

Article

Humic Substances Enhance Waterlogging Tolerance in Eggplant Through Differential Regulation of Redox Homeostasis and Nutrient Acquisition

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Highlights

What are the main findings?

- Humic acid and fulvic acid alleviated waterlogging-induced growth inhibition and improved nutrient acquisition in eggplant.
- Humic acid enhanced waterlogging tolerance by reducing oxidative damage and restoring redox homeostasis, whereas fulvic acid promoted growth through reactive oxygen species signaling.
- Humic substances improved chlorophyll content, nutrient uptake and antioxidant responses, resulting in enhanced tolerance to waterlogging stress.

What are the implications of the main findings?

- Humic substances represent sustainable bio-stimulants for improving crop resilience under climate change-induced waterlogging conditions.
- Differential physiological responses to humic and fulvic acids provide new insights for developing targeted strategies to enhance waterlogging tolerance in vegetable crops.



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Abstract

Eggplant (*Solanum melongena* L.), the world's fifth most important vegetable crop, is highly sensitive to waterlogging (WL), which limits its productivity under climate-driven flooding events. This study evaluated whether humic acid (HA) and fulvic acid (FA) alleviate WL-induced stress by improving nutrient acquisition and antioxidant defense. Eggplant seedlings were subjected to WL stress for 10 days with and without bio-stimulant treatments, under controlled conditions using a simple, completely randomized design. Waterlogging deteriorated soil properties, reducing pH from 7.7 to 5.4, organic matter by ~75%, soil nutrients 73%, resulting in a decline in plant growth (61%), nutrients (23–74%), chlorophyll concentrations (25–45%), and increased oxidative stress indicators (33–314%).

Both HA and FA significantly mitigated these stress effects, restoring plant growth by 11–43%, increasing chlorophyll by ~42%, and enhancing nutrients by 10–17-fold. However, HA and FA differentially modulated stress responses. HA reduced H₂O₂ and MDA by 40–75% and normalized antioxidant enzyme activities, indicating redox homeostasis, while FA maintained relatively higher H₂O₂ levels (133.7 mmol kg^{−1}) while improving growth and chlorophyll (~32–67%), suggesting a stress priming rather than stress suppression mechanism. Overall, HA conferred WL tolerance by limiting oxidative damage, whereas FA promoted growth through reactive oxygen species signaling, supporting the potential of humic substances as sustainable bio-stimulants for climate-resilient crop production.

Keywords: bio-stimulants; flooding tolerance; fulvic acid; humic acid; oxidative stress; redox homeostasis; *Solanum melongena*

1. Introduction

Climate change, especially extreme temperatures, rising sea levels, and an imbalanced hydrological cycle, results in an uneven distribution of rainfall and increased water stress (drought and floods) globally [1]. The frequency of floods and waterlogging conditions has been increasing due to land degradation, poor drainage, and climate change [2]. It is estimated that around 12% of the arable land is affected by waterlogging. It alters soil pH, oxygen, and nutrient availability, and redox potential, creating an anoxic environment for plants and reducing root respiration and overall growth [3]. Hence, irregular water supply negatively impacts plant growth and survival [4].

Waterlogging stress is a condition in which the plant's stem and leaves are partially submerged [5]. As a result, the plant shifts from aerobic respiration to anaerobic respiration, which is less efficient and leads to energy loss and the accumulation of toxic metabolites, such as aldehydes and alcohols, as by-products, further exacerbating the plant's stress condition [6]. Waterlogging can reduce crop yield up to 20–33% due to a reduction in plant height (−11%), biomass (−29%), net photosynthesis (−39%), and leaf area index (−23%) [7]. Plants experience compound stresses, including limited gas exchange and low light intensity, which impair photosynthesis, reduce growth (root and shoot development), and chlorophyll content [8], and increase vulnerability to pest attack, especially fungal diseases [5]. Additionally, it disrupts nutrient uptake, causing deficiencies of essential nutrients including nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca), as well as micronutrients like iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) [9]. However, a plant's response may vary from crop to crop, necessitating the assessment of food crops under waterlogging conditions.

Previous studies have reported negative impacts of waterlogging in major food crops, including maize [10], rapeseed [11], and mung bean [12]. Vegetable crops are more prone to and sensitive to waterlogging conditions, as reported for onion [13], cucumber [14], cabbage, tomato [15], and chili pepper [16]. Among solanaceous crops, eggplant (*Solanum melongena* L.) is the fifth most popular worldwide [17], after potato, tomato, pepper, and tobacco [18]. It is a warm-weather crop cultivated across ~2 million hectares in tropical and subtropical regions, with global production of 57.5 million tons (Mt) in 2024 [19]. It is a low-calorie vegetable rich in fiber, minerals, phenolics, proteins, vitamins, and antioxidants [20]. Although eggplant is known among vegetables for drought tolerance, it is highly sensitive to waterlogging conditions [21]. The seasonal crop water requirement for eggplant is 380 mm [20]. Excessive water reduces eggplant growth and development, significantly affecting plant height, fruit yield, and chlorophyll content [8,21]. Jiang et al. [22] found

that during early growth stages, chlorophyll a content is reduced under waterlogging stress. Moreover, they observed that prolonged stress led to degradation of chlorophyll b, whereas the carotenoids remained stable throughout the stress. Thus, it indicates that oxygen deprivation from waterlogging may degrade the photosynthetic machinery in eggplant, thereby reducing photosynthesis and ultimately affecting yield. As a protection mechanism against this stress, the plant was found to increase the root lignin content after 48 h [22].

Altogether, waterlogging stress poses a serious threat not only to crop production and food security but also to the soil profile, further endangering productivity. Hence, it is important not only to understand plant responses but also to identify effective strategies to mitigate this stress in plants and in the edaphic environment. To this end, humic substances have received increasing attention as natural bio-stimulants [23] to maintain plant growth, especially under waterlogging conditions, due to their positive effects on soil fertility and water-holding capacity [24]. Humic substances are complex organic compounds derived from decomposed plant and animal residues and are found in organic wastes such as peat, compost, leonardite, and lignite [25]. They are characterized as dark in color, carbon-rich, and include humic acids (HA), fulvic acids (FA), and humin [24]. As bio-stimulants, these compounds enhance nutrient uptake, fruit quality, and stress tolerance to disease and abiotic stresses, promote growth and early flowering, and support beneficial microorganisms [25]. Recently, they have been found to enhance plant growth and yield in many vegetable crops, including broccoli [23], cabbage [6], cucumber [26], and potato [27]. Under waterlogging conditions, plants supplemented with HA and FA showed a reduction in reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2 ; -35%), malondialdehyde (MDA; -30%), while a 1.4–2.0-fold increase in antioxidant enzymes like catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in cucumber [26]. Similarly, in cabbage, fulvic acid was found to be more proficient in reducing nutrient losses under waterlogging conditions [6]. Given eggplant's sensitivity to waterlogging, understanding its physiological response to waterlogging and identifying eco-friendly mitigation strategies are essential for improving crop resilience and ensuring sustainable production and food security. In particular, the use of humic substances may help mitigate the adverse effects of waterlogging, yet their role in eggplant remains poorly understood. This study thus hypothesized that exogenous application of humic and fulvic acid may enhance waterlogging tolerance in eggplant by improving physiological responses, scavenging reactive oxygen species, and modulating antioxidant enzymes. Eggplant seedlings were subjected to waterlogging stress, followed by exogenous application of HA and FA. Standard morphological traits and stress indicators were recorded, and enzyme concentration was measured to assess seedling responses, with a focus on alleviating stress and improving plant growth and resilience.

2. Materials and Methods

2.1. Experimental Material

Seeds of *Solanum melongena* L. cv. Aydın Siyahı were sown in a peat–perlite mixture and grown for 30 days under controlled conditions to obtain uniform seedlings. The seedlings were then transplanted into 1.5 L pots containing the experimental growing medium and allowed to acclimate before treatment application. Humic acid and fulvic acid were applied according to the experimental design, after which waterlogging stress was imposed for 10 days. Following the stress period, the respective bio-stimulant treatments were continued for an additional 7 days before plants were harvested for morphological, physiological, biochemical, and nutrient analyses. Thus, the complete experimental period from sowing to final harvest lasted approximately 90 days. The potting medium was profiled for physiochemical properties and nutrients, and was found suitable for eggplant

cultivation with 7.1 pH, 161.1 $\mu\text{mhos cm}^{-1}$ EC, 1.9% organic matter (O.M), 3.6% lime, macronutrients including 0.001% total nitrogen with 2.1 ppm ammonium nitrogen ($\text{NH}_4\text{-N}$) and 1.8 pp nitrate nitrogen ($\text{NO}_3\text{-N}$), 2.7 mg kg^{-1} P, 220.4 mg kg^{-1} K, 1738.3 mg kg^{-1} Ca, 115.4 mg kg^{-1} Mg, and 12.5 mg kg^{-1} Na, as well as micronutrients including 1.8 mg kg^{-1} Fe, 0.6 mg kg^{-1} Zn, and 0.3 mg kg^{-1} Mn.

2.2. Experimental Design and Growth Conditions

The experiment was conducted under controlled conditions maintained as 22–25 °C for a 16 h day period and 16–18 °C for 8 h nighttime, 50–55% relative humidity, and 150–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, following a triplicated completely randomized design (CRD) with six treatments viz. control, waterlogging (WL), humic acid (HA), WL+ HA, fulvic acid (FA), and WL + FA. The experiment involved a total of 108 plants (six plants per replicate, per treatment). The HA was sourced from Powhumus[®], while Fulvital[®] was used for fulvic acid, both derived from leonardite (Humintech, Am Pösenberg, Grevenbroich, Germany). Powhumus contained 55–60% HA, 73% O.M, 10% K_2O , while Fulvital had 33% FA along with Mg (5%), and 80% O.M. Before stress (WL) application, the rootzone of the seedlings (respective treatment set) was subjected to Powhumus and Fulvital at the rate of 10 kg ha^{-1} , the recommended dose as per the manufacturer's instructions. The dosage per pot and aqueous solution was prepared following Ekinci et al. [26].

To apply waterlogging stress, plastic-lined large containers were filled with water, and pots were placed in them. To ensure saturation and waterlogging (hypoxic) conditions, the water level was maintained at approximately 2–3 cm above the soil surface for 10 days [28]. Subsequently, the HA and FA treatments were continued for a week before terminating the experiment (50 days after transplantation).

2.3. Soil Nutrient Profile

To determine the effects of HA and FA treatments on soil, samples were collected from each pot after the experiment ended, oven-dried until constant weight, and homogenized for analysis. The analyses targeted soil pH (1:1), electrical conductivity (EC, $\mu\text{S cm}^{-1}$), lime concentration (CaCO_3 ; %), O.M (%), total N (N, %), available P (mg kg^{-1}), exchangeable K (cmol kg^{-1}), sulfur (S, mg kg^{-1}), Ca (mg kg^{-1}), Mg (mg kg^{-1}), Na (cmol kg^{-1}), boron (B, mg kg^{-1}), copper (Cu, ppm), Fe (ppm), Zn (ppm), and Mn (ppm). All the analyses were performed following the standard methods of soil analysis provided by the International Centre for Agricultural Research in the Dry Areas (ICARDA) [29]. The micronutrients were measured using the diethylene triamine pentaacetic acid (DTPA) method and quantified using an atomic absorption spectrophotometer (NovAA400P, Analytik Jena GmbH, Jena, Germany).

2.4. Morphological and Growth Parameters

Plant height (PH) was measured as the distance from the soil surface at the base of the plant to the apical growing point and expressed in centimeters (cm). Stem diameter (SD; mm) was recorded using a digital vernier caliper (500-196-30, Mitutoyo America Corporation, Aurora, IL, USA). Measurements were obtained at three locations on the stem and averaged. Samples were collected to measure shoot and root fresh weight (SFW, RFW, respectively; g) after washing with distilled water. Samples were then oven-dried at 65 °C for 48 h with periodic weighing until a constant weight was reached, and dry weight was recorded (shoot dry weight—SDW; root dry weight—RDW).

2.5. Mineral Nutrient Analysis

Oven-dried leaf samples were used for mineral nutrients determination including N (%), P (%), K (%), Ca (%), Mg (%), S (%), Mn (mg kg^{-1}), Fe (mg kg^{-1}), Zn (mg kg^{-1}),

B (mg kg^{-1}), Cu (mg kg^{-1}), and Na (mg kg^{-1}). Samples were ground to a fine powder, and nutrients were extracted using the wet digestion method described by Ryan et al. [30]. One gram of leaf sample was dissolved in a 10 mL mixture of nitric and perchloric acid (2:1) overnight and digested until white fumes appeared. Digested samples were diluted to a final volume of 50 mL with ultrapure water, double-filtered, and then analyzed for all minerals except N, using an atomic absorption spectrophotometer (NovAA400P, Analytik Jena GmbH, Jena, Germany). Nitrogen concentration was determined using the Kjeldahl method [31].

2.6. Chlorophyll Concentrations

Fresh leaf samples were homogenized in 80% acetone solution, and concentrations of total chlorophyll (Chl (T)), chlorophyll a (Chl a), and b (Chl b), were determined using spectrophotometer (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Massachusetts, United States) at an absorbance of 645 nm [32] and 663 nm [33] and expressed as mg g^{-1} of fresh weight.

2.7. Quantification of Stress Markers

Oxidative stress markers, including hydrogen peroxide (H_2O_2 ; mmol kg^{-1}) and malondialdehyde (MDA; mmol kg^{-1}), were determined using fresh leaf samples. The samples were homogenized in a shaking mixer for 30 s and measured at 390 nm. The MDA was measured at 532 and 600 nm. Proline content was measured at 520 nm by absorbance, following Bates and Waldren [34]. All compounds were quantified using a spectrophotometer (Multiskan GO Microplate, Thermo Scientific, Massachusetts, United States) as described by Ekinici et al. [26].

2.8. Antioxidant Enzyme Assays

Fresh leaf samples were homogenized in an extraction buffer (ice cold) containing phosphate buffer (50 mM, pH 7.0) and EDTA (1 mM), followed by centrifugation (12,000 rpm, 15 min, 4 °C). The resulting supernatant was used for the enzymatic assays, and readings were obtained using a spectrophotometer (Multiskan GO Microplate, Thermo Scientific, Waltham, MA, USA).

2.8.1. Catalase (CAT)

The CAT activity (EU g^{-1} leaf) was determined [35] based on the spectrophotometric method reported by Lück [36]. The method is based on H_2O_2 decomposition by catalase; as a result, absorption decreases over time, allowing enzyme activity to be measured. The reaction mixture contained phosphate buffer (50 mM, pH 7.0), H_2O_2 (10 mM), and the enzyme extract (sample). The enzyme activity was measured at 240 nm for 1 min.

2.8.2. Peroxidase (POD)

The POD activity (EU g^{-1} leaf) was quantified based on guaiacol oxidation measured as an increase in absorbance due to a colored compound resulting from a reaction where guaiacol and H_2O_2 are substrates. The increase in absorbance was monitored at 470 nm [37].

2.8.3. Superoxide Dismutase (SOD)

SOD activity (units per gram of fresh leaf) was measured by the nitro blue tetrazolium (NBT) method, in which photochemical reduction of this compound is inhibited [38]. The amount of enzyme required to inhibit 50% of NBT reduction at 560 nm was defined as one unit of SOD activity.

2.9. Statistical Analysis

To screen the best-performing treatment based on the combined effects of all traits under study, a clustering heatmap was generated using TBtools (v2.476). All other statistical analyses were performed using R software v. 4.1.2 [39]. A one-way analysis of variance (ANOVA, $p < 0.001$) was performed using the ‘agricolae’ package [40] to assess treatment effects on morphological, biochemical, nutrient, and stress-related traits. To evaluate the response of each treatment to the traits under study, a principal component analysis (PCA) was performed using the ‘ggbiplot2’ package, with scaling and centering enabled [41]. To evaluate the association of traits, Pearson’s correlation was performed using the package ‘corrplot’ [42]. Means were separated using Tukey’s Honest Significant Difference (HSD) post hoc using the ‘agricolae’ package. Results were presented as mean $\pm$ standard error through bar graphs generated using packages ‘tidyverse’, ‘ggplot2’, ‘agricolae’, and combined into single panels using ‘patchwork’ package [43].

3. Results

3.1. Effect on Soil Properties

Humic substances markedly altered the soil properties compared to the control (Table 1). Soil was alkaline (pH 7.7), but under WL conditions, it became acidic (pH 5.4). Similarly, O.M was reduced to approximately half (0.5%) under the WL conditions. Humic acid (HA), however, maintained the neutral soil pH comparable to the control. The EC increased under both HA and FA treatment, reaching $> 67 \mu\text{mhos cm}^{-1}$, compared to $23 \mu\text{mhos cm}^{-1}$ under WL conditions, indicating higher soluble nutrient availability. However, humic substances did not restore O.M under WL conditions.

Table 1. Effect of humic substances on physio-chemical properties of soil under normal and waterlogging conditions.

Treatment	pH	EC	Lime	OM	N	P	K	S	Ca	S	Mg	Na	B	Cu	Fe	Zn	Mn
C	7.7	64.9	0.4	1.2	0.03	25.2	1.7	9.4	8.6	2.7	7.3	1.8	0.01	0.2	1.0	0.1	0.1
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.1	3.8	0.1	0.1	0.01	0.1	0.1	0.1	0.4	0.2	0.3	0.2	0.01	0.04	0.2	0.01	0.01
WL	5.4	23.2	0.1	0.3	0.02	10.5	0.7	3.2	4.3	1.8	2.3	0.6	0.01	0.3	0.3	0.05	0.05
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.1	1.3	0.02	0.01	0.00	1.0	0.03	0.01	0.3	0.2	0.2	0.1	0.00	0.04	0.04	0.00	0.00
HA	7.6	76.0	1.0	1.6	0.05	49.1	2.2	10.4	14.6	3.5	7.6	2.0	0.02	0.2	0.8	0.2	0.1
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.1	2.6	0.03	0.1	0.01	1.9	0.2	0.55	1.4	0.2	0.3	0.1	0.00	0.01	0.07	0.02	0.00
FA	6.5	67.1	0.5	1.7	0.08	24.9	2.0	11.9	10.7	4.5	10.9	2.0	0.04	0.3	1.2	0.3	0.2
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.2	3.3	0.02	0.1	0.01	2.9	0.2	0.4	0.3	0.2	0.6	0.01	0.00	0.01	0.10	0.03	0.15
WL + HA	5.5	70.7	0.2	0.6	0.02	19.4	1.0	6.5	4.3	2.6	3.4	1.1	0.01	0.1	0.3	0.07	0.05
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.1	0.3	0.02	0.1	0.00	0.3	0.2	0.4	0.3	0.1	0.5	0.1	0.00	0.02	0.04	0.01	0.01
WL + FA	5.7	49.0	0.2	0.5	0.02	13.8	0.9	8.4	3.6	2.8	4.6	1.2	0.02	0.1	0.3	0.05	0.06
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.2	3.1	0.03	0.02	0.00	1.7	0.01	0.5	0.2	0.4	0.7	0.1	0.00	0.01	0.05	0.00	0.01

EC: electrical conductivity ($\mu\text{mhos cm}^{-1}$), OM: organic matter (%), N: nitrogen (%), P: phosphorus (ppm), K: potassium (cmol kg^{-1}), S: sulfur (mg kg^{-1}), Ca: calcium (mg kg^{-1}), Mg: magnesium (mg kg^{-1}), Na: sodium (cmol kg^{-1}), B: boron (ppm), Cu: copper (ppm), Fe: iron (ppm), Zn: zinc (ppm), Mn: manganese (ppm), C: control, WL: waterlogging, HA: humic acid, FA: Fulvic acid.

Micronutrients, especially Fe and Mn, increased significantly in response to FA treatment under WL conditions. Fe increased from 1.00 ± 0.17 ppm (control) to 1.15 ± 0.10 ppm (FA), Zn from 0.14 ± 0.01 to 0.28 ± 0.03 ppm, and Mn from 0.12 ± 0.01 to 0.22 ± 0.15 ppm. HA also improved several secondary nutrients, including Ca ($14.57 \pm 1.40 \text{ mg kg}^{-1}$ vs.

$8.57 \pm 0.44 \text{ mg kg}^{-1}$ in control) and Mg, while FA enhanced Mg ($10.92 \pm 0.56 \text{ cmol kg}^{-1}$) and S ($4.51 \pm 0.17 \text{ mg kg}^{-1}$). Overall, humic substances improved soil fertility indicators, with HA being more effective for macronutrient and pH stability, while FA showed stronger effects on organic matter, nitrogen, and micronutrient availability.

3.2. Effects on Plant Growth Attributes

Significant differences among treatments were observed for all morphological traits, including plant height (PH), stem diameter (SD), and fresh and dry root and shoot weights (Figure 1, Table S1). Waterlogging (WL) caused significant reductions in all the traits (Figure 2). Compared to the control (C), WL reduced PH, SD, SFW, RFW, SDW, and RDW by 34, 19, 53, 43, 61, and 56%, respectively (Figure 1a–f).

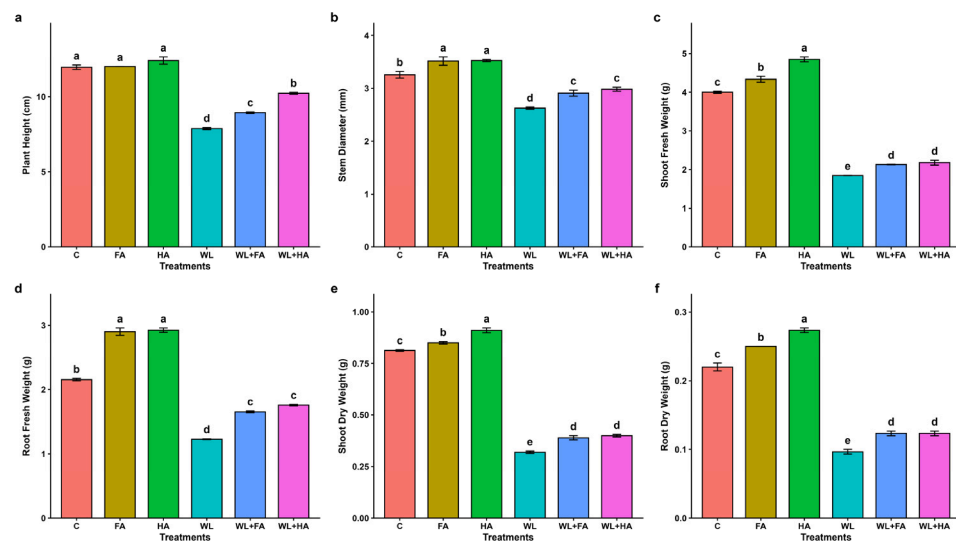


Figure 1. Impact of bio-stimulants (humic acid and fulvic acid) on plant growth attributes under normal and waterlogging conditions, including plant height (a), stem diameter (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), and root dry weight (f). Data are presented as mean $\pm$ standard error (SE), while SE is represented by error bars. C: control, WL: waterlogging, HA: humic acid, FA: fulvic acid. Letters above the bars are based on Tukey's Honest Significant Difference (HSD) post hoc test ($p < 0.05$); different letters among two bars indicate significant differences among respective treatments.



Figure 2. Impact of humic acid and fulvic acid on growth parameters of eggplant under control and waterlogging conditions. C: control, WL: waterlogging, HA: humic acid, FA: fulvic acid.

Under normal (control) conditions, HA and FA had positive impacts on plant growth, although HA had more profound effects compared to FA, causing an increase of up to 4% (plant height)–35% (root fresh weight) in different traits. The FA treatment also increased all the traits (8–34%) except for the PH, which remained almost the same as control plants (Figure 1a).

Application of HA and FA under waterlogging conditions partially improved growth parameters, but these improvements were substantially lower than those of the control plants. For instance, when comparing PH under WL alone, it decreased by ~30% compared to the control; with HA treatment under WL (HA + WL), the decrease was 10%, thereby restoring 20% PH in HA-treated plants under WL conditions (Figure 1a). A similar trend was observed for other traits, except for stem diameter, where the effects were less pronounced (Figure 1b). Likewise, FA improved plant traits under WL conditions, with growth increasing by 11% (SD; Figure 1b) to 34% (RFW; Figure 1d). Notably, the impact of HA was more prominent on PH under WL conditions than FA, i.e., PH was 13% higher than when treated with FA (Figure 1a). WL reduced the plant biomass (fresh and dry), in the range of 42–60%, and was recovered to some extent by HA and FA application. Under the HA application, SFW, RFW, SDW, and RDW increased to 2.2, 1.8, 0.4, and 0.2 g, respectively, compared to WL, which had 1.8, 1.2, 0.3, and 0.1 g, respectively, corresponding to 17.9, 42.8, 25.0, and 27.6% increases in biomass, respectively; similarly, FA-treated plants showed increases of 15.3%, 34.4%, 21.9%, and 27.6%, respectively.

3.3. Effects on Chlorophyll Concentrations

Significant differences were found among treatments for chlorophyll content (Table S1), particularly between HA and FA and the control under normal conditions, as indicated by statistical lettering (Figure 3a–c). Similar results were found under waterlogging conditions (WL), except in the case of chlorophyll b where FA treatment did not significantly vary from the WL condition (Figure 3b), and HA and FA had a non-significant difference among themselves under WL conditions for chlorophyll a and total chlorophyll, i.e., both treatments had a similar effect for these traits (Figure 3a,c).

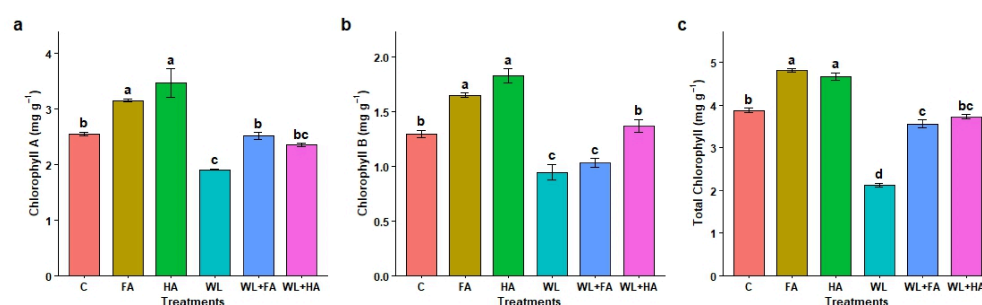


Figure 3. Impact of bio-stimulants (humic acid and fulvic acid) on photosynthetic pigments (chlorophyll) under normal and waterlogging conditions, including chlorophyll A (a), chlorophyll B (b), and total chlorophyll (c). Data are presented as mean $\pm$ standard error (SE), while SE is represented by error bars. C: control, WL: waterlogging, HA: humic acid, FA: fulvic acid. Letters above the bars are based on Tukey's Honest Significant Difference (HSD) post hoc test ($p < 0.05$); different letters among two bars indicate significant differences among respective treatments.

Waterlogging significantly reduced photosynthetic pigments, with reductions of 25%, 27%, and 45% in Chl. a, b, and Chl. (T), respectively, compared to the control. Contrastingly, both treatments (HA and FA) enhanced chlorophyll accumulation under normal conditions (Figure 3a,b, and Figure 3c, respectively). The HA application enhanced chl. a (36%) and b (41%) more than the FA application (24 and 27%, respectively) (Figure 3a,b), while for total chlorophyll, the FA application increased 3% more in concentration than the HA application (Figure 3c). The treatment of these bio-stimulants also partially alleviated the impacts of WL stress by restoring pigment levels to near-control levels (only 2–11% reduction with HA treatment and 1–20% reduction with FA treatment). Compared with WL, HA increased chlorophyll concentrations by 23, 44, and 75%. Similarly, FA treatment increased chlorophyll up to 32, 9, and 67%.

3.4. Effects on Plant Nutrients

The concentrations of micro- and macro-nutrients were significantly affected by waterlogging stress and the application of HA and FA (Table S2, Figure 4a–l). Each nutrient responded differently to the treatments under normal conditions. For instance, FA reduced concentration of some nutrients (N—15% and Ca—38%) (Figure 4a and d, respectively), while it increased concentrations of the rest of the nutrients including P (0.2 to 1.0%, +357%; Figure 4b), K (0.4 to 1.5%, +253%), Mg (0.2 to 0.3%, +65%), S (0.1 to 0.3%, +107%), Mn (18.4 to 46.4 mg kg^{−1}, +151%), Fe (17.9 to 52.7 mg kg^{−1}, +195%), Zn (9.2 to 34.4 mg kg^{−1}, +272%), B (4.6 to 5.0 mg kg^{−1}, +9%), Cu (0.1 to 9.2 mg kg^{−1}, +16,632%), and Na (26.4 to 277.8 mg kg^{−1}, +951%) (Figure 4b,c,f–l). HA consistently increased all nutrients under normal conditions, especially Fe, Zn, B, and Cu, with increases of 406, 709, 177, and 117,911% in concentrations compared to control conditions.

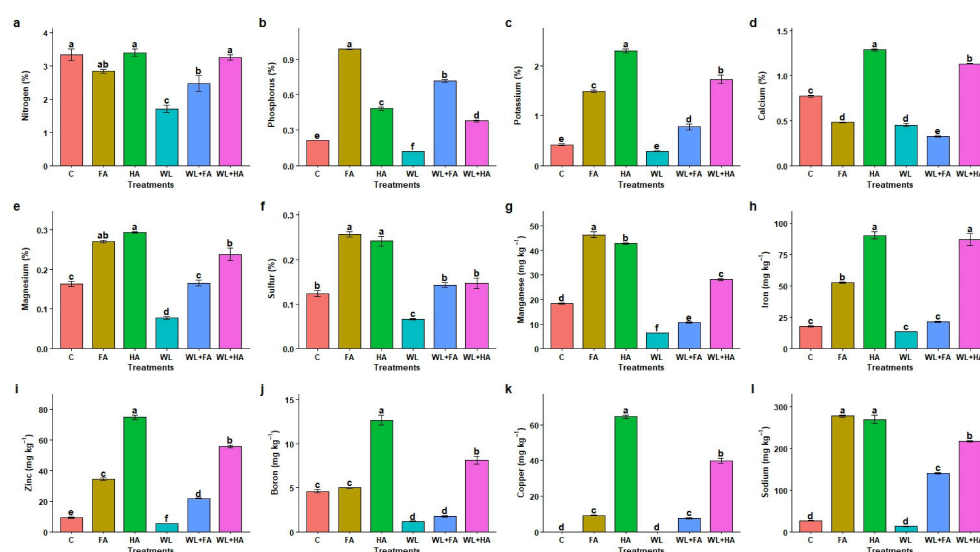


Figure 4. Impact of bio-stimulants (humic acid and fulvic acid) on micro- and macro-nutrients, including nitrogen (a), phosphorus (b), potassium (c), calcium (d), magnesium (e), sulfur (f), manganese (g), iron (h), zinc (i), boron (j), copper (k), and sodium (l) under normal and waterlogging conditions. Data are presented as mean $\pm$ standard error (SE), while SE is represented by error bars. C: control, WL: waterlogging, HA: humic acid, FA: fulvic acid. Letters above the bars are based on Tukey's Honest Significant Difference (HSD) post hoc test ($p < 0.05$); different letters between two bars indicate significant differences between respective treatments.

On the other hand, WL drastically reduced nutrient contents ranging from -23 (Fe, mg kg^{−1}) to -74 (B, mg kg^{−1}). Application of FA and HA substantially restored nutrient levels; however, HA treatment was more effective for most nutrients, except P (Figure 4b). The HA application increased N (+1.6%), P (+0.3%), K (+1.4%), Ca (+0.7%), Mg (+0.2%), S (+0.1%), Mn (+21.6 mg kg^{−1}), Fe (+73.2 mg kg^{−1}), Zn (+55.6 mg kg^{−1}), B (+7.0 mg kg^{−1}), Cu (+39.8 mg kg^{−1}), and Na (+204.5 mg kg^{−1}). This increase was almost twice that of the FA treatment except for P, where FA-treated plants had 0.7% P, while HA-treated plants had 0.4% (Figure 4c). Sulfur showed a similar increase in concentrations by both treatments (0.1%) (Figure 4f).

3.5. Effects on Oxidative Stress Markers

Waterlogging conditions significantly altered oxidative stress markers, including reactive oxygen species (H₂O₂; Figure 5a), lipid peroxidation indicator (MDA; Figure 5b), and osmo-protectant (proline; Figure 5c). Significant differences were found among treatments for the oxidative stress markers (Table S3).

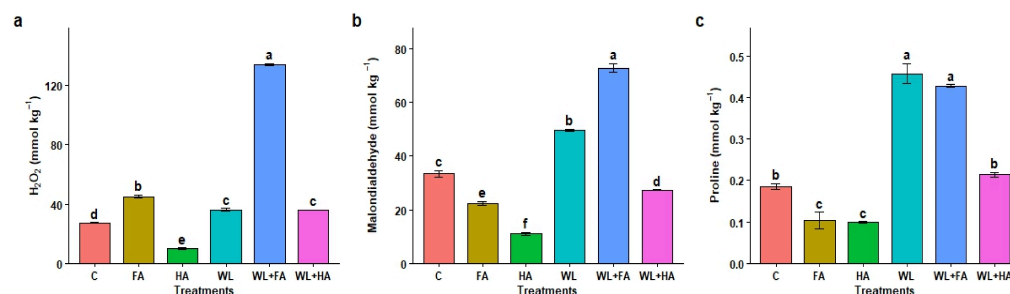


Figure 5. Response of oxidative stress markers including hydrogen peroxide (H₂O₂; (a)), malondialdehyde (b), and proline (c) to the application of bio-stimulants (humic acid and fulvic acid) under normal and waterlogging conditions. Data are presented as mean $\pm$ standard error (SE), while SE is represented by error bars. C: control, WL: waterlogging, HA: humic acid, FA: fulvic acid. Letters above the bars are based on Tukey's Honest Significant Difference (HSD) post hoc test ($p < 0.05$); different letters among two bars indicate significant differences among respective treatments.

Under normal conditions, the concentration of H₂O₂ was 27.3 mmol kg⁻¹ and increased to 36.2 mmol kg⁻¹ (+32.7%) under WL conditions (Figure 5a). Similarly, MDA also increased under stress conditions (from 33.3 to 49.4 mmol kg⁻¹—48.4% increase) (Figure 5b). The HA application markedly reduced these stress indices; for instance, H₂O₂ was reduced to 10 mmol kg⁻¹, and MDA was reduced to 11.1 mmol kg⁻¹. While FA increased H₂O₂ to 44.9 mmol kg⁻¹, it decreased MDA to 22.3 mmol kg⁻¹. Both treatments reduced proline to 0.1 mmol kg⁻¹ (Figure 5c).

Under WL conditions, HA maintained H₂O₂ up to 35.8 mmol kg⁻¹. In contrast, FA application increased H₂O₂ to 133.7 mmol kg⁻¹ (+169.6%) and MDA to 72.6 mmol kg⁻¹ (46.9% increase compared to WL alone). Proline increased from 0.19 to 0.46 mmol kg⁻¹ (+142.1%). The HA application reduced proline concentrations to control levels of approximately 0.21 mmol kg⁻¹, while FA maintained them at WL levels (0.43 mmol kg⁻¹; a 6.5% reduction nonetheless) (Figure 5c).

3.6. Effects on Antioxidant Enzymes

The antioxidant enzyme activities were significantly influenced by the treatments under WL conditions (Table S3, Figure 6a–c), except for catalase, which was not significantly affected by FA under WL, as indicated by the lettering (Figure 6a).

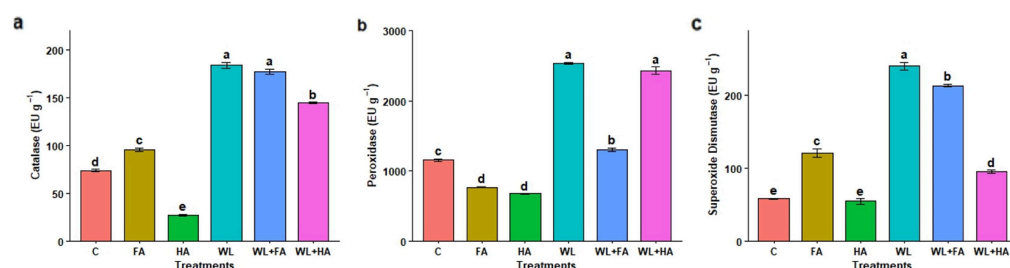


Figure 6. Response of antioxidant enzymes including catalase (a), peroxidase (b), superoxide dismutase (c), to the application of bio-stimulants (humic acid and fulvic acid) under normal and waterlogging conditions. Data are presented as mean $\pm$ standard error (SE), while SE is represented by error bars. C: control, WL: waterlogging, HA: humic acid, FA: fulvic acid. Letters above the bars are based on Tukey's Honest Significant Difference (HSD) post hoc test ($p < 0.05$); different letters among two bars indicate significant differences among respective treatments.

Catalase activity under control conditions averaged 73.9 EU g⁻¹. While FA increased its concentration (95.4 EU g⁻¹), HA decreased it up to 27.1 EU g⁻¹ (63% reduction). Under WL conditions, catalase concentration increased to 183.8 EU g⁻¹ (+148.8%) (Figure 6a),

and FA maintained these levels, while HA reduced it to 144.7 EU g⁻¹. The opposite was found for peroxidase, where HA maintained levels of POD, the same as WL concentrations (Figure 6b), while FA reduced POD to 1302 EU g⁻¹, a 48.6% reduction compared to WL (2534.7 EU g⁻¹). However, under normal conditions, both HA and FA reduced POD concentrations (673.71 and 767.21 EU g⁻¹, respectively), compared to the control (1151.7 EU g⁻¹).

Likewise, superoxide dismutase showed a concentration pattern like that of catalase. Where FA increased SOD activity (120.5 EU g⁻¹) under normal conditions (Figure 6c), while HA reduced it (54.4 EU g⁻¹), compared to the control having 57.9 EU g⁻¹ activity. Waterlogging significantly increased the concentrations to 240.1 EU g⁻¹. Treatment with FA maintained these levels (213.2 EU g⁻¹), whereas HA substantially reduced SOD activity to 95.1 EU g⁻¹ (−64.1%) relative to WL conditions.

3.7. Selection of Treatment Based on Association Analysis and Heatmap

The principal component analysis (PCA) of all traits under study, including plant growth and nutrient traits, oxidative stress indicators, and antioxidant enzyme activities, revealed treatment-dependent responses (Figure 7a). PC1 and PC2 explained 70.8% and 13.4% of the variation in the trait-environment interaction, respectively, for a total of 84.2%. Overall, the traits showed a wide range of variability among the treatments (HA and FA) under normal and stress conditions. The HA had the highest values for the growth parameters and nutrient traits, followed by FA, while the enzyme activities and stress indices were highest under WL and WL + FA treatments. Interestingly, HA under WL conditions (WL + HA) exhibited contrasting behavior relative to the control, showing opposite trait responses.

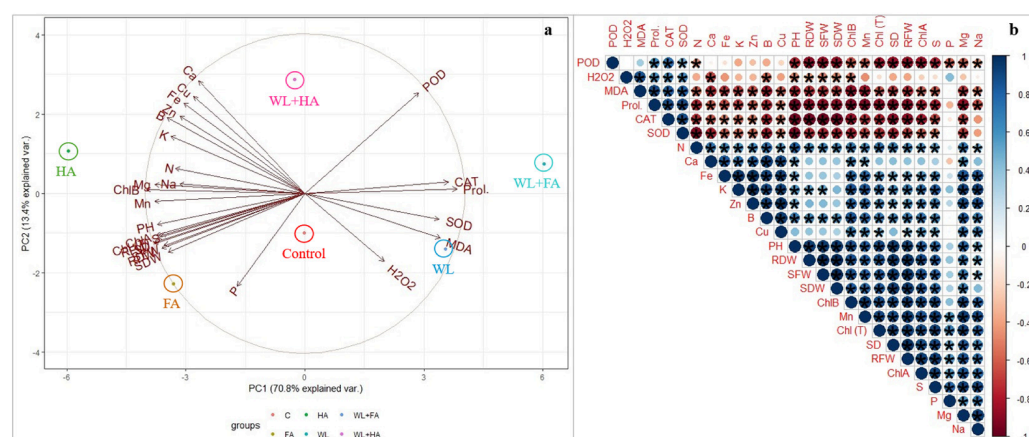


Figure 7. Principal component analysis (a) and Pearson correlation plots (b) for the plant growth traits, nutrients, photosynthetic pigments, oxidative stress markers, and antioxidant enzymes under normal and waterlogging conditions. In the PCA plot, the circle indicates the 68% confidence interval, which explains the theoretical maximum extent of the arrows. In a correlation plot, an asterisk (*) indicates significance at $p < 0.05$. Blue color indicates positive association, red indicates negative. C: control, WL: waterlogging, HA: humic acid, FA: fulvic acid, PH: plant height (cm), SD: stem diameter (mm), SFW: stem fresh weight (g), RFW: root fresh weight (g), SDW: stem dry weight (g), RDW: root dry weight (g), ChlA: Chlorophyll A (mg g⁻¹), ChlB: Chlorophyll B (mg g⁻¹), Chl (T): total chlorophyll (mg g⁻¹), N: nitrogen (%), P: phosphorus (%), K: potassium (%), Ca: calcium (%), Mg: magnesium (%), S: sulfur (%), Mn: manganese (mg kg⁻¹), Fe: iron (mg kg⁻¹), Zn: zinc (mg kg⁻¹), B: boron (mg kg⁻¹), Cu: copper (mg kg⁻¹), Na: sodium (mg kg⁻¹), H₂O₂: hydrogen peroxide (mmol kg⁻¹), MDA: malondialdehyde (mmol kg⁻¹), ProL: proline (mmol kg⁻¹), CAT: catalase (EU g⁻¹), POD: peroxidase (EU g⁻¹), SOD: superoxide dismutase (EU g⁻¹).

The correlation analysis indicated a significant positive association between almost all antioxidant enzymes and oxidative stress markers, except for POD with H_2O_2 and MDA (Figure 7b), while these stress indicators had a negative association with growth and nutrient traits, with few exceptions in POD and H_2O_2 . Notably, N had a significant positive association with all growth and nutrient traits except P. In fact, P showed a positive association only with Chl. a, Chl. (T) (photosynthetic pigment), four nutrients including Mn, S, Mg, and Na, and with SD among growth traits (Figure 7b). The remaining nutrients and growth traits showed positive associations with one another.

A heatmap was generated to select the best-performing treatment based on phenotypic combinations of all the traits (Figure 8). The heatmap displayed variation among treatments based on color and its intensity. The change in values shifted from red to blue, with red indicating higher values and blue indicating lower values. Overall, treatments were divided into two major and three minor groups: control and WL + HA were clustered together in one major group, while HA and FA, and C and WL + HA were clustered together in two minor subgroups. Close clustering of C and WL + HA indicated that trait values under these treatments were comparable. The WL and HA treatments showed contrasting behavior, i.e., HA had the highest values (indicated by red color) for growth, nutrients, and chlorophyll concentrations, while lower values for stress indices, and vice versa for WL. FA had a similar response as WL except for P, which was higher under FA treatment.

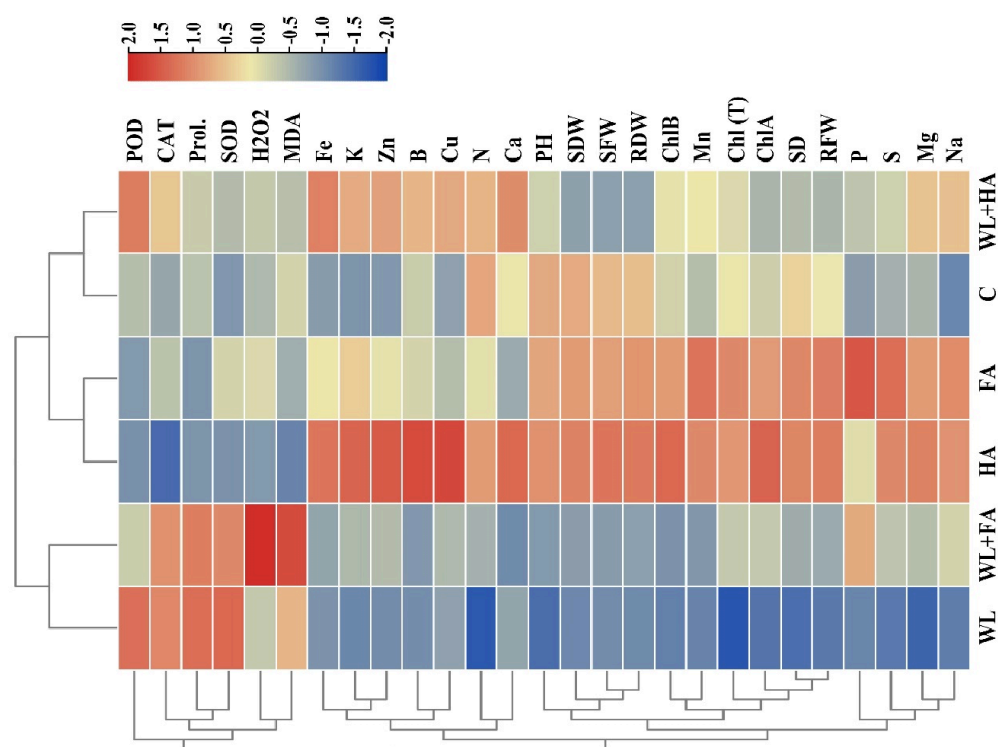


Figure 8. Heatmap for the plant growth traits, nutrients, photosynthetic pigments, oxidative stress markers, and antioxidant enzymes under normal and waterlogging conditions. C: control, WL: waterlogging, HA: humic acid, FA: fulvic acid, PH: plant height (cm), SD: stem diameter (mm), SFW: stem fresh weight (g), RFW: root fresh weight (g), SDW: stem dry weight (g), RDW: root dry weight (g), ChlA: Chlorophyll A ($mg\ g^{-1}$), ChlB: Chlorophyll B ($mg\ g^{-1}$), Chl (T): total chlorophyll ($mg\ g^{-1}$), N: nitrogen (%), P: phosphorus (%), K: potassium (%), Ca: calcium (%), Mg: magnesium (%), S: sulfur (%), Mn: manganese ($mg\ kg^{-1}$), Fe: iron ($mg\ kg^{-1}$), Zn: zinc ($mg\ kg^{-1}$), B: boron ($mg\ kg^{-1}$), Cu: copper ($mg\ kg^{-1}$), Na: sodium ($mg\ kg^{-1}$), H_2O_2 : hydrogen peroxide ($mmol\ kg^{-1}$), MDA: malondialdehyde ($mmol\ kg^{-1}$), Prol.: proline ($mmol\ kg^{-1}$), CAT: catalase ($EU\ g^{-1}$), POD: peroxidase ($EU\ g^{-1}$), SOD: superoxide dismutase ($EU\ g^{-1}$).

4. Discussion

Regardless of irrigation or rainfall cultivation, plants are affected by waterlogging (WL) stress, either short or long term [44]. The situation, however, is further exacerbated by climate change, with intensified rainfall, heat, and soil degradation [45]. Excessive soil moisture creates hypoxic conditions for plants, leading to anaerobic respiration and affecting plant growth, morphology, metabolism, and physiological functions [44], especially in waterlogging-sensitive plants such as eggplant [22]. The current study also demonstrated the sensitivity of eggplant to waterlogging conditions, as reflected by reduced plant growth, impaired photosynthetic pigments, reduced nutrient accumulation, increased plant stress markers, and increased antioxidant enzyme activity (Figures 1–6). To modulate WL conditions, humic substances, including humic acid (HA) and fulvic acid (FA), were applied, and the results indicated that both nutrients effectively restored plant growth under WL conditions (Figure 1). However, the HA application was found to be more efficient (Figure 8) by enhancing plant nutrient uptake (Figure 4), scavenging reactive oxygen species (H_2O_2 ; Figure 5a), lipid peroxidation indicator (MDA, Figure 5b), and osmo-protectant (proline, Figure 5c), and maintaining the levels of antioxidant enzymes (Figure 6).

4.1. Humic Substances Alleviated Waterlogging-Induced Growth Inhibition

Waterlogging significantly reduced plant growth, represented by reduced plant height (−34%), stem diameter (−19%), stem fresh and dry weight (54 and 61%, respectively), and root fresh and dry weight (43 and 56%, respectively) (Figure 1a–f). This indicated a more than 50% reduction in biomass due to WL conditions, as previously reported by Zohura et al. [44] in eggplant, where fruit number and yield were more severely affected by WL than by drought. Similar results were also reported by Sarker et al. [46], who found that WL stress reduced biomass (plant height, number of leaves, stem diameter, and leaf area) by more than 50% 12 days after WL stress. These adverse effects on growth are caused by oxygen deprivation in the rhizosphere, which restricts aerobic respiration and ATP production, impairs root function, and limits water and nutrient uptake [47]. Moreover, WL conditions drastically changed the soil from alkaline to acidic, thereby limiting nutrient uptake and reducing organic matter by ~80% (Table 1). Such changes likely impaired root metabolic activity, thereby limiting biomass accumulation [48].

Application of humic substances alleviated these detrimental effects. Under normal conditions, HA enhanced growth by 4–35%, with the highest increase observed in root fresh weight (Figure 1d), as also evident by the neutralization of the soil pH (Table 1). Previous studies also concluded that humic substances enhance root elongation and lateral root growth, suggesting stimulation of root growth [49]. Hence, the enhanced nutrient uptake observed in this study with HA application (Figure 4) may also be attributed to improved root growth and function. Similarly, humic substances partially compensated for the effects on plant growth under WL conditions. For example, HA and FA applications increased PH by 30 and 14%, respectively, compared to the WL condition (Figure 1a). A similar pattern was observed for the other traits as well, with the efficiency of HA and FA in restoring plant growth being comparable.

4.2. Humic Substances Improved Photosynthetic Pigments

Waterlogging conditions significantly reduce chlorophyll a, b, and total chlorophyll (Figure 3a–c, respectively), indicating disruption of photosynthetic machinery. Previous studies have reported a decline in chlorophyll concentration under flooding conditions, attributed to chlorophyll degradation, reduced biosynthesis, and damage to chloroplast structure due to oxidative stress [50,51]. The observed reductions of 25, 27, and 45% in chlorophyll pigments under WL conditions are comparable to previous research [52],

which demonstrated reduced photosynthetic activity under hypoxic conditions. This also explains the reduced plant growth observed in this study under WL conditions (Figure 1). The FA application was more effective in mitigating WL effects for chlorophyll a (+32%; Figure 3a), while HA was more efficient for chlorophyll b and total chlorophyll (+44 and 75%, respectively) (Figure 3b,c). The positive impact of humic substances on chlorophyll concentrations can be attributed to enhanced accumulation of N, Mg, and Fe (Figure 4b,f, and Figure 4h, respectively), as also reported by Nardi et al. [53]. Similarly, Lotfi et al. [54] found that fulvic acid effectively enhanced the fluorescence of chlorophyll a under water stress conditions in *Brassica napus*. Hence, both humic substances were found to be effective in preserving photosynthetic activity, thereby contributing to improved plant growth performance under WL conditions.

4.3. HA and FA Differentially Improved Nutrient Accumulation

One of the most pronounced effects of waterlogging was a significant reduction in macro- and micro-nutrients (Figure 4a–l). A reduction in all nutrients was observed and is likely due to impaired root growth, nutrient-absorption capacity, and ion-transport mechanisms [55]. The reduction in nutrient concentrations is comparable to those previously reported for vegetable crops such as snake cucumber [26] and cabbage [6], which reported 32% to 80% decreases in essential micro- and macronutrients. Moreover, in this study, the soil profile under the WL condition also supports this reduction in nutrients, owing to reduced nutrient availability from acidic pH, which leads to a lower redox potential. Soil nutrient availability was reduced for P (58%), K (57%), S (66%), Mg (68%), Fe (73%), Zn (64%), and Mn (58%) (Table 1). Nutrient uptake from the soil requires energy, while hypoxic conditions that limit ATP production restrict ion transport, thereby disrupting nutrient acquisition [56].

Both humic substances increased nutrient concentrations under WL conditions; however, HA was more efficient in alleviating the WL effect on nutrients. Under waterlogging, HA restored almost all nutrient levels to levels higher than those in untreated WL plants, except for S, which remained nearly the same. However, concentrations of N, P, K, Ca, Mg, S, Mn, Fe, Zn, B, Cu, and Na increased by 1.9, 4.0, 5.7, 2.2, 2.0, 4.3, 6.3, 10.3, 6.8, 39.8, and 16.5-fold compared to the untreated WL plants (Figure 4a–e,g–l, respectively). This enhancement in nutrient availability may be attributed to improved membrane permeability, as HA is known to stimulate root growth, chelate nutrients, and increase their availability, thereby increasing nutrient uptake [57]. HA approximately doubled P (19.4 ppm), exchangeable K (1.0 cmol kg^{-1}), increased S by 100%, Mg by 45%, and Na by 71%. This increase also corresponds to the improved soil nutrient status with HA treatment (Table 1).

Interestingly, FA was more effective in enhancing P concentrations than HA (Figure 4b). This may be because FA has a lower molecular weight and greater solubility than HA, which may facilitate P mobilization and transport in plants [58]. But overall, HA performed better in nutrient accumulation than FA. Although FA improved soil nutrient status, particularly O.M (+88%), S (+159%), and Mg (+95%) under WL conditions (Table 1), these improvements were less consistently reflected in plant growth than with HA, suggesting that HA had stronger bio-stimulatory effects on root functioning. This also supports the view that both substances act through distinct physiological mechanisms and that their complementary effects warrant further exploration. Although the present study demonstrated that humic acid significantly improved soil nutrient availability and plant nutrient uptake, the underlying microbial mechanisms were not investigated. Future studies integrating soil enzyme activities, rhizosphere bacterial and fungal community analyses (e.g., 16S rRNA and ITS sequencing), and functional microbiome profiling will be essential to clarify the contribution of soil microorganisms to the beneficial effects of

humic substances under waterlogging stress. A limitation of the present study is that mineral nutrient concentrations were determined only in leaf tissues. Future studies should quantify nutrient accumulation separately in roots and shoots and integrate analyses of ion transport, nutrient partitioning, and transporter gene expression to better elucidate the mechanisms by which humic substances regulate nutrient uptake and translocation under waterlogging stress.

4.4. HA Protected Against Oxidative Damage Under Waterlogging Conditions

Waterlogging stress significantly increased H_2O_2 , MDA, and proline accumulation by 1.3-, 1.5-, and 2.5-fold, respectively (Figure 5a–c), indicating enhanced oxidative and membrane damage [59]. Increases in H_2O_2 and MDA can be corroborated by previous studies [6,26]. However, in contrast to Anee and Nahar [60], who reported a reduction in proline concentration in response to WL stress in sesame, our study reported an increase in osmo-protectant accumulation under stress; this has also been reported in *Physalis Peruviana* [61] and *Cucumis melo* [26]. Oxygen deprivation during flooding disrupts mitochondrial electron transport chains, leading to excessive production of ROS, including superoxide radicals and hydrogen peroxide [22]. Similarly, the elevated MDA levels (Figure 5b) indicated lipid peroxidation and membrane deterioration leading to disrupted nutrient uptake and cellular compartmentalization [44]. Chávez-Arias et al. [61] also reported an increasing trend in MDA with increasing WL duration in goldenberry. The increased proline concentration further corroborates a response to oxidative damage and lipid peroxidation. Proline is a well-known stress marker and osmo-protectant that maintains plant water status and cellular homeostasis, thereby facilitating the synthesis of stress-adaptive enzymes and biomolecules [52,61]. Furthermore, the negative association between oxidative stress markers and plant growth traits (Figure 7b) underscored the detrimental effects of oxidative damage on plant performance.

The HA application reduced these stress indicators under normal and WL conditions. Under WL conditions, HA restored H_2O_2 to the control levels and reduced MDA levels to 45% compared to untreated WL plants (Figure 5a,b). Moreover, reduced proline accumulation in HA-treated plants further supported the idea that HA alleviated plant stress, as proline synthesis is induced under cellular stress [23]. These results thus supported the hypothesis that humic substances stabilized plant cells under waterlogging conditions in eggplant.

However, FA treatment increased H_2O_2 concentrations under both normal and stress conditions, despite reducing MDA under WL conditions. This could be explained by the fact that an increase in ROS may be an early signaling response that activates defense responses and stress adaptation [62]. Secondly, FA may stimulate metabolic activity and respiration, thereby increasing ROS. Nevertheless, the concurrent reduction in MDA under normal conditions suggested that ROS levels during FA treatment may represent a controlled signaling response rather than severe oxidative damage, as FA positively impacted plant growth traits (Figures 1 and 2).

4.5. Differential Regulation of Antioxidant Enzymes by HA and FA

The enzyme activities were influenced by both waterlogging and the application of bio-stimulants (Figure 6). Waterlogging significantly increased catalase, SOD, and POD by 2.5-, 2.2-, and 4.15-fold, respectively, reflecting activation of the antioxidant enzymatic system to scavenge ROS. Increased levels of antioxidant enzymes under flooding stress have been widely documented as an adaptive mechanism for maintaining redox homeostasis [28,60,63].

Under normal conditions, HA application significantly reduced antioxidant activities (−63% catalase and −42% POD), except for SOD, which was reduced by only 6% (Figure 6c). This reduction was likely due to reduced ROS and other oxidative stresses under normal conditions, modulated by HA, thereby diminishing the antioxidant system's requirement. Similarly, under WL conditions, HA reduced POD, SOD, and CAT activities to 1-, 0.9-, and 0.4-fold, respectively. These reductions corresponded to lower H₂O₂ levels (Figure 5a) and MDA concentrations (Figure 5b), suggesting that HA not only reduced antioxidant activity but also modulated ROS-scavenging capacity [6].

Contrary to HA, FA treatment maintained relatively higher CAT and SOD activities under WL conditions up to 1.2- and 2.4-fold higher than the HA-treated plants under WL (Figure 6a,c). This increase, however, was in line with higher H₂O₂ concentrations (Figure 5a), indicating continued stress despite partial mitigation of its effects. Unexpectedly, this was not reflected in plant growth as FA improved plant growth under stress conditions (Figures 1 and 2). This suggests an alternate differential mechanism of stress modulation by FA, as POD concentrations were reduced in FA-treated plants under WL stress (Figure 6b). This is also supported by Lotfi et al. [54] in *Brassica napus*, where FA treatment improved chlorophyll a in the inflorescence, as reported in our study (Figure 3a), and increased POD activity, which is consistent with current research (Figure 6b). Hence, FA may opt to protect against oxidative damage by lignifying and crosslinking cell walls. A similar trend has also been reported in wheat [52] and rapeseed [64]. Strong positive correlations between oxidative stress markers and antioxidant enzymes (Figure 7b) further supported these results, indicating that enhanced enzyme activities were a response to increased oxidative stress.

4.6. Multivariate Analyses Identified HA as the Most Effective Treatment

Principal component analysis and heatmap clustering provided integrated insights into the comparative performance of treatments across all traits under study (Figures 7a and 8, respectively). The PCA explained ~84% of the total variation, indicating distinct separation of the treatments. HA treatment alone clustered more closely with plant growth traits, nutrients, and chlorophyll concentrations, while WL and FA + WL treatments were closely associated with oxidative stress markers and antioxidant enzymes. Notably, the control and WL + HA clustered closely (Figures 7a and 8), indicating that the HA treatment effectively restored the morpho-physiological and biochemical processes disrupted by WL to near-normal levels. Moreover, HA treatment under normal conditions had the most favorable profile, indicated by better plant growth, enzyme profiles, and chlorophyll machinery, combined with minimal oxidative damage, indicated by oxidative stress indicators. Another limitation of the present study is that plant responses were evaluated only after a fixed 10-day waterlogging period. Future investigations should incorporate multiple sampling time points to elucidate the temporal dynamics of physiological, biochemical, and molecular responses to humic and fulvic acids across the early, intermediate, and late stages of waterlogging stress.

5. Conclusions

This study provides the first integrated research on comparative effects of two humic substances on morpho-physiological, nutrient, and oxidative stress profiles under normal and waterlogging conditions in eggplant. Eggplant growth, as measured in terms of nutrient uptake, chlorophyll accumulation, and biomass production, showed severe impairment due to waterlogging that was associated with increased oxidative stress status and increased activities of antioxidant enzymes. Humic acid (HA) and fulvic acid (FA) have been shown to mitigate these negative effects, but HA has been reported to provide

greater protection than FA. These findings show that, compared to FA, HA potentially exerted greater increases in both stress tolerance and the amelioration of detrimental effects by enhancing nutrient acquisition under salt stress and preserving redox homeostasis and photosynthetic pigments, thereby preventing oxidative damage. In conclusion, our results show that humic substances, especially HA, are promising candidates for sustainable bio-stimulants to enhance the waterlogging tolerance of eggplant. Molecular and rhizosphere-based studies will be required to determine the signaling pathways responsible for the differential responses of plants to HA and FA. A limitation of the present study is that only a single agronomically recommended application rate for humic acid and fulvic acid was evaluated. Future studies should investigate multiple application rates, potential dose-dependent responses, possible inhibitory effects at excessive concentrations, and the effectiveness of combined HA + FA applications under waterlogging stress.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae12080974/s1>, Table S1: Analysis of variance (ANOVA) for the plant growth parameters and chlorophyll content; Table S2: Analysis of variance (ANOVA) for the micro and macro-nutrient content; Table S3: Analysis of variance (ANOVA) for the oxidative stress markers and antioxidant enzymes.

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Abbreviations

The following abbreviations are used in this manuscript:

C	control
WL	waterlogging
HA	humic acid
FA	fulvic acid
PH	plant height (cm)
SD	stem diameter (mm)
SFW	stem fresh weight (g)
RFW	root fresh weight (g)
SDW	stem dry weight (g)
RDW	root dry weight (g)
ChlA	Chlorophyll A (mg g^{-1})
ChlB	Chlorophyll B (mg g^{-1})
Chl (T)	total chlorophyll (mg g^{-1})
N	nitrogen (%)
P	phosphorus (%)

K	potassium (%)
Ca	calcium (%)
Mg	magnesium (%)
S	sulfur (%)
Mn	manganese (mg kg ⁻¹)
Fe	iron (mg kg ⁻¹)
Zn	zinc (mg kg ⁻¹)
B	boron (mg kg ⁻¹)
Cu	copper (mg kg ⁻¹)
Na	sodium (mg kg ⁻¹)
H ₂ O ₂	hydrogen peroxide (mmol kg ⁻¹)
MDA	malondialdehyde (mmol kg ⁻¹)
Prol.	proline (mmol kg ⁻¹)
CAT	catalase (EU g ⁻¹)
POD	peroxidase (EU g ⁻¹)
SOD	superoxide dismutase (EU g ⁻¹)

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