	37.0 ± 2.1b	35.7 ± 2.2b
ANOVA *p* values	***	***	***	***	***
Interaction effects					
S0H0	330.9 ± 2.7c	69.4 ± 0.6e	475.9 ± 0.37c	35.5 ± 0.3e	34.2 ± 0.5e
S0H1	408.3 ± 0.6b	88.8 ± 0.3b	513.5 ± 0.6b	50.9 ± 0.4b	62.2 ± 0.5a
S0H2	416.3 ± 0.6a	94.4 ± 0.4a	533.3 ± 0.6a	63.2 ± 0.5a	50.0 ± 0.4b
S0H3	333.3 ± 0.4c	74.3 ± 0.5d	485.8 ± 0.2c	45.2 ± 0.5c	44.2 ± 0.5c
S1H0	284.3 ± 0.5d	63.2 ± 0.6f	333.4 ± 0.6f	21.1 ± 0.4g	20.1 ± 0.4h
S1H1	274.4 ± 0.7e	85.2 ± 0.5c	444.4 ± 0.6d	35.9 ± 0.3e	34.9 ± 0.3e
S1H2	244.3 ± 0.5g	86.5 ± 0.7bc	454.6 ± 0.7d	40.2 ± 0.6d	39.3 ± 0.6d
S1H3	253.4 ± 0.6f	71.9 ± 0.1de	359.8 ± 7.4e	35.0 ± 0.4e	34.0 ± 0.4e
S2H0	116.5 ± 0.6k	34.4 ± 0.6h	212.3 ± 0.6i	12.6 ± 0.7h	11.6 ± 0.7i
S2H1	186.5 ± 0.5i	45.1± 0.4g	274.4 ± 0.6h	30.0 ± 0.3f	29.9 ± 0.1fg
S2H2	194.5 ± 0.6h	46.7 ± 0.6g	285.9 ± 0.3g	33.4 ± 0.5e	32.4 ± 0.5ef
S2H3	174.6 ± 0.6j	34.6 ± 0.5h	266.2 ± 0.5h	30.9 ± 0.1f	29.0 ± 0.3g
ANOVA *p* values	***	***	***	***	***

Significance levels are denoted by *** $p \leq 0.001$. Mean values ± standard error in a column for each characteristic, with dissimilar letters are significantly different (Tukey test at $p \leq 0.05$).

Table 5. Effect of humic acid (HA), salinity and their interactions on Ivy geranium micro-nutrient concentration (mg/total dry weight) at 90 days from planting.

Treatments	Manganese	Copper	Iron	Zinc	Sodium
NaCl salinity (mg/L)					
230 tap water (S0)	0.95 ± 0.00a	0.18 ± 0.01a	1.55 ± 0.03b	0.37 ± 0.00a	47.8 ± 1.3c
2000 (S1)	0.82 ± 0.02b	0.20 ± 0.00b	2.24 ± 0.10a	0.30 ± 0.02b	67.5 ± 3.2b
4000 (S2)	0.60 ± 0.02c	0.12 ± 0.00c	2.25 ± 0.07a	0.24 ± 0.01c	77.1 ± 3.0a
ANOVA *p* values	***	***	***	***	***
HA (mg/L)					
0 (H0)	0.71 ± 0.06c	0.13 ± 0.01c	1.65 ± 0.05c	0.24 ± 0.02d	77.9 ± 5.8a
500 (H1)	0.81 ± 0.05b	0.17 ± 0.01b	2.07 ± 0.14b	0.34 ± 0.01b	60.4 ± 3.9b
1000 (H2)	0.87 ± 0.04a	0.20 ± 0.01a	2.28 ± 0.13a	0.37 ± 0.01a	55.7 ± 3.5c
2000 (H3)	0.78 ± 0.04b	0.16 ± 0.01b	2.06 ± 0.14b	0.27 ± 0.02c	62.4 ± 4.0b
ANOVA *p* values	***	***	***	***	***
Interaction effects					
S0H0	0.92± 0.00ab	0.14 ± 0.00d	1.47 ± 0.04g	0.34 ± 0.00c	55.1± 0.0g
S0H1	0.97 ± 0.00a	0.20 ± 0.00b	1.51 ± 0.06g	0.38 ± 0.00b	44.9± 0.3hi
S0H2	0.98 ± 0.00a	0.23 ± 0.00a	1.74 ± 0.00ef	0.42 ± 0.00a	44.3 ± 0.61i
S0H3	0.95 ± 0.00a	0.17 ± 0.00c	1.50 ± 0.00g	0.37 ± 0.00bc	46.8 ± 0.2h
S1H0	0.74 ± 0.00de	0.17 ± 0.00c	1.66 ± 0.00f	0.23 ± 0.00f	84.2 ± 0.5b
S1H1	0.85 ± 0.03bc	0.19 ± 0.00bc	2.33 ± 0.00c	0.35 ± 0.00c	65.2 ± 0.5f
S1H2	0.92 ± 0.00ab	0.25 ± 0.00a	2.56 ± 0.00a	0.38 ± 0.00b	54.0± 0.7g
S1H3	0.80 ± 0.00cd	0.19 ± 0.00bc	2.44 ± 0.00bc	0.24 ± 0.00f	66.4 ± 0.3ef
S2H0	0.49 ± 0.03g	0.10 ± 0.00e	1.84 ± 0.00e	0.15 ± 0.00g	94.5 ± 0.6e
S2H1	0.61 ± 0.00f	0.13 ± 0.00d	2.38 ± 0.00c	0.28 ± 0.00e	71.1 ± 0.4d
S2H2	0.71 ± 0.01e	0.13 ± 0.00d	2.54 ± 0.00ab	0.32 ± 0.00d	68.8 ± 0.1de
S2H3	0.61 ± 0.00f	0.12 ± 0.00de	2.24 ± 0.00d	0.22 ± 0.00f	74.0 ± 0.3c
ANOVA *p* values	***	***	***	***	***

Significance levels are denoted by *** $p \leq 0.001$. Mean values ± standard error in a column for each characteristic, with dissimilar letters are significantly different (Tukey test at $p \leq 0.05$).

On the other hand, HA concentrations foliar spraying increased all ion concentrations except Na which decreased in the shoot of the Ivy geranium plant over non-treated plants. The most effective concentration of HA in increasing N, P, K, Ca, Mg, Mn, Cu, Fe and Zn, as well as decreased Na, was 1000 mg/L over nontreated plants (Tables 4 and 5).

Foliar spray with HA concentrations under all levels of saline water distinctly invalidates their determinantal impact on ion content. The foremost effective concentration was 1000 mg/L HA, which increased N (66.9%), P (35.8%), K (34.6%), Ca (164.6%), Mg (179.2%), Mn (44.8%), Cu (36.0%), Fe (38.0%) and Zn (113.3%), meanwhile declining Na (27.1%) compared with unsprayed severe salt-affected plants.

4. Discussion

Reduced agricultural water consumption and increased water use efficiency are needed globally to protect water for human use [37]. This disorder encourages the adoption of some alternative water resources for irrigation, such as saline water, which hastens the onset of salinized soil. Accordingly, some researchers have shown that foliar spraying of HA concentrations is able to play a substantial function in boosting plant establishment by increasing a plant's aptitude to endure stress tolerance [38,39]. Results from the present study show that salinity stress reduced Ivy geranium growth and flowering as well as ion (N, P, K, Ca, Mg, Mn, Cu and Zn) and chlorophyll but raised Fe, Na and proline. Alternatively, HA in special 1000 mg/L considerably boosted all examined characteristics while minimizing Na accumulation.

The injury impact of salinity on vegetative growth trials was established previously [12,14]. These depressive effects might be attributed to the distribution of biophysiological pathways and molecular modifications, i.e., photosynthesis, nutrient balance,

reactive oxygen species (ROS) buildup and alterations in salt injury in different plants. Additionally, salinity stress may be hindering ion absorption resulting from the occurrence of Na and chloride ions in irrigation water or the permeability of these ions to the plant tissues, which consecutively causes ionic toxicity alongside a decline in the vegetative growth characteristics of plants [40]. Additionally, salinity induces hormonal imbalance that participates in cellular division and enlargement that negatively affects plant growth [41,42]. HA supplementation normally increases plant growth and lessens salinity injury [43,44]. The motivating impact of HA under normal or stressful circumstances is devoted to hastening photosynthesis pathways and enhancing photo-assimilation translocation in plants [45]. HA application improved hormonal and ROS balance [46]; as well as activation of antioxidant enzymes and accelerating organic solute accumulation [47], which was ultimately reflected in plant growth [22]. Moreover, the encouraging role of HA can be linked to its impact on boosting interior carbon dioxide concentration and leaf thickness, improving cell water maintenance and boosting water use efficiency [30]. Additionally, HAs' positive effect could be due to the hormone-like activity or may be connected to encouraging indole acetic acid assimilation, which accelerates cell division and enlargement as well as eradicating ROS [48]. Additionally, gibberellic acid (GA)-like substances and activity in humic substances have been reported since the 1990s [49], accordingly, the accumulation of gibberellin may accelerate the cell elongation-related genes that are induced cell elongation [50], raise cell permeability and recover the absorption of nutrients [51,52].

A current study proved that flowering attributes markedly decreased with saline water, which was confirmed previously in different plants [13,53]. Therefore, salt-affected plants may lessen flowering intensity, delay flowering and shorten the flowering period [8]. Flower stalk length is an energetic quality feature of OFP as it impacts the commercial importance of cut flower crops. Flower length and diameter, stem thickness and length were considerably decreased by raising the salinity level over control, which was approved by Kucukahmetler [54]. According to Ahmad et al. [55], the application of saline water blocks the vascular system and eventually restricts water uptake. The reduction in flowering due to salinity may be attributable to the decline of plant photosynthesis through the variations in chlorophyll levels and components and the destruction of chloroplasts [56]. Moreover, it hinders photochemical activities and reduces the Calvin cycle enzyme activities [57], modifying the concentration of hormones straight intricate in flowering, such as abscisic and jasmonic acids [58]. HA not only encouraged vegetative growth but also floral attributes as a greater number of florets per spike were formed by plants treated with HA. Current findings are harmonized with the results of Nofal et al. [29] and Baldotto and Baldotto [44] who stated that HA improved the flowering of several ornamental and flowering plants when used at higher concentrations. These findings confirmed that HA spraying improved spike length, which established the function of HA in enhancing ion uptake and sequentially improved spike length and whole flower quality. Parallel outcomes of enhancement in ion uptake, particularly of N, P and S by the activity of HA, have also been stated by Atiyeh et al. [59] and Arancon et al. [60]. Additionally, given the presence of GA-like compounds and activity in HS that have been reported since the 1990s, the stimulating effect of HA on blooming may be the result of the buildup of GA that accelerates flowering development [49].

It could be concluded that, from the current outcomes, chlorophyll content considerably declined under saline water up to 4000 mg/L. The degeneration in chlorophyll, once salinity occurs, could result from the drop in chlorophyll biosynthetic or boosted enzymatic chlorophyll deprivation [61], in addition, the degeneration of the thylakoid membranes and devastation of chlorophyll by diverse ROS, and alterations in chlorophyll protein complexes [62]. Moreover, salinity may induce a deterioration in chlorophyll biosynthesis intermediation and decrease the expression of ChlD, Chl Hand Chl I-1 gene encoding subunits of Mg-chelatase [63,64]. As indicated in the current findings and earlier research, utilization of HA has been recorded to improve chlorophyll accumulation in plants within stress or non-stress circumstances [65,66]. This increase may be attributed to the rise

in cytokinin assimilation, which accelerates chloroplast differentiation and chlorophyll biosynthesis and declines its degradation [67]. Additionally, HA probably keeps chlorophyll biosynthesis via the protection of the sulphydryl group and boosts Mg absorption and accumulation. The rise in chlorophyll levels by HA spraying might be caused by the hastening of N and NO_3uptake, improving N metabolism and assembly of protein [68]. Humic acid additionally increased N and K uptakes, which are elaborated in chloroplast differentiation and chlorophyll assimilation [69].

Within stress conditions, plants possess several strategies including a hyperaccumulation of organic osmolytes like proline without interfering with metabolic pathways to withstand stress conditions [70,71]. Current findings proved that irrigation with saline water with or without HA spraying significantly increased proline accumulation in plant tissues. Numerous occupations are anticipated for proline buildup within stress factor and/or HA spraying including osmotic adjustment, protein and enzyme stabilization, and ROS scavenging, besides acting as a reservoir of energy and N for exploitation [72]. Moreover, Bellinger et al. [73] suggested that the rise of proline in salt-affected plants might be deduced as a tolerance strategy of osmotic regulation and/or buildup of the extra ammonium created by salinity. Proline buildup can be explicated by the greater inhibitory rate of proline dehydrogenase and proline oxidase [71]. Yet, it is also possible to find a lessening in the proline production in plants caused by its fast breakdown upon stress reprieve. The breakdown products deliver reducing agents that support mitochondrial oxidative phosphorylation and generation of adenosine triphosphate (ATP) for rescue from stress and repairing stress-induced injury [74]. Moreover, HA appears to have an encouraging effect on enzyme activity and secondary plant metabolism [22], as well as plant respiration and photosynthesis, which in turn affect carbohydrate levels [75] and amino acid metabolism [76].

Salinity normally induces ion imbalance by declines in N, P, K, Mg, Ca, Mn, Cu and Zn associated with excess accretion of Fe and Na, which was confirmed previously [11,12,20]. The drastic impacts of salinity on plant nutritional status may be attributed to a decline in nutrient uptake and/or transport as well as ion toxicity [77]. The deterioration of root development within salinity could be one of the reasons behind the decline in plants' ion content [78]. Under salinity, the decrease in either N or P may result from the antagonism between both chloride and nitrate [79] or phosphate [80] molecules, respectively. The prevention of plant K uptake is chiefly caused by the physical and chemical similarities between K and Na that induced the competition on major binding sites [81]. Additionally, there is an antagonism between Ca and Mg with Na which affects membrane properties and causes a degeneration of membrane integrity and selectivity [82]. The current findings displayed that ions' content was progressively increased by HA concentrations, nevertheless, Na was reduced. These findings were consistent with those achieved by Ennab et al. [33] and Sahar et al. [83]. Additionally, HA maintained an extraordinary level of acid phosphate activity that increased phosphate activity holds for improved plant P uptake [84]. HA has been described to improve plant nutrient uptake due to the improving permeability of root membranes [85]. The findings additionally revealed that HA spraying possibly will lessen the destructive impact of salinity by maintaining leaf water status, dropping the uptake of Na and Cl [86], increasing Ca and K, motivating chloroplast development and improving phloem loading [87]. Also, HA has been displayed to improve plant membrane permeability, stimulating the uptake and translocation of nutrients and increasing root development [88]. Additionally, HA usually retained ATPase and Na/H antiport, which facilitate Na compartmentation under salinity [23]. Fernandez et al. [89] revealed that foliar spraying of leonardite extracts (as a natural source of HA) motivated shoot growth and promoted the buildup of several ions. It was stated that HA encourages H^+-ATPase activity in the plasma membrane and stimulates plant growth via the rise in lateral root emergence and whole root absorbance [90]. The rise in nitrogen by HA application may be attributed to the enhancement of nitrogen assimilation enzymes like nitrate reductase and nitrite reductase [91].

5. Conclusions

The current findings indicated that irrigating Ivy geranium plants with saline water up to 4000 mg/L NaCl adversely affected vegetative and flowering growth attributes, alongside decreasing nutrient contents (except Fe and Na). However, HA application special at 1000 mg/L helped in recovering plant growth and flowering to levels comparable to those of control plants.

Author Contributions: Conceptualization, K.M.E., F.A.A. and M.A.A.-Y.; methodology, K.M.E. and M.A.A.-Y.; software, K.M.E.; validation, K.M.E., F.A.A. and M.A.A.-Y.; formal analysis, K.M.E., F.A.A. and M.A.A.-Y.; investigation, K.M.E. and M.A.A.-Y.; resources, K.M.E., F.A.A. and M.A.A.-Y.; data curation, K.M.E.; writing—original draft preparation, K.M.E., F.A.A. and M.A.A.-Y.; writing—review and editing, K.M.E. and F.A.A.; visualization, K.M.E.; supervision, F.A.A.; project administration, F.A.A.; funding acquisition, K.M.E., F.A.A. and M.A.A.-Y. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Deputyship for Research and Innovation, "Ministry of Education" in Saudi Arabia for funding this research (IFKSUOR3-576-1).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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