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Exogenous Application of Humic Acid Mitigates Salinity Stress on Pittosporum (*Pittosporum tobira)* Plant by Adjusting the Osmolytes and Nutrient Homeostasis

Fawzy F. Lasheen[1] · Mohammed Hewidy[1] · Ahmed N. Abdelhamid[1] · Ramez S. Thabet[2] · Mohamed M. M. Abass[2] · Asmaa A. Fahmy[3] · Hani S. Saudy[4] · Karim M. Hassan[1]

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Abstract

Improving the physiological status expressed in adjusting the osmolytes and nutrients balance of plant cell is a crucial matter for ameliorating the hazards of salinity. In this context, humic substances have a significant role for stimulating the plant tolerance to various stresses. Therefore, the current study aimed to assess the importance of foliar spray of humic acid (0 and 150 mg L^{-1}) for avoiding the effect of salt stress (0, 4000 and 8000 mg L^{-1}) on vegetative growth of pittosporum plant, protein, proline, peroxidase activity and nutrient components related to salinity. The treatments were arranged in a randomized complete block design with 3 replicates. Results revealed that the highest vegetative growth was recorded with mg L^{-1} humic acid. While, salinity levels of 4000, and 8000 mg L^{-1} led to increases in protein, proline peroxidase activity, and chloride and sodium inions. Compared to humic acid-untreated plants, application of humic acid under salinity level of 4000 mg L^{-1} enhanced plant height, root fresh weight $plant^{-1}$, root dry weight $plant^{-1}$, shoot fresh weight $plant^{-1}$ and shoot dry weight $plant^{-1}$ by 12.6, 10.9, 17.7, 43.4, 19.4%, respectively, in the second season. Humic acid application under all salinity levels showed favorable effect for keeping leaves in both seasons, since fallen leaves number was reduced. The increases in potassium (K) content reached about 12.0 and 22.4% under 4000 mg L^{-1} and 8000 mg L^{-1}, respectively, owing to humic acid application. Protein, proline content and peroxidase activity showed the minimal values under humic acid × salinity level of 4000 mg L^{-1}. It could be concluded that application of humic mitigates the harmful effect of salinity and improves the vegetative growth parameters and physiological status of pittosporum plants while increases the uptake of beneficial nutrients.

Keywords Free radical quenching · Ionic balance · Osmo-protectants · Pittosporum physiology · Soil salinity

Introduction

Pittosporum is an ornamental greenhouse plant widely used in landscaping and along roadsides, as well as in parks and gardens (Abou Kubaa et al. 2020). *Pittosporum* genus belongs to the family Pittosporaceae which includes about 200 species, the *Pittosporum tobira* plants typified by about 2–3 m high, the leaves are dark green (dark green with white zones in *Pittosporum tobira* var. variegate type) (Tian-Tain et al. 2011). The genus *Pittosporum* is considered a source of essential oils such as monoterpenes, aliphatic hydrocarbons, sesquiterpenes. Also, α-pinene as major compound was obtained from the flowers of pittosporum plants (Nickavar et al. 2004). Furthermore, El-Dib et al. (2015) reported that the *n*-butanol fractions from pittosporum leaves possess antimicrobial activity and cytoprotective effects against breast carcinoma, hepatocellular carcinoma colon carcinoma cancer cell lines. Besides pittosporum is regarded as one of popular ornamental decorative shrubs in landscape purposes because of its beautiful shape and leave colors and white odor flowers.

✉ Hani S. Saudy
hani_saudy@agr.asu.edu.eg

[1] Horticulture Department, Faculty of Agriculture, Ain Shams University, 68-Hadayek Shoubra, 11241 Cairo, Egypt

[2] Department. of Ornamental Plant Research Institute, Agriculture Research Center, Giza, Egypt

[3] Horticulture Department of, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

[4] Agronomy Department, Faculty of Agriculture, Ain Shams University, 68-Hadayek Shoubra, 11241 Cairo, Egypt

Distinctive adverse effects on crop yield and quality are realized owing to eco-stresses occurrence (Saudy et al. 2020a; Mubarak et al. 2021; El-Metwally and Saudy 2021; El-Bially et al. 2022a; El-Metwally et al. 2022a; Saudy et al. 2023a). Under various environmental stresses, several changes in plant physiology expressed in nutrient uptake and osmolytes accumulation occur (Hadid et al. 2023), affecting crop growth and productivity (Saudy and El-Metwally 2019, 2023). Salinity is one of the most widespread abiotic stresses which results in significant losses in agricultural crop production, especially in arid and semiarid areas (Salem et al. 2021; Hernández 2019; Alsamadany et al. 2022; El-Bially et al. 2022b). Saline conditions (consists of Cl and or Na ions) results in retarding growth, chlorophyll damage with low crop quantity and quality (Abd El-Mageed et al. 2022; Salem et al. 2022; Shaaban et al. 2023a).

Humic acid is a natural soil organic matter compound resulted from either plant, animal or microbial residues decomposition as well as from metabolic activity of microbes (Du jardin 2015). Several studies showed that humic acid not only enhances root, leaf and shoot growth, but also, stimulates the germination of many plant species (Trevisan et al. 2010). Humic acids are the main fractions of humic substances and the most active components of soil and compost organic matter. Humic acids have been shown to stimulate plant growth and consequently yield by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities (Chen et al. 2004a; Dinçsoy and Sönmez 2019; Ramadan et al. 2023). Because of their potential to stimulate nutrient content, phenols, flavonoids and antioxidant activity in plant leaves, humates compounds considered natural antioxidants (Bayat et al. 2021). It is well known that the increase in sodium and chloride ions accumulation in plant tissues associates salt stress, causing disruption ion homeostasis, with physiological disturbance, and reduction in potassium uptake (Ketehouli et al. 2019). Also, the accumulation of osmolytes such as proline and soluble proteins increased in salt-stressed plants (Rahman et al. 2016). Contrary, humic acid reduced the accumulation of sodium and salinity-induced increase in proline while increased the antioxidant enzymes, protecting the plant from hazards of salinity (Abbas et al. 2022; Abu-Ria et al. 2023). The optimal concentrations able to affect and stimulate plant growth have been generally found in the range of 50–300 mg L^{-1}, but positive effects have been also exerted by lower concentrations Chen et al. (2004b). A distinction on the effects of humic acid should be made between indirect and direct effects on plants growth. Indirect effects are mainly exerted through properties such as enrichment in soil nutrients, increase of microbial population, higher cation exchange capacity, improvement of soil structure (Roudgarnejad et al. 2021). Whereas direct effects are various biochemical actions exerted at the cell wall, membrane or cytoplasm and mainly of hormonal nature (Varanini and Pinton 2001). The hormone like activities of humic acids is well documented in various papers, in particular auxin-, cytokinin- and gibberellins like effects (Nardi et al. 2016).

Based on the distinctive properties and mode of action of humic acid, the current study hypotheses that the negative impacts of salinity on pittosporum could be mitigated by exogenous supply of humic acid via adjusting the osmolytes and nutrient homeostasis. Therefore, the main objective of the research was to assess the change in physiology and nutrient contents f pittosporum plants owing to application of humic acid under different salt stress levels.

Material and Methods

Plant Material and Practical Procedures

This study was conducted at the Ornamental Farm, Department of Horticulture, Faculty of Agriculture, Ain Shams University, Cairo, Egypt during two seasons of 2020 and 2021. The basal physico-chemical analysis of the used experimental soil was determined by the standard methods of Page et al. (1982) and Klute (1986). The analysis showed that the soil classified as loamy sandy, comprising 86.0% sand, 12.0% silt, and 2.0% silt while having 0.44% organic matter, 1.48% calcium carbonate, pH of 7.44, 1.05 dS m^{-1} electric conductivity, 17.64 mg kg^{-1} total nitrogen, 13.60 mg kg^{-1} available phosphorus, and 254.78 mg kg^{-1} available potassium.

One month old, healthy and uniform in shape, *pittosporum tobira* var. *variegata* transplants (20 cm, length) were purchased from a private nursery, Giza, Egypt. Each single transplant was cultivated in the first week of April (in both seasons) in a plastic pot (35 cm diameter) filled with loam and sand (1:1). The irrigation was regularly done 2–3 times a week after calculating the decrease in water-holding capacity using the weight method. Fertilization was done by using a half-strength Hoagland's nutrient solution (one time every 10 days). After 2 months of cultivation, all pots (72 pots) were divided into 2 groups, in the first week of June, to apply the foliar applications of humic acid treatments (distilled water as a control, without humic acid) and spraying of 150 mg L^{-1} with a rate of 50 ml solution per pot (technical grade, Sigma-Aldrich Chemical Co. Washington, USA). Pittosporum plants were subjected to three salinity levels (0, 4000, 8000 mg L^{-1} NaCl salt. The experiment layout in a randomized complete block design with 3 replicates.

Assessments

Vegetative Growth

On August 15 in each year, plant height, lateral branches number plant^{-1}, leaves number plant^{-1}, stem diameter, fallen leaves number plant^{-1}, root fresh and dry weight plant^{-1} and shoots fresh and dry weight plant^{-1} were measured.

Plant Mineral Content

Representative pittosporum shoot samples were oven dried at 70 °C and milled into fine powder before determining the ions of chloride (Cl), potassium (K) and sodium (Na) on a dry weight basis. Cl ions were determined by Mohr method described by Sheen and Kahler (1938). K and Na ions were assessed by using Phillips Unicum Atomic absorption spectrometer as described by Chapman and Pratt (1982). Furthermore, K/Na Ratio was calculated.

Proteins, Proline and Peroxidase Activity

Total proteins, according to AOAC (2012), and proline based on the methods of Bates et al. (1973) were determined. The peroxidase enzyme (POD) activity was determined using 4-methylcatechol as substrate. The increase in the absorption caused by oxidation of 4-methylcatechol by H_2O_2, was measured at 420 nm spectrophotometrically. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 5 mM 4-methylcatechol, 5 mM H_2O_2 and 500 µl of crude extract in a total volume of 3.0 m. at room temperature. One unit of enzyme activity was defined as 0.001 change in absorbance per min, under assay conditions (Chance and Maehly 1955; Onsa et al. 2004).

Data Analysis

A 2–way analysis of variance (ANOVA) was performed for the obtained data according to Casella (2008), using Costat software program, Version 6.303 (2004). Salinity and humic acid treatments were considered fixed effects while replications (blocks) were considered random effects. Using to Duncan's multiple range test, means were separated only when the F–test indicated significant ($P < 0.05$) differences among the treatments.

Results

Growth Response

Results data related to the growth of pittosporum clarified the adverse effect of salinity on plant height, lateral branches number plant^{-1}, stem diameter, leaves number plant^{-1} (Table 1), as well as fresh and dry weights of roots and shoots (Table 2) in growing seasons of 2020 and 2021 Plant height and Leaves number plant^{-1} in both seasons, fresh and dry weights of plant roots in the second season as well as dry weight of plant shoots in the first season showed the maximum reduction with salinity level of 8000 mg L^{-1}. Salinity level of 8000 along 4000 recorded reductions of lateral branches number plant^{-1} stem diameter and shoot weight plant^{-1} in both seasons as well as fresh and dry weights of plant roots in the first season and dry weight of plant shoots in the second season compared to the con-

Table 1 Effect of humic acid as foliar application and salinity stress and their interaction on plant height, lateral branch number, stem diameter and leaves number of pittosporum plant during 2020 and 2021 seasons

Variable		Plant height (cm)		Lateral branches number plant^{-1}		Stem diameter (cm)		Leaves number plant^{-1}	
		2020	2021	2020	2021	2020	2021	2020	2021
Salinity (mg L^{-1}), S									
0		40.16a	41.16a	2.66a	2.16a	1.24a	1.24a	149.33a	163.33a
4000		32.33b	33.66b	1.66ab	1.58b	1.11ab	1.05b	115.83b	124.50b
8000		29.33c	29.50c	1.50b	1.33b	0.98b	0.98b	105.50c	107.50c
Humic acid (mg L^{-1}), H									
0		35.44a	32.55b	1.66a	1.66a	1.15a	1.04a	114.00b	126.88b
150		32.44b	37.00a	2.22a	2.00a	1.07a	1.14a	133.11a	136.66a
S x H									
0	0	37.66b	40.00b	2.33ab	2.00ab	1.23a	1.18ab	139.33ab	161.66b
	150	42.66a	42.33a	3.00a	2.66a	1.25a	1.30a	159.33a	165.00a
4000	0	31.33cd	31.66d	1.33b	1.50b	1.05ab	1.05b	110.00cd	119.00b
	150	33.33c	35.66c	2.00ab	1.66b	1.18a	1.06b	121.66bc	130.00b
8000	0	28.33d	26.00e	1.33b	1.33b	0.93b	0.90c	92.66d	100.00c
	150	30.33cd	33.00d	1.66ab	1.33b	1.03ab	1.06b	118.33bcd	115.00bc

Means sharing the same letter for each variable in each column are not significantly different by Duncan's multiple range test at $p \leq 0.05$

Table 2 Effect of humic acid as foliar application and salinity stress and their interaction on root and shoot weights of pittosporum plant during 2020 and 2021 seasons

Variable		Root weight plant^{-1} (g)				Shoot weight plant^{-1} (g)			
		Fresh		Dry		Fresh		Dry	
		2020	2021	2020	2021	2020	2021	2020	2021
Salinity (mg L^{-1}), S									
0		54.83a	56.40a	32.86a	30.05a	127.53a	122.62a	47.23a	43.98a
4000		44.09b	39.60b	24.34b	22.50b	88.44b	89.01b	40.50b	35.21b
8000		41.58b	36.52c	23.69b	20.16c	69.35b	77.72b	34.59c	32.25b
Humic acid (mg L^{-1}), H									
0		47.28a	42.54b	26.17a	22.47b	88.81a	86.33b	40.41a	34.72b
150		46.39a	45.82a	27.76a	26.00a	101.40a	106.57a	41.13a	39.57a
S x H									
0	0	54.66a	55.01a	31.66a	27.76b	122.97ab	119.85a	46.63a	42.90ab
	150	55.00a	57.79a	34.06a	32.33a	132.09a	125.39a	47.82a	45.06a
4000	0	45.66b	37.54c	23.78b	20.66d	83.70cd	73.13c	40.60b	32.09de
	150	42.52b	41.66b	24.90b	24.33c	93.19bc	104.89ab	40.40b	38.33bc
8000	0	41.51b	35.05c	23.06b	19.00d	59.78d	66.02c	34.00c	29.16e
	150	41.66b	38.00c	24.33b	21.33d	78.93cd	89.42bc	35.18c	35.33cd

Means sharing the same letter for each variable in each column are not significantly different by Duncan's multiple range test at $p \leq 0.05$

trol treatment. On the other hand, the lowest fallen leaves number was obtained under non-saline conditions (Fig. 1). While, salinity levels of 4000 and 8000 mg L^{-1} increased the leaves fall than no salinity level by 2.13 and 3.45 in the first season and 1.92 and 3.32 times in the second one, respectively.

The beneficial effect of humic acid on pittosporum growth was more pronounced in the second season. In this regard, the increases due to humic acid application in plant height, leaves number plant^{-1}, root fresh weight plant^{-1}, root dry weight plant^{-1}, shoot fresh weight plant^{-1} and shoot dry weight plant^{-1} were 13.7, 7.7, 7.7, 15.7, 23.4 and 13.9%, respectively, compared to the humic acid-untreated plants. Humic acid application coped the leaves fall by 64.5 and 65.5% in the first and second seasons, respectively (Fig. 1).

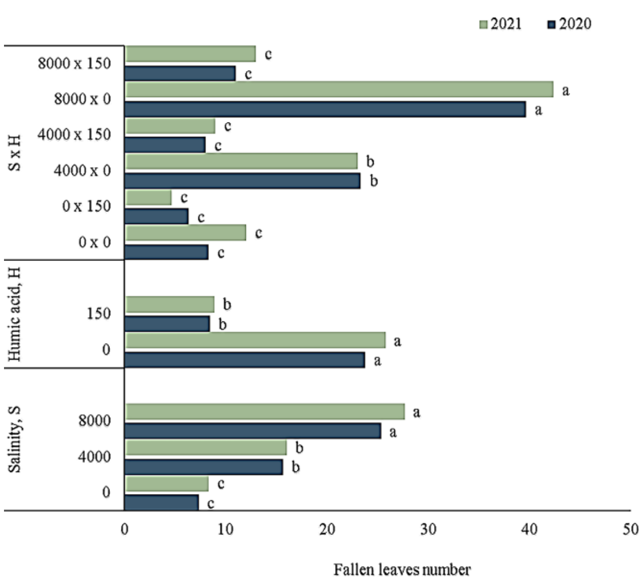

Fig. 1 Effect of humic acid as foliar application and salinity stress and their interaction on fallen leaves number of pittosporum plant during 2020 and 2021 seasons. Means sharing the same letter for each variable in each bar are not significantly different by Duncan's multiple range test at $p \leq 0.05$

From the interaction data, it is interesting to note that humic acid had favorable role act for alleviating the hazards of salinity stress on pittosporum plants especially in 2021 season. Compared to humic acid-untreated plants, application of humic acid under salinity level of 4000 mg L^{-1} enhanced plant height, root fresh weight plant^{-1}, root dry weight plant^{-1}, shoot fresh weight plant^{-1} and shoot dry weight plant^{-1} by 12.6, 10.9, 17.7, 43.4, 19.4%, respectively, in the second season. Moreover, plant height, stem diameter, and shoot dry weight plant^{-1} were showed increased values with humic acid application higher than no humic acid application under salinity level of 8000 mg L^{-1}. Humic acid application under all salinity levels showed favorable effect for keeping leaves in both seasons, since fallen leaves number was reduced (Fig. 1).

Mineral Content

As shown in Table 3, increase in salinity level increased chloride (Cl) and sodium (Na) as well as decreased potassium (K) and K/Na ratio. Thus, the maximum Cl and Na values were recorded with salinity level of 8000 mg L^{-1}. Unlike, without salinity effect K and K/Na ratio gave the highest values. On the other site, addition of humic acid-

Table 3 Effect of humic acid as foliar application and salinity stress and their interaction on chloride (Cl), potassium (K), sodium (S) and K/Na ratio of pittosporum plant during 2020 and 2021 seasons

Variable		Nutrient (%)			K/Na ratio
		Chloride	Potassium	Sodium	
Salinity (mg L^{-1}), S					
0		0.23c	3.01a	0.20c	15.81a
4000		1.00b	2.57b	0.87b	3.45b
8000		2.23a	2.44c	1.93a	1.30c
Humic acid (mg L^{-1}), H					
0		1.41a	2.45b	1.17a	7.01a
150		0.96b	2.89a	0.83b	6.70a
S × H					
0	0	0.17f	2.74b	0.15f	17.98a
	150	0.29e	3.27a	0.24e	13.64b
4000	0	1.37c	2.42e	1.18c	2.05d
	150	0.62d	2.71c	0.56d	4.84c
8000	0	2.68a	2.19f	2.18a	1.00d
	150	1.96b	2.68d	1.68b	1.59d

Means sharing the same letter for each variable in each column are not significantly different by Duncan's multiple range test at $p \leq 0.05$

treated plants had lower Cl and Na content (31.9 and 29.1% decreases, respectively, and higher K (17.9% increase) compared to humic acid-untreated plants.

Under saline conditions, humic acid caused reductions in Cl and Na amounted to 54.7 and 52.5% under 4000 mg L^{-1} and 26.9 and 22.9% under 8000 mg L^{-1}, respectively (Table 3). Furthermore, the increases in K content reached about 12.0 and 22.4% under 4000 mg L^{-1} and 8000 mg L^{-1} owing to humic acid application compared to the counterpart control treatment (without humic acid).

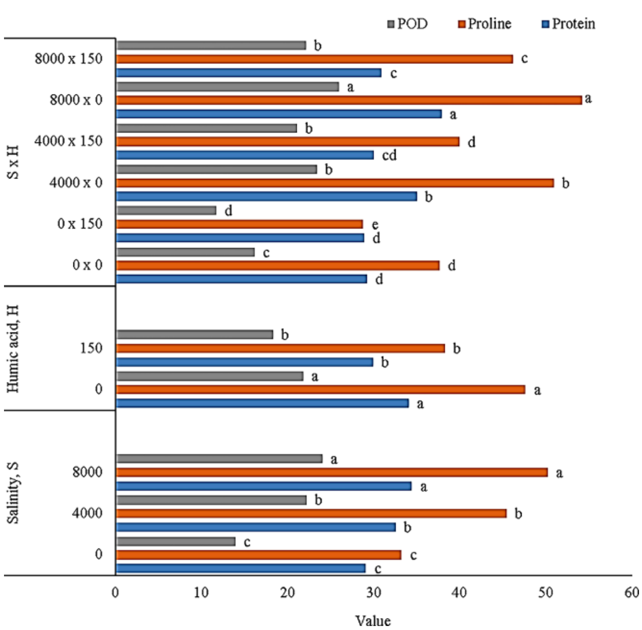

Fig. 2 Effect of humic acid as foliar application and salinity stress and their interaction on total protein (mg g^{-1} fw), proline content (mg g^{-1} fw) and peroxidase (POD) enzyme activity (U g^{-1}) in pittosporum plant. Means sharing the same letter for each variable in each bar are not significantly different by Duncan's multiple range test at $p \leq 0.05$

Osmolytes and Antioxidant Activity

As depicted in Fig. 2, protein, proline and peroxidase activity significantly responded to salinity level and humic acid and their interaction. Salinity levels of 4000 mg L^{-1} and 8000 mg L^{-1} showed increases in protein (11.8 and 18.3%), proline (36.9 and 51.2%) and peroxidase activity (59.6 and 72.4%) higher than the control treatment. With application of humic acid, protein content, and proline content and peroxidase activity were decreased. With exception of the normal condition (no salinity), protein content, and proline content and peroxidase activity showed the minimal values under humic acid × salinity level of 4000 mg L^{-1}.

Discussion

Huge efforts have been exerted to overcome the issues and sustain the crop productivity and quality under harsh growth conditions (El-Bially et al. 2018; El-Metwally et al. 2021; Saudy et al. 2021; El-Metwally et al. 2022b; Abd–Elrahman et al. 2022; Shaaban et al. 2023b). Plants exposed to salinity stress exhibited a reduction in growth and yield potential (Liu et al. 2020; Shahid et al. 2020). However, applications of humic acid mitigated the negative effects of salinity, as evidenced by the increases in plant height, lateral branches number, stem diameter, leaves number as well as root and shoot weights of pittosporum (Table 1 and 2) and decrease in fallen leaves (Fig. 1). To explain the beneficial effect of humic acid-mediated salinity stress mitigation, the current study assessed several features that could be used to improve pittosporum response under salinity conditions. It has been observed that subjecting plants to salt stress led to significant decreases in all vegetative growth parameters (plant height, lateral branches number, leaves number, stem diameter, fresh and dry weights of plant parts (root and shoot), while fallen leaves and toxic ions increased. Salinity had serious unfavorable effects on plant growth, as evidenced by the significant reduction in growth and biomass of tomato (Alam et al. 2020) and faba bean (Abdel Latef et al. 2021). In the current study, salinity resulted in an ionic imbalance, since potassium was reduced while chloride and sodium were increased under salt stress.

Due to induction of osmotic stress with over-accumulation of Na^+ and Cl^- ions in cells by salinity, ionic imbalance and toxicity were obtained (Khan et al. 2018). Salinity causes handicapping the absorption of needed nutrients (Khan et al. 2019). Owing to the ionic misbalance in plants, negative changes in different morpho-physiological and biochemical were reported (Makhlouf et al. 2022; Saudy et al. 2023b). Moreover, mung bean plants suffer from water deficiency with low nutrients absorption under saline conditions, hence photosynthesis disruption and oxidative stress occurred (Rahman et al. 2019). Owing to salinity, reactive oxygen species (ROS) generated in plant cells (Khan et al. 2018; Hadid et al. 2023), causing oxidative damage expressed in lipid membranes deterioration of the plant cells (Guimarães et al. 2011). Accordingly, salinity leads to reduction of plant leaf area, gas exchange through stomata, and plant pigments concentrations (Netondo et al. 2004; Shaaban et al. 2023a). Due to high accumulation of sodium as a toxic ion and decrease in potassium, calcium and magnesium as beneficial ions, the distinctive association between weak growth of salt-stressed plants and ionic toxicity was observed (Abdel Latef et al. 2021). This might be attributed to the injuries of the cell membrane and ion leakage (Abd El-Mageed et al. 2022), and turmoil in uptake of beneficial ions (Liu et al. 2019; Mubarak et al. 2021).

For adapting to salt stress, plants synthesize specific proteins to be more tolerant under salinity situations (Bavei et al. 2011). Various plants produced certain proteins under stress conditions (Parker et al. 2006; Younis et al. 2009; Hadid et al. 2023). This effect has been proved in the current study, since protein, proline and peroxidase activity increased with increasing salinity level (Fig. 2). Plants could combat the osmotic stress of salinity by accumulating a diverse osmolytes in their cells such as proline (Hayat et al. 2012; Zulfiqar et al. 2020), hence water balance and osmotic potential maintained (Mansour and Ali 2017; Hasanuzzaman et al. 2019). The physiological upregulation of the defensive actions for oxidative pressure has been recorded in different plants under abiotic stresses (Latif et al. 2016; Tahjib-Ul-Arif et al. 2018).

Several attempts have been adopted to mitigate the impact of salinity and increase the tolerance of plants to salt stress (Wang et al. 2019). It is well known that nutrients are readily absorbed by plant roots when the soil minerals are available (Ouni et al. 2014). Therefore, as obtained in the current study, soil addition of humic acid significantly increased the beneficial nutrient contents in the leaves of pittosporum plants. This useful finding may be because humic acid participates to an enhancement in organic acids (Liu et al. 2019), while plant phytohormones released by microorganisms (Cheng et al. 2020; Sun et al. 2021) to shrink the unwholesome impacts of salts in the soil. Additionally, the generated phytohormones motivate the proliferation of plant roots (Nunes et al. 2019), promoting the nutrient absorbing surfaces and organic acids production which increase the solubility for various mineral forms, hence enhance plant responses (Rady et al. 2016; Belal et al. 2019; Saudy et al. 2020b). Humic increases accumulation of leaf nutrients and chlorophyll biosynthesis (Kaya et al. 2018). Furthermore, humic substances can display gibberellin- and cytokinin-like activities (Nardi et al. 2016), with improvement in cell membrane permeability (Osman and Rady 2014). Therefore, the saline soil fertility and plants nutrients uptake were ameliorated in favor of plant growth and yield with humic acid application (Table 1, 2 and 3).

Generally, humic acid ameliorated the negative effects of salinity on pittosporum plant through adjusting the osmolytes and nutrient homeostasis. Thus, pittosporum growth and development were relatively improved under saline conditions by application of humic acid.

Conclusion

Humic acid is a good anti-stress for a salinity level of 4000 mg L^{-1} where most pittosporum traits of are improved. The beneficial effects of humic acid for coping the salinity injury are associated with its significant role in adjusting various physiological aspects expressed in nutritional status and osmo-protectant composition. Accordingly, it is advisable to introduce humic acid as a useful practice pittosporum plants cultivated under saline conditions.

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Conflict of interest F.F. Lasheen, M. Hewidy, A.N. Abdelhamid, R.S. Thabet, M.M.M. Abass, A.A. Fahmy, H.S. Saudy and K.M. Hassan declare that they have no competing interests concerning the current research publication.

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Hani Saber Saudy Professor of Agronomy