Ornamental Horticulture
ISSN 2447-536X | WWW.ORNAMENTALHORTICULTURE.COM.BR

SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

ARTICLE

Drench of humic acid mitigate the adverse impacts of alkalinity on rose

Aplicação de ácido húmico via solo atenua os impactos adversos da alcalinidade na rosa

Sahar Mordai[1], Jafar Amiri[1*], Zohreh Jabbarzadeh[1*], MirHassan Rasouli-Sadaghiani[2], and Ali Shaygan[3]

[1] Urmia University, Faculty of Agriculture, Department of Horticultural Science, Urmia, Iran.
[2] Urmia University, Faculty of Agriculture, Department of Soil Science, Urmia, Iran.
[3] Parks and Landscape Organization of Urmia, Iran

Abstract: Alkaline stress is a major environmental factor that limits the growth and productivity of rose plants. Humic acid (HA) is a natural substance that has been shown to have various beneficial effects on plant growth and stress tolerance. Roses (*Rosa hybrida* L.) are among the world's most important and popular cut flowers, dominating the cut flower export market. This study investigated the effects of HA application on the physiological and biochemical properties of rose plants grown under alkaline conditions. A randomized complete design with three replicates was used. Each replicate consisted of two pots containing a single rose plant. Humic acid was applied at 0, 500, 1,000, and 2,000 mg L^{-1} through drenching at 15-day intervals for two months. Plants treated with 1,000 mg L^{-1} HA exhibited significantly higher levels of total protein, proline, catalase activity, guaiacol peroxidase activity, and antioxidant capacity. Control plants (receiving no HA) showed the highest levels of malondialdehyde and electrolyte leakage, indicating greater cellular damage. Plants treated with 500 mg L^{-1} HA displayed the highest sugar content and ascorbate peroxidase activity. Overall, the results of this study suggest that HA application can be an effective strategy to improve the tolerance of rose plants to alkaline stress and enhance their growth and productivity in alkaline soils.
Keywords: antioxidant enzyme, electrolyte leakage, malondialdehyde, proline, protein, sugar content.

Resumo: O estresse alcalino é um importante fator ambiental que limita o crescimento e a produtividade das roseiras. O ácido húmico (AH) é uma substância natural que demonstrou ter vários efeitos benéficos no crescimento das plantas e na tolerância ao estresse. As rosas (*Rosa hybrida* L.) estão entre as flores de corte mais importantes e populares do mundo, dominando o mercado de exportação de flores de corte. Este estudo investigou os efeitos da aplicação de AH nas propriedades fisiológicas e bioquímicas de roseiras cultivadas em condições alcalinas. Foi utilizado delineamento inteiramente casualizado com três repetições. Cada repetição consistiu de dois vasos contendo uma única planta de rosa. O ácido húmico foi aplicado nas doses de 0, 500, 1.000 e 2.000 mg L^{-1} via solo em intervalos de 15 dias durante dois meses. Plantas tratadas com 1.000 mg L^{-1} de HA exibiram níveis significativamente mais elevados de proteína total, prolina, atividade de catalase, atividade de guaiacol peroxidase e capacidade antioxidante. As plantas controle (que não receberam AH) apresentaram os maiores níveis de malondialdeído e vazamento de eletrólitos, indicando maior dano celular. Plantas tratadas com 500 mg L^{-1} de HA apresentaram maior teor de açúcar e atividade de ascorbato peroxidase. No geral, os resultados deste estudo sugerem que a aplicação de AH pode ser uma estratégia eficaz para melhorar a tolerância das roseiras ao stress alcalino e aumentar o seu crescimento e produtividade em solos alcalinos.
Palavras-chave: enzima antioxidante, extravasamento de eletrólitos, malondialdeído, prolina, proteína, teor de açúcar.

Introduction

Plant growth frequently encounters a range of stressful conditions, including soil acidity and alkalinity. Soil pH is a crucial factor that significantly influences soil biology, chemistry, and physical processes, directly impacting plant growth and development. Soil and crop productivity are interconnected with pH. In all soils, solubility, mobility, and bioavailability of trace elements are influenced by pH. However, soils that fall outside of the range of optimal nutrient availability are classified as either acidic or alkaline, presenting a variety of challenges to plants. While plants differ in their tolerance to extreme pH levels, most agricultural plants perform optimally at a pH near neutrality (Msimbira and Smith, 2020).

Rose (*Rosa hybrida* L.) from the Rosaceae family is the world's most popular flower, renowned for its extensive distribution, flowering habits, and traits. Roses are typically utilized as cut flowers, pot flowers, or single plants in gardens and landscapes, serving as a source of fragrance and vitamin C. The optimal day/night temperature regime of 25-27 °C/16-18 °C and the optimal relative humidity of 60%-70% are ideal for the growth and production of rose flowers. While some roses can tolerate a pH level up to 7.5, when the pH exceeds 7.5, many plants struggle to obtain sufficient phosphorus, iron, and manganese. As a result, a less-than-ideal pH can prevent our plants from effectively absorbing nutrients, even from nutrient-rich soil (Dole and Wilkins, 2005).

Humus is defined as fixed organic matter. Humic materials encompass three groups: humic acid, fulvic acid, and humin. Humic acid is generated from the decomposition of organic matter, particularly plant-derived matter. It can be found in soils, coals, and peats. Humic acid can form stable, insoluble complexes with micronutrients (Vikram et al., 2022). As a natural organic compound, humic acid comprises 50%-90% organic matter of both hydrophilic and hydrophobic types. It also contains a broad range of chemical groups, including carboxyl, phenol, carbonyl, and hydroxyls, bound to aliphatic or aromatic carbons. It also enhances the physical, chemical, and biological properties of soil and restores soil health. Humic compounds can mitigate the effects of environmental stresses such as salinity and drought on plants by producing proline and reducing the uptake of alkaline ions and can contribute to increased photosynthesis and enzymatic activity in plants (Yang et al., 2023).

The most significant feature of humic acid is that it aids in the dissolution and release of fixed elements, particularly in alkaline soils, on the one hand, and stores excess elements as a reservoir to release them to the roots as needed to balance the plant, on the other hand (Ampong et al., 2022). Given the properties of humic acid, the present study aims to investigate the influence of humic acid on the growth and physiological traits of roses grown in alkaline soil.

Materials and Methods

Rosa hybrida cv. Herbert Stevens stem cuttings were taken and planted in sand. The initial soil was alkaline, with the analysis results shown in Table 1. Once roots developed, the cuttings were transplanted into pots 14 cm in diameter and 11 cm tall. Inside a greenhouse, the plants received light at an intensity of 15,500 lux and experienced day/night temperatures of 24-27 °C and 20-23 °C, respectively. Watering occurred three times per week.

[*] **Corresponding author:** j.amiri@urmia.ac.ir; z.jabbarzadeh@urmia.ac.ir | https://doi.org/10.1590/2447-536X.v30.e242710 | Editor: Michele Valquiria dos Reis (Universidade Federal de Lavras, Brasil) | Received Dec 19, 2023, Accepted May 3, 2024 Available online June 13, 2024 |

Table 1. The results of soil analysis

Soil texture	pH	EC (dS m^{-1})	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	Organic C (%)	K (mg kg^{-1})	P (mg kg^{-1})
Loam-clay-sand	8.6	3.3	33	20	47	93	54	499	14.1

The experiment used a completely randomized design with four treatments, each replicated three times. Each replicate consisted of two pots. The treatments involved humic acid (HA) applied as a drench solution at concentrations of 0, 500, 1,000, and 2,000 mg L^{-1} at 15-day intervals for two months. Physiological and biochemical measurements were taken two weeks after the final treatment.

Soluble sugar content was evaluated following the method of Irigoyen et al. (1992). Fresh leaf tissue (0.5 g) was crushed with 5 mL of 70% ethanol. The homogenate was then centrifuged at 3,500 rpm for 15 minutes at 4 °C. To 0.1 mL of the supernatant, 3 mL of 0.2% anthrone reagent was added. The total soluble sugar content was measured using a spectrophotometer at 625 nm.

The amount of protein in mature rose leaves was measured. Leaves were ground in a buffer solution, with a pH of 6.5, spun in a centrifuge to isolate soluble proteins, and then analyzed with a spectrophotometer at a wavelength of 595 nm. Protein concentration was estimated using the Bradford method (1976) with bovine serum albumin as a standard. Finally, the protein content was expressed in mg of protein per g of fresh leaf weight (FW).

Malondialdehyde (MDA) content, an indicator of lipid peroxidation in plant tissues, was measured in the rose leaves. 0.2 g of rose leaves were ground with TCA solution. The resulting mixture was centrifuged, then was mixed with 20% TCA and 0.5% TBA. The samples were incubated in a hot water bath at 95 °C for 30 minutes. The samples were then immediately cooled. The clear supernatant was collected, and its absorbance was measured at two wavelengths: 600 nm and 532 nm using a spectrophotometer. The MDA content was calculated based on a formula established by Horst and Cakmak (1991).

$$\text{MDA } (\mu\text{mmol g}^{-1}\text{ FW}) = A_{532} - A_{600}/155) \times 100$$

Proline content, an indicator of plant stress tolerance, was measured in rose leaves. Leaf tissue was crushed in ethanol, centrifuged to isolate the extract, and then reacted with ninhydrin to produce a colored compound. The intensity of this color, measured by a spectrophotometer, was used to quantify proline levels (Paquin and Lechasseur, 1979).

The Kang and Saltiveit's (2002) method was used to provide plant extracts to determine the activity of catalase, ascorbate peroxidase and guaiacol peroxidase. 0.5 g of leaf tissues were homogenized with buffer solution (pH=7.5). The homogenate was then centrifuged at 4000 rpm for 20 minutes at 4 °C. Activity of catalase was measured by Aebi's (1984) method. The absorbance was read at 240 nm. The activity of ascorbate peroxidase was evaluated by Nakano and Asada's (1981) method. 50 mM phosphate buffer and hydrogen peroxide were added to extract. The change in the absorbance was read at 290 nm. The activity of guaiacol peroxidase was found out by Updhyaya et al. (1985) method at 420 nm.

Antioxidant capacity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical degradation method (Espin et al., 2000). A quantity of 1,000 ml of DPPH solution was added to 50 mL of leaf extract solution and after 50 min the absorbance was measured at 517 nm.

Electrolyte leakage (EL) was determined following the method described by Lutts et al. (1995). Small leaf discs were placed in water for 24-h, and the electrical conductivity of the solution was measured. This reflects the number of electrolytes (ions) leaking from damaged cells. A higher conductivity indicates more cell damage. To account for all electrolytes, the samples were then heat-treated, and the final conductivity was measured. Electrolyte leakage was calculated as a percentage based on these two conductivity readings using the formula: EL = (EC1/EC2) × 100.

Analysis of variance and means comparison were performed using SAS 9.2 software package, with means compared by Duncan's multiple range test at the $p < 0.01$ significance level.

Results and Discussion

The highest soluble sugar content was achieved in plants treated with 500 mg L^{-1} HA, demonstrating a significant difference compared to other HA rates and the control (Fig. 1).

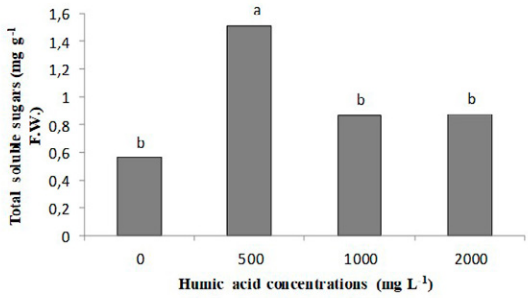

Fig. 1. Effect of different concentrations of humic acid (HA) on total soluble sugars of rose (*Rosa hybrida* cv. Herbert Stevens) under alkaline soil conditions. (Dissimilar letters show significant differences at the $p < 0.01$ level).

Sugars are considered compatible osmolytes in osmotic adjustment. They function as an osmotic agent in cell turgor maintenance, osmoprotection, carbon storage, and free radical scavenging. Moreover, they contribute to stabilizing proteins and membranes. According to previous studies, sugars play a more prominent role in osmotic adjustment than proline. Under stress conditions, the amount of sugars increases in leaf cells due to the impaired ability of sugar mobilization in the phloem. Sugars protect membranes and proteins through a specific mechanism. Hydroxyl groups (OH) of sugars replace water to preserve hydrophilic reactions in membranes and proteins, thereby binding to hydrogen ions. This can inhibit protein degradation, which is crucial for osmotic protection and adjustment. It is worth noting that the decrease in soluble sugar content in alkaline conditions is not a response to osmotic stress or ion toxicity but rather a consequence of abnormal metabolism caused by ion imbalance or impaired root function (Zhang et al., 2022).

One possible explanation for the accumulation of soluble sugars is the enhanced water uptake and relative water content of leaves. It could also be attributed to the provision of energy to bolster antioxidant enzyme activity in plants. Due to its hormonal-like activity, HA can enhance the uptake of minerals such as N, P, and K by plants (Ampong et al., 2022). Additionally, HA might have been able to counteract the increase in total soluble sugars by preserving antioxidant capacity, increasing antioxidant enzyme activity, alleviating cell injuries, and inhibiting electrolyte leakage (Lasheen et al., 2024).

Protein content was increased with HA application and all treatments used in this study were able to increase total protein compared to the control, although no significant difference was observed between HA treatments (Fig. 2). These results support the positive impact of HA on this species under alkaline soil conditions.

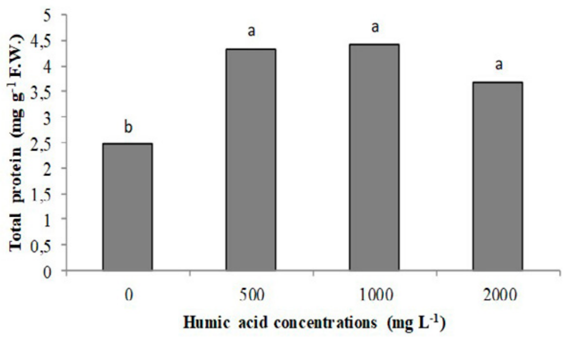

Fig. 2. Effect of different concentrations of humic acid (HA) on the total protein of rose (*Rosa hybrida* cv. Herbert Stevens) under alkaline soil conditions. (Dissimilar letters show significant differences at the $p < 0.01$ level).

Some of the major effects of stress on plants include the generation of free radicals and the impairment of cell structures such as membranes, amino acids, and proteins. Moreover, photosynthesis is reduced under these conditions, and the decreased activity of meristematic tissues can lead to reduced auxin and cytokinin synthesis. These growth hormones protect structures like proteins, in addition to influencing cell division and enlargement (Rai and Kaushik, 2023). The loss of protein content under alkaline stress may be linked to these factors. The higher protein yield observed with HA incorporation in the soil may be associated with the increased availability of minerals, particularly elements like N and Zn that enhance the ratio of proteins to carbohydrates. Proteins are not only crucial for osmotic adjustment but may also participate in ROS scavenging, improving antioxidant capacity, stabilizing biological macromolecule structures, reducing cell pH, and combating NH^{4+} toxicity (Athar et al., 2022).

In this study, the highest MDA content was observed in control plants, and HA application significantly reduced MDA levels compared to the control (Fig. 3).

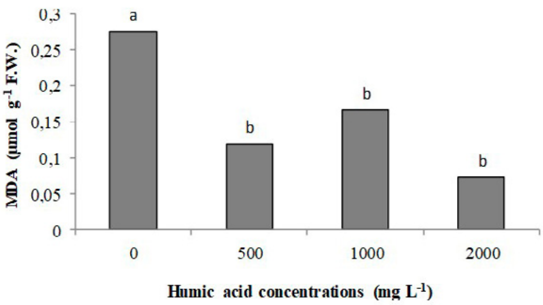

Fig. 3. Effect of different concentrations of humic acid (HA) on malondialdehyde of rose (*Rosa hybrida* cv. Herbert Stevens) under alkaline soil conditions. (Dissimilar letters show significant differences at the $p < 0.01$ level).

MDA increases significantly under alkaline stress. When plants are exposed to stress, reactive oxygen species (ROS) are generated, causing secondary oxidative injuries such as lipid peroxidation of membranes. This leads to the impairment of membrane semipermeability and increased MDA content (Hnilickova et al., 2021). Alkalinity as a stressor, can elevate MDA levels in plants. HA treatment contributed to reducing MDA content in plants as compared to the control. This suggests that HA partially alleviates oxidative stress by providing water to stressed plants, thereby reducing lipid peroxidation and degradation. The accumulation of MDA is an indicator of membrane damage. HA reduces MDA content and improves the physiological quality of flowers by enhancing antioxidant activities and participating in other enzymatic processes (Shukry et al., 2023).

A probable cause of membrane damage in control plants in this study is the generation of free radicals within the cells. These free radicals attack the fatty acids of the membranes, leading to the formation of peroxy and hydroperoxy radicals from lipid peroxidation (Sharma et al., 2023). These newly synthesized radicals can escalate oxidation reactions of lipids, further increasing MDA production. This persistent process can degrade cell membranes, leading to the outflow of water and dissolved material from the intracellular to the extracellular space. As a result, electrolyte leakage increases, and cells lose their ability to conserve water, resulting in decreased leaf water content (Sachdev et al., 2021). As shown in Table 2, proline levels increased with HA application, although all treatments caused this increase, only the 1,000 mg L^{-1} HA treatment significantly increased proline content compared to the control.

Under normal conditions, about 8% of the synthesized proline is decomposed. However, in stressful conditions, this decomposition is reduced to 4.7% due to the decrease in its oxidation to glutamate. Furthermore, since plant growth is reduced under stress, less proline is utilized for protein synthesis. When a plant encounters stress, it increases the concentration of its osmolytes to maintain osmotic adjustment and water uptake. Among compatible dissolved compounds, proline is one of the most common osmolytes. Although proline accumulates in various plant parts under stress, the highest accumulation occurs in leaves. Proline accumulation is less significant in roots. It has been documented that root proline accumulation is attributed to the synthesis of proline in leaves and its subsequent mobilization to roots (Zhang et al., 2022).

Table 2. The effect of different rates of humic acid (HA) on some biochemical traits of rose (*Rosa hybrida* cv. Herbert Stevens) under alkaline soil conditions. (Dissimilar letters in each column show significant differences at the $p < 0.01$ level)

HA rate (mg L^{-1})	Proline (mg g^{-1} FW)	Catalase (µmol min^{-1} g^{-1} FW)	Guaiacol peroxidase (µmol min^{-1} g^{-1} FW)	Ascorbate peroxidase (µmol min^{-1} g^{-1} FW)	Antioxidant capacity (%)	Electrolyte leakage (%)
0	2.27 b	3.73 c	3.11 b	5.12 b	43.33 c	41.91 a
500	2.82 ab	4.42 bc	3.22 b	9.07 a	70.23 a	34.24 a
1,000	3.45 a	7.75 a	8.77 a	6.73 ab	78.06 a	32.38 a
2,000	2.62 ab	5.66 b	7.83 a	5.12 b	59.76 b	16.28 b

As mentioned earlier, HA significantly increased proline levels in treated plants (Table 2). This may be related to the fact that dissolved sugars increase, enabling the plant to maintain its osmotic potential under various conditions, thereby maintaining carbohydrate reserves at an optimal level for basic cell metabolism and enhancing yield. Proline is an amino acid stored in the cytoplasm and is likely to play a role in effectively protecting the structure of intracellular macromolecules during stress. Studies have shown that HA enhances nitrate uptake, ATPase enzyme activity, and the synthesis of organic N-containing compounds in plants (Cavalcanti Filho et al., 2023). It appears that the initial response to HA in nutrient uptake and increased synthesis of organic protein-containing compounds like proline is the activation of membrane proteins (Ghasemi et al., 2022). HA plays a positive and effective effect in increasing the activity of several enzymes involved in the synthesis of organic compounds in plants. Additionally, the use of organic compounds and minerals essential for growth can partially contribute to plant defense systems (Chen et al., 2022).

The application of 1,000 and 2,000 mg L^{-1} HA in the present study enhanced catalase enzyme activity in roses, but the most significant improvement was observed at a concentration of 1,000 mg L^{-1} (Table 2). Also, application of 1,000 and 2,000 mg L^{-1} HA significantly influenced GPX activity (Table 2).

As shown in Table 2, while the application of humic acid positively affected ascorbate peroxidase activity, only the 500 mg L^{-1} concentration demonstrated a significant difference compared to the control, contributing to free radical scavenging and alleviating plant damage.

This study found that humic acid can effectively enhance the antioxidant capacity of rose plants under alkaline conditions. Different HA concentrations displayed efficacy, but 500 and 1,000 mg L^{-1} exhibited the most significant impact on antioxidant capacity.

Alkaline stress elevates the production of reactive oxygen species (ROS), including superoxide (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). These ROS severely disrupt the natural metabolism of plants and inflict damage on lipids, proteins, and nucleic acids. During photosynthesis, internal oxygen concentrations rise, leading chloroplasts to generate ROS. The synthesized superoxide quickly transforms into hydrogen peroxide and oxygen. Additionally, hydrogen peroxide may interact with certain metal ions and/or chelates, resulting in the generation of reactive hydroxyls (Zhang et al., 2022). Different oxygen radical species can directly harm the fats in cell membranes, inactivate enzymes, and injure nucleic acids (Mishra et al., 2023). To safeguard themselves from active oxygen damage, plants synthesize antioxidants. Superoxide dismutase converts superoxides into hydrogen peroxides.

Catalases and/or peroxidases break down hydrogen peroxides. In the absence of catalase in chloroplasts, hydrogen peroxide is decomposed by peroxidase-specific ascorbate, which is always present within these organelles. Ascorbate is synthesized in chloroplasts via the ascorbate-glutathione cycle. Furthermore, ascorbate can be directly oxidized by superoxide (Zhang et al., 2022). GPX is one of the enzymes involved in oxidizing phenol compounds and plays a crucial role in strengthening antioxidant defense. It functions as a peroxide receiver and scavenger, mitigating its detrimental effects on molecules (Rajput et al., 2021).

The findings of this study demonstrate that humic acid can effectively enhance catalase, guaiacol peroxidase, ascorbate peroxidase, and total antioxidant capacity in rose plants under alkaline conditions. HA is probably involved in scavenging free radicals by elevating the antioxidant system, thereby protecting plants from oxidative stress. It is likely that the application of HA, which improves plant nutritional status and nutrient uptake, can enhance the activity of antioxidant enzymes (Ampong et al., 2022). Humic acid's ability to alleviate oxidative stress in rose plants by increasing their antioxidant capacity makes it a promising soil amendment for cultivation in alkaline soils.

The application of humic acid in this study led to a decrease in ion leakage under alkaline conditions, with the 1,000 mg L^{-1} concentration demonstrating the most significant reduction, effectively halving ion leakage compared to the control group (Table 2).

Environmental stressors often disrupt membrane permeability and increase electrolyte leakage by stimulating reactive oxygen species (ROS) signaling pathways. Stressful conditions lead to lipid peroxidation through ROS synthesis, making membrane leakage a reliable indicator of increased electrolyte leakage. Several studies have established the crucial role of unsaturated fatty acids in cell membranes for maintaining membrane fluidity (Hasanuzzaman et al., 2020). ROS can react with fatty acids under stressful conditions, causing the peroxidation of key membrane lipids and ultimately leading to cell content leakage, rapid cell dehydration, and cell death (Ologundudu et al., 2021).

The reduced cytoplasmic material leakage observed in HA-treated plants (Table 2) suggests that humic acid provides favorable conditions for the plant and promotes cell wall stability. A similar study found that HA application enhanced pittosporum cell membrane stability, leading to a substantial decrease in electrolyte leakage (Lasheen et al., 2024).

Correlation analysis were carried out to find the factors affecting these results (Table 3). The relationship between all traits is positive. In other words, under alkaline conditions, plant protectants such as enzymatic and non-enzymatic antioxidants such as antioxidant enzymes, proline and soluble sugars were increased.

Table 3. Correlation between total soluble sugars (TSS), total protein (TP), malondialdehyde (MDA), proline, CAT, GPX, APX, total antioxidant capacity, and ion leakage of rose according to Pearson analysis.

Pearson Correlation Coefficients, N=4 Prob > ‖r‖ under H0: Rho-0									
	TSS	**TP**	**MDA**	**Proline**	**CAT**	**GPX**	**APX**	**Antioxidant**	**Ion leakage**
TSS	1.00000	0.77195 0.2281	0.98536 0.0146	0.75612 0.2439	0.96035 0.0397	0.77317 0.2268	0.92870 0.0713	0.87891 0.1211	0.88935 0.1106
TP	0.77165 0.2281	1.00000	0.66277 0.3372	0.94963 0.504	0.82270 0.1773	0.83973 0.1603	0.73965 0.2604	0.97832 0.0217	0.96117 0.0388
MDA	0.98536 0.0146	0.66277 0.3372	1.00000	0.67919 0.3208	0.94471 0.0553	0.74007 0.2599	0.93661 0.0634	0.79860 0.2014	0.79886 0.2011
Proline	0.75612 0.2439	0.94963 0.0504	0.67919 0.3208	1.00000	0.87691 0.1231	0.96212 0.0379	0.83769 0.1623	0.95816 0.0418	0.87459 0.1254
CAT	0.96035 0.0397	0.82270 0.1773	0.94471 0.0553	0.87691 0.1231	1.00000	0.91679 0.0832	0.98788 0.0121	0.92198 0.0780	0.87232 0.1277
GPX	0.77317 0.2268	0.83973 0.1603	0.74007 0.2599	0.96212 0.0379	0.91679 0.0832	1.00000	0.91855 0.0815	0.89563 0.1044	0.77694 0.2231
APX	0.92870 0.0713	0.73965 0.2604	0.93661 0.0634	0.83769 0.1623	0.98788 0.0121	0.91855 0.0815	1.00000	0.85927 0.1407	0.78616 0.2138
Antioxidant	0.87891 0.1211	0.97832 0.0217	0.79860 0.2014	0.95816 0.0418	0.92198 0.0780	0.89563 0.1044	0.85927 0.1407	1.00000	0.97485 0.0252
Ion leakage	0.88935 0.1106	0.96117 0.0388	0.79886 0.2011	0.87459 0.1254	0.87232 0.1277	0.77694 0.2231	0.78616 0.2138	0.97485 0.252	1.00000

Conclusions

Humic acid significantly mitigates the adverse effects of alkaline stress, making it a promising soil amendment for countering the negative impacts of alkaline soils on rose plants. Furthermore, 1,000 mg L^{-1} humic acid effectively improved total protein, proline, catalase activity, guaiacol peroxidase activity, and antioxidant capacity, while also reducing malondialdehyde and electrolyte leakage. Additionally, 500 mg L^{-1} HA treatment resulted in the highest sugar content and ascorbate peroxidase activity.

Acknowledgments

The authors express their thanks to the Parks and Landscape Organization of Urmia and Department of Horticultural Sciences, Faculty of Agriculture, Urmia University, who respectively provided the necessary Greenhouse and laboratory facilities to conduct this research.

Author Contribution

SM: performed the experiment, chemical analysis, statistical analysis and preparing the manuscript. JA: assistance in designing the experiment, chemical and statistical analysis and writing the manuscript. ZJ: assistance in designing the experiment, chemical and statistical analysis and writing the manuscript. MHRS: assistance in soil analysis. ASh.: provided greenhouse and plant cultivation facilities.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability Statement

Data will be made available on request.

References

AEBI, H. Catalase *in vitro*. **Methods in Enzymology**, v.105, p.121-126, 1984. https://doi.org/10.1016/s0076-6879(84)05016-3

AMPONG, K.; THILAKARANTHNA, M.S.; GORIM, L.Y. Understanding the role of humic acids on crop performance and soil health. **Frontiers in Agronomy**, v.4, p.848621, 2022. https://doi.org/10.3389/fagro.2022.848621

ATHAR, H-U-R.; ZULFIQAR, F.; MOOSA, A.; ASHRAF, M.; ZAFAR, Z.U.; ZHANG, L.; AHMED, N.; KALAJI, H.M.; NAFEES, M.; HOSSAIN, M.A.; ISLAM, M.S.; EL SABAGH, A. SIDDIQUE K.H.M. Salt stress proteins in plants: An overview. **Frontiers in Plant Science**, v.13, p.999058, 2022. https://doi.org/10.3389/fpls.2022.99905

BRADFORD, M.M.; A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v.72, n.1-2, p.248-254, 1976. https://doi.org/10.1006/abio.1976.9999

GHASEMI, Z.; JAHANBIN, S.; LATIFMANESH, H. Effects of humic acid foliar application on millet (*Panicum miliaceum* L.) yield and some of the biochemical and physiological parameters under drought stress condition in Ramjerd region of Fars. **Environmental Stresses in Crop Sciences**, v.15, n.1, p.137-147, 2022. https://doi.org/10.22077/escs.2020.3666.1890

CAVALCANTI FILHO P.F.M.; BAIA, D.C.; RIBEIRO, R.C.; ROSA, R.C.C.; CANELLAS, L.P. Humic acids induce the expression of nitrate transporters in passion-fruit seedlings. **Soils and Plant Nutrition**, v.45, e–941, 2023. https://doi.org/10.1590/0100-29452023941

CHEN, Q.; QU, Z.; MA, G.; WANG, W.; DAI, J.; ZHANG, M.; WEI, Z.; LIU, Z. Humic acid modulates growth, photosynthesis, hormone and osmolytes system of maize under drought conditions. **Agricultural Water Management.** v.263, p.107447, 2022. https://doi.org/10.1016/j.agwat.2021.107447

DOLE, J.M.; WILKINS, H.F. 2005. **Floriculture: Principles and Species**. New Jersey: Prentice Hall, 2005.1023p.

ESPIN, J.C.; SOLER-RIVAS, C.; WICHERS, H.J. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-2-picrylhydrazyl radical. **Journal of Agricultural and Food Chemistry**, v.48, p.648-656, 2000. https://doi.org/10.1021/jf9908188

HASANUZZAMAN, M.; BHUYAN, M.; ZULFIQAR, F.; RAZA, A.; MOHSIN, S.M.; MAHMUD, J.A.; FUJITA, M.; FOTOPOULOS, V. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. **Antioxidants**, v.9, p.681, 2020. https://doi.org/10.3390/antiox9080681

HNILICKOVA, H.; KRAUS, K.; VACHOVA, P.; HNILICKA, F. Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in *Portulaca oleracea* L. **Plants**, v.10, p.845, 2021. https://doi.org/10.3390/plants10050845

HORST, J.H.; CAKMAK, I. Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycin max*). **Physiologia Plantarum**, v.83, p.463-468, 1991. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

IRIGOYEN, J.J.; EMERICH, D.W.; SANCHEZ-DIAZ, M. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. **Physiologia Plantarum**, v.84, p.55-60, 1992. https://doi.org/10.1111/j.1399-3054.1992.tb08764.x

KANG, H.M.; SALTVEIT, M.E. Chilling tolerance of maize, cucumber and rice Seedling (leaves and roots) and differentially affected by salicylic acid. **Physiologia Plantarum**, v.115, p.571-576, 2002. https://doi.org/10.1034/j.1399-3054.2002.1150411.x

LASHEEN, F.F.; HEWIDY, M.; ABDELHAMID, A.N.; THABET, R.S.; ABASS, M.M.M.; FAHMY, A.A.; SAUDY, H.S.; HASSAN, K.M. Exogenous application of humic acid mitigates salinity stress on Pittosporum (*Pittosporum tobira)* plant by adjusting the osmolytes and nutrient homeostasis. **Journal of Crop Health**, v.76, p.317-325, 2024. https://doi.org/10.1007/s10343-023-00939-9

LUTTS, S.; KINET, J.M.; BOUHARMONT, J. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. **Journal of Experimental Botany**, v.46, n.12, p.1843-1852, 1995. https://doi.org/10.1093/jxb/46.12.1843

MISHRA, N.; JIANG, C.; CHEN, L.; PAUL, A.; CHATTERJEE, A.; SHEN, G. Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. **Frontiers in Plant Science**, v.14, p.1110622, 2023. https://doi.org/10.3389/fpls.2023.1110622

MSIMBIRA, L.A.; SMITH, D.L. The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. **Frontiers in Sustainable Food Systems**, v.4, p.106, 2020. https://doi.org/10.3389/fsufs.2020.00106

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. **Plant and Cell Physiology**, v.22, p.867-880, 1981. https://doi.org/10.1093/oxfordjournals.pcp.a076232

OLOGUNDUDU, F. Antioxidant enzymes and non-enzymatic antioxidants as defense mechanism of salinity stress in cowpea (*Vigna unguiculata* L. Walp) - Ife brown and Ife bpc. **Bulletin of the National Research Centre**, v.45, p.152, 2021. https://doi.org/10.1186/s42269-021-00615-w

RAJPUT, V.D.; HARISH SINGH, R.K.; VERMA, K.K.; SHARMA, L.; QUIROZ-FIGUEROA, F.R.; MEENA, M.; GOUR, V.S.; MINKINA, T.; SUSHKOVA, S.; MANDZHIEVA, S. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. **Biology (Basel)**, v.10, n.4, p.267, 2021. https://doi.org/10.3390/biology10040267

PAQUIN, R.; LECHASSEUR, P. Observationssur une methode de dosage de la praline libre dans les extraits de plantes. **Canadian Journal of Botany**, v.57, p.1851-1854, 1979. https://doi.org/10.1139/b79-233

RAI, K.K.; KAUSHIK, P. Free radicals mediated redox signaling in plant stress tolerance. **Life**, v.13, n.1, p.204, 2023. https://doi.org/10.3390/life13010204

SACHDEV, S.; ANSARI, S.A.; ANSARI, M.I.; FUJITA, M.; HASANUZZAMAN, M. Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. **Antioxidants**, v.10, p.277, 2021. https://doi.org/10.3390/antiox10020277

SHARMA, P.; LAKRA, N.; GOYAL, A.; AHLAWAT, Y.K.; ZAID, A.; SIDDIQUE, K.H.M. Drought and heat stress mediated activation of lipid signaling in plants: a critical review. **Frontiers in Plant Science**, v.14, p.1216835, 2023. https://doi.org/10.3389/fpls.2023.1216835

SHUKRY, W.M.; ABU-RIA, M.E.; ABO-HAMED, S.A.; ANIS, G.B.; IBRAHEEM, F. The efficiency of humic acid for improving salinity tolerance in salt sensitive rice (*Oryza sativa*): growth responses and physiological mechanisms. **Gesunde Pflanzen**, v.75, p.2639-2653, 2023. https://doi.org/10.1007/s10343-023-00885-6

UPDHYAYA, A.; SANKHLA, D.; DAVIS, T.D.; SANKHLA, N.; SMITH, B.N. Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in sensing soybean leaves. **Journal of Plant Physiology**, v.121, p.453-467, 1985. https://doi.org/10.1016/S0176-1617(85)80081-X

VIKRAM, N.; SAGAR, A.; GANGWAR, C.; HUSAIN, R.; NARAYAN KEWAT, R. **Properties of humic acid substances and their effect in soil quality and plant health. Humus and Humic Substances - Recent Advances**. London: IntechOpen, 2022.

YANG, F.; YUANG, Y.; LIU, Q.; ZHANG, X.; GAI, SH.; JIN, Y.; CHENG, K. Artificial humic acid promotes growth of maize seedling under alkali conditions. **Environmental Pollution**, v.327, p.121588, 2023. https://doi.org/10.1016/j.envpol.2023.121588

ZHANG, H.; ZHU, J.; GONG, Z.; ZHU, J-K. Abiotic stress responses in plants. **Nature Reviews Genetics**, v.23, p.104–119, 2022. https://doi.org/10.1038/s41576-021-00413-0