

The Effect of Humic Acid Use on Reducing Salt Stress in Purple Basil (*Ocimum basilicum* L.)

Mor Fesleğende (*Ocimum basilicum* L.) Tuz Stresinin Azaltılmasında Hümik Asit Kullanımının Etkisi

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Abstract

Salt stress is a significant environmental stressor limiting plant development, yield potential and overall plant performance worldwide. Humic acid, a biostimulant agent, encourages plant growth and boosts stress tolerance to against the adverse impacts of salt stress. The current research intends to examine the activity of humic acid application in alleviating the detrimental influence of saltinity in purple basil (*Ocimum basilicum* L. cv. midnight). To achieve this goal, a completely randomised factorial experiment with three replications was designed. Two different humic acid doses (0 and 1000 mg L⁻¹) and three varying salt treatments (0, 75 and 150 mM NaCl) were administered to the plants. Purple basil plants growth traits (plant height, leaf length, leaf fresh and dry weights), chlorophyll fluorescence (Fv/Fm), electrolyte leakage and photosynthetic pigment (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) levels were identified. Plant height, leaf length, fresh leaf weight, dry leaf weight, chlorophyll fluorescence (Fv/Fm), chlorophyll a and total chlorophyll level declined in salt stressed plants subjected in relative to the control group. Humic acid treatment helped with the elimination of the harmful influence of salinity on these parameters (except leaf length, chlorophyll a and total chlorophyll at 150 mM NaCl concentration). However, chlorophyll b and total carotenoid content in salt-treated plants showed no significant difference at 75 mM NaCl, whereas they decreased considerably at 150 mM NaCl. While the influence of humic acid treatment on chlorophyll b and total carotenoid content was statistically insignificant (p>0.05) at 75 mM NaCl, it was found to have decreasing effect at 150 mM NaCl. Furthermore, electrolyte leakage increased linearly with increasing salinity, while humic acid application only showed an ameliorating effect at 75 mM NaCl concentration. The results of this study showed that humic acid was successful in removing the impact of salt stress in purple basil at 75 mM NaCl.

Keywords: Abiotic stress, Humic acid, Photosynthetic pigments, Purple basil, Salinity

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Öz

Tuz stresi, dünya genelinde bitki gelişimini, verim potansiyelini ve genel bitki performansını sınırlayan önemli bir çevresel stres faktörüdür. Hümik asit, bir biyostimülan olarak, bitki büyümesini teşvik etmekte ve tuz stresinin olumsuz etkilerine karşı bitki direncini artırmaktadır. Bu çalışma, mor fesleğende (*Ocimum basilicum* L. cv. midnight) tuzluluğun zararlı etkisini hafifletmede humik asit uygulamasının etkinliğini incelemeyi amaçlamaktadır. Bu amaca ulaşmak için, üç tekrarlı tamamen rastgele faktöriyel bir deney tasarlanmıştır. Bitkilere iki farklı humik asit konsantrasyonu (0 ve 1000 mg L⁻¹) ve üç farklı tuz uygulaması (0, 75 ve 150 mM NaCl) yapılmıştır. Mor fesleğen bitkisinin büyüme parametreleri (bitki boyu, yaprak uzunluğu, yaprak taze ve kuru ağırlıkları), elektrolit sızıntısı, klorofil floresansı (Fv/Fm) ve fotosentetik pigment (klorofil a ve b, toplam klorofil ve karotenoidler) seviyeleri belirlenmiştir. Bitki boyu, yaprak uzunluğu, yaprak taze ağırlığı, yaprak kuru ağırlığı, klorofil floresansı (Fv/Fm), klorofil a ve toplam klorofil seviyesi tuz stresine maruz kalan bitkilerde kontrol grubuna kıyasla azalmıştır. Hümik asit uygulaması, tuzluluğun bu parametreler üzerindeki zararlı etkilerinin ortadan kaldırılmasına katkıda bulunmuştur (150 mM NaCl konsantrasyonunda yaprak uzunluğu, klorofil a ve toplam klorofil hariç). Bununla birlikte, tuz stresi altındaki bitkilerde klorofil b ve toplam karotenoid içeriği 75 mM NaCl'de önemli bir farklılık göstermezken, 150 mM NaCl'de belirgin şekilde azalmıştır. Hümik asit uygulamasının klorofil b ve toplam karotenoid içeriği üzerindeki etkisi 75 mM NaCl'de istatistiksel olarak önemsizken (p>0.05), 150 mM NaCl'de azaltıcı etkiye sahip olduğu bulunmuştur. Ayrıca, elektrolit sızıntısı artan tuzlulukla doğrusal olarak artarken, hümik asit uygulaması sadece 75 mM NaCl konsantrasyonunda iyileştirici etki göstermiştir. Bu çalışmanın sonuçları, humik asidin mor fesleğende tuz stresinin etkisini gidermede 75 mM NaCl'de başarılı sonuçlar verdiğini ortaya koymuştur.

Anahtar Kelimeler: Abiyotik stres, Hümik asit, Fotosentetik pigmentler, Mor fesleğen, Tuzluluk

1. Introduction

Purple basil (*Ocimum basilicum* L. cv. midnight) is an annual, highly valued herb from the mint family (*Lamiaceae*), widely cultivated worldwide (Zaman et al., 2022). Purple basil contains monoterpenes, sesquiterpenes, phenylpropanoids and especially the red-blue plant pigment anthocyanins (McCance et al., 2016; Mishra et al., 2022). The use of basil is not limited to a single field of endeavour; rather, it is employed in a number of different areas, including traditional medicine, cosmetics, pharmaceuticals and the food industry (Bravo et al., 2021). Furthermore, among the most important uses of purple basil include anticancer, antimicrobial, anti-inflammatory, antidiabetic and antioxidant properties (Shahrajabian et al., 2020; Mishra et al., 2022).

Salinity is a critical problem threatening agricultural productivity on an international level, particularly in arid and semi-arid areas (Majeed and Muhammad, 2019). Globally, salinity affects 20% cultivated land and 33% of irrigated areas and salinity level of arable land are expected to increase to 50 % by 2050 (Soda et al., 2016; Machado and Serralheiro, 2017). The primary causes of this process are climate change, the over exploitation of groundwater, consumption of poor-quality irrigation water and the application of chemical fertilisers (Machado and Serralheiro, 2017; Eswar et al., 2021). Salinity, which indicates soil salt levels, is determined in desi Siemens (dS) and is often stated as electrical conductance (Hasan et al., 2024). Saline soil is expressed as soil with a saturated soil solution in which the reaches 4 dS m⁻¹ (approximately 40 mM NaCl) and concentration of ions exceeding 4 dS m⁻¹ in the rhizosphere is indicative of stress circumstances (Demirbas and Balkan, 2018; Bernstein, 2019; Majeed and Muhammad, 2019). Soil salinity leads to a range of physiological and metabolic disorders in plants, affecting numerous processes, including seed germination, osmotic stress, ion toxicity, reduced water availability for plants, decreasing photosynthetic activity, accumulation of reactive oxygen species (Naveed et al., 2020; Balasubramaniam et al., 2023; Beyaz and Kazankaya, 2024; Stefanakis et al., 2024).

To increase salt stress resistance in plants, several methods are used. Among these methods, plant growth regulators, osmolytes, mineral supplements and supplements made from organic materials have an important place (Ashraf et al, 2008; Wichern et al., 2020). Organic fertilisers increase productivity by improving the structural and chemical features of the soil as well as meeting the nutritional needs of the plant with its structure consisting of plant and animal wastes (Tunçtürk et al., 2020; Turhan and Ozmen, 2021). The main component of organic fertiliser is humic substances (Canellas et al., 2015). Humic matters are analysed in three main groups according to their content: humins, humic acids and fulvic acids and humic acids are the main constituents of these substances (Kaya et al., 2018; Ukalska-Jaruga et al., 2021). Humic acid is a natural organic substances of large molecular weight, distributed in terrestrial soil, sediment and natural water (de Melo et al., 2016; Alomar et al., 2023). Humic acid contributes to plant development and yield by enhancing soil fertility and nutrient availability (Maiwan et al., 2023). To illustrate, humic acid addresses nutrient deficiency by enabling the binding of essential nutrients, including sodium, potassium, magnesium, zinc, iron, copper and calcium (Busato et al., 2023). In addition, humic acid increases water holding capacity by creating large pore spaces in the soil and prevents water evaporation by binding with water molecules (Amerian et al., 2024). Rakkammal et al. (2024) found that a lower dose of humic acid (100 mg L⁻¹) alleviated the negative effects of salt stress in finger millet. In the research examining the impact of humic acid (0.05%) on switchgrass plants in a salty environment, it was demonstrated that humic acid treatment elevated plant height (41.1%) and dry weight (26.9%) and improved photosynthetic functions (Zhang et al., 2024). Investigation performed on tomato plants, applying humic acid (3 ml/l) under salt stress (150 mM) was found to increase chlorophyll values while reducing ion leakage (Ural et al., 2023). Similarly, increasing humic acid concentrations (0, 500, 1000 mg l⁻¹) provided tolerance of Italian basil to salinity conditions (Hoseini et al., 2023). However, the response of physiological and morphological characteristics of purple basil to humic acid in salt stressed conditions remains unclear.

The prevalence of soil salinity is on the rise, largely due to the increased utilisation of chemical inputs in crop production and the adoption of non-conservative irrigation techniques. In light of the detrimental impact of salinity on crop growth and yield, and the pivotal role of humic acid application in mitigating salt stress, the external application of humic acid may prove to be a crucial strategy for the cultivation of purple basil under conditions of salt stress. For this reason, this research has examined the potential of humic acid to decline the deleterious effects of salt stress on purple basil.

2. Materials and Methods

2.1. Plant samples and growth medium

This survey was executed under controlled greenhouse conditions (25°C, 70% humidity) at Duzce University Faculty of Forestry from 15 February to 15 June 2022. The research design was performed in a completely randomised set up with three repetitions. The plant used in the study was purple basil (*Ocimum basilicum* L. cv. Midnight) (Vilmorin). ‘K-Humate’ purchased commercially from Gübretaş (Istanbul, Türkiye) was used as stress alleviator in the study. K-Humate contains a high proportion of organic matter (25%) with potassium and a high proportion of fulvic and humic acids. In the experimental, the plants were treated with two levels of humic acid (0 and 1000 mg L⁻¹) and three varying salt treatments (0, 75 and 150 mM NaCl). Seeds were sown in pots containing peat:perlite (1:1) (Table 1) with three seeds in each pot (diameter, 14.5 cm, depth, 10.5 cm). After germination, two plant was cultivated in each pot. Seedlings received irrigation with fresh half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). When the plants reached the four leaf stage, all pots were divided 2 groups and seedlings were treated with or without humic acid for seven days. Humic acid solution prepared at a concentration of 1000 mg L⁻¹ was mixed with full Hoagland's solution (NH₄H₂PO₄, Ca(NO₃)₂, MgSO₄, KNO₃, Fe₂(C₄H₄O₆), H₃BO₃, CuSO₄.5H₂O, MnCl₂.4H₂O, MoO₃, ZnSO₄.7H₂O; EC: 1006.0 µS cm⁻¹) and applied to the plants as irrigation water every two days. After, humic acid applications were completed and subsequently, salt treatments were applied to the plants. Salt doses (0, 75 and 150 mM NaCl) were implemented to seedlings combined with Hoagland's solution. Hence, the experimental design included the six treatment groups: (1) Control, normal conditions without humic acid and salt applications (2) 75 NaCl, 75 mM NaCl application alone without humic acid treatment (3) 150 NaCl, 150 mM NaCl application alone without humic acid treatment (4) HA, humic acid pretreatment for seven days without salt applications (5) HA+75 NaCl, 75 mM NaCl application for six weeks after humic acid pretreatment for seven days (6) HA+150 NaCl, 150 mM NaCl application for six weeks after humic acid pretreatment for seven days. Salt treatment was applied every other day for six weeks and physiological problems such as leaf yellowing were observed at 150 mM NaCl concentration. Following the observation of these symptoms, salt application was discontinued and plants (four months) were harvested for analysis.

Table 1. Some physical characteristics of the growing medium

Parameters	Values
pH	6.3
EC (µS cm ⁻¹)	1200.0
Water holding capacity (v/v, %)	50-70
Organik matter (%)	50.4
C (%)	25
N (%)	1
Ca (%)	0.3
P (%)	0.1
K (%)	0.1
Fe (ppm)	300
Zn (ppm)	50
Mn (ppm)	200
Cu (ppm)	20

2.2. Growth parameters

Plant height (cm) is the measurement distance from the base to the plant's highest part. Leaf length (cm), the distance from the base to the leaf apex was determined by measuring with a ruler. Leaf fresh weight (g) was measured by weighing the leaf samples taken after harvesting with a digital weighting balance. Then, leaf specimens were desiccated at 80°C for 72 hours and reweighed to find out leaf dry weight.

2.3. Electrolyte leakage

The method of Lutts et al. (1996) was employed to determine electrolyte leakage in leaf tissue. Leaf samples 1 cm in diameter were taken from all experimental groups and inserted into tubes with containing 10 mL distilled

water at 25°C for 24 hours. Thereafter, the initial electrical conductivity (EC1) of the liquids within the tube was measured utilizing an EC meter (Mettler Toledo, Seven Compact). The same samples were then autoclaved at 120 °C for 20 min and the final electrical conductivity (EC2) was measured after cooling the sample. Electrolyte leakage in leaf tissue was calculated by the formula Electrolyte leakage (%) = [EC1/EC2] x 100.

2.4. Chlorophyll fluorescence

For the determination of the chlorophyll fluorescence value, the leaves of the plant samples of all treatment groups acclimatized to darkness with clips for 15 min. Then, the maximum efficiency of photosystem II (Fv/Fm) was measured using a fluorometer (Plant Efficiency Analyser, Hansatech, UK).

2.5. Photosynthetic pigments

The chlorophyll a and b level of the leaves was established following Arnon (1949) and the total carotenoids content according to Jaspars (1965). 0.1 g fresh leaf specimens were taken from the experimental groups were extracted with 80% acetone. The obtained homogenate was subjected to centrifugation at 5000 rpm for a period of five minutes. Then, the absorbance readings of the supernatant were recorded with spectrophotometer to determine the chlorophyll a (663 nm), chlorophyll b (645 nm) and carotenoid (450 nm) contents. The content of photosynthetic pigments was calculated using the following formula (Eq. 1)-(Eq. 4):

$$\text{Chlorophyll a (mg g}^{-1}\text{ FW)} = (\Delta A_{663} \times 12.7) - (\Delta A_{645} \times 2.69) \quad (\text{Eq. 1})$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ FW)} = (\Delta A_{645} \times 22.9) - (\Delta A_{663} \times 4.68) \quad (\text{Eq. 2})$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ FW)} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (\text{Eq. 3})$$

$$\text{Carotenoid (mg g}^{-1}\text{ FW)} = (\Delta A_{450} \times 4.07) - [(0.0435 \times \text{Chlorophyll a}) + (0.3367 \times \text{Chlorophyll b})] \quad (\text{Eq. 4})$$

2.6. Statistical analyses

The statistical calculations of the obtained data were performed using SPSS for Windows 22.0 software. The experimental treatments were processed through two-way analysis of variance (ANOVA) in compliance with the factorial experiment, repeated three times and based on a completely randomised design. The Duncan Multiple Comparison Test was used to make comparisons of means at a significance level of $p < 0.05$. To assess the magnitude of differences, p -values and effect sizes (eta squared; η^2) were calculated.

3. Results and Discussion

3.1. Growth traits of plants

Salt stress was found to have a significant effect on the plant height of purple basil seedlings ($F(2,12) = 615.174$, $p < 0.01$, $\eta^2 = 0.990$). While the main effect of humic acid was found to be statistically insignificant ($F(1,12) = 3.522$, $p > 0.05$, $\eta^2 = 0.227$), the interaction between salt stress and humic acid was determined to have a significant effect on plant height ($F(2,12) = 9.783$, $p < 0.01$, $\eta^2 = 0.620$). For plant height, the highest value was determined in the untreated plants, while the smallest value was founded in the 150 mM NaCl application (Table 2). The height of purple basil seedlings decreased with increasing salt concentration. Plant height decreased by 28.5% at 75 mM NaCl and 47.04% at 150 mM NaCl relative to control plants. The decreasing salt stress effects on plant height was also reported in studies on purple basil (Lopes et al., 2024) and cauliflower (Turhan, 2019) plants. Salt stress limits the plant's access to water by reducing root length, resulting in slowed shoot growth (Yıldız et al., 2020). However, humic acid application caused increase 6.9% at 75 mM NaCl and 9.4% at 150 mM NaCl in plant height compared to salt treatments alone. The beneficial influence of humic acid on plant height in salt treated plants was also shown in soybean (Matuszak-Slamani et al., 2017). Likewise, while salt stress caused a reduction in plant height in cucumber plants, humic acid application alleviated this negative effect in comparison to unstressed plants (Amerian et al., 2024). Humic acid is effective in alleviating the detrimental effects of salinity by promoting root growth and regulating mineral uptake (Çimrin et al., 2010). Humic acid may also contribute to plant protection against salt stress by improving nutrient uptake and transport through metabolic changes, hormonal effects and chelation (Amerian et al., 2024).

The main effect of salt concentration on the leaf length of purple basil is statistically significant ($F(2,12) = 192.583, p < 0.01, \eta^2 = 0.970$). While the main effect of humic acid was found to be statistically insignificant ($F(1,12) = 4.687, p > 0.05, \eta^2 = 0.281$), the combined effect of salt and humic acid on leaf length was found to be statistically significant ($F(2,12) = 9.25, p < 0.01, \eta^2 = 0.607$). The highest leaf length was obtained in the control sample and the lowest in the 150 mM NaCl treatment (Table 2). Leaf length decreased with increasing salt concentration. Compared to control plants, leaf length decreased by 19.7% at 75 mM NaCl concentration and 27.6% at 150 mM NaCl concentration. Similarly, it was reported that leaf length decreased with increasing salt concentration (0, 50, 100, 200, 300, 400 mM) in lettuce seedlings (Shin et al., 2020). When the effect of humic acid application on leaf length was evaluated, an increase of 8.2% was observed at 75 mM NaCl compared with salt application alone. The effect of humic acid on increasing leaf length was also reported in a study on lettuce seedlings (Ozdemir, 2019). However, humic acid did not cause a significant difference on leaf length at 150 mM NaCl concentration. High salinity at 150 mM NaCl may be associated with damage to root cells, which may prevent effective utilisation of humic acid by the plant.

Salt concentrations had a significant effect ($F(2,12) = 118.425, p < 0.01, \eta^2 = 0.952$) on the fresh weight of purple basil leaves. Similarly, the application of humic acid also had a significant effect on fresh leaf weight ($F(1,12) = 12.892, p < 0.01, \eta^2 = 0.518$), and when evaluated together with salt stress, it was found to produce a statistically significant interaction ($F(2,12) = 9.705, p < 0.01, \eta^2 = 0.618$). It was observed that the greatest leaf fresh weight was obtained from the control group, while the smallest was obtained from the 150 mM NaCl concentration (Table 2). Salt exposed plant's leaf fresh weight decreased by 30.4% at 75 mM NaCl and 49.3% at 150 mM NaCl compared with plants without salt stress. Similarly, negative relationship was identified between saline water application and the fresh weight of leaves in the *Allium schoenoprasum* plant (Arslan et al., 2018). This might be because of salinity limiting cell expansion in young leaves, causing a decline in leaf area and consequent reduction in photosynthesis through stomatal closure (Munns and Tester, 2008), which can bring out about a decline in leaf fresh weight. This study, the influence of humic acid on leaf fresh weight was evaluated, demonstrating a significant increase of 16.7% at 75 mM NaCl and 31.4% at 150 mM NaCl when the humic acid treated plants were compared with plants subjected to salt treatment. Yildiztekin et al. (2018) stated that humic acid treatment boosted leaf fresh weight of plants affected by salinity in comparison with the control on pepper plants. This may be attributed to humic acid improves the root system and increases the root surface area, thus improving water and mineral uptake in plants (Schmidt et al., 2007). Also, it has been mentioned humic acid application against salt stress reduces sodium toxicity by regulating ion balance, balances osmolyte concentrations and reduces oxidative stress by boosting the activities of antioxidant enzymes (Shukry et al., 2023).

Salt stress was found to have a significant effect on leaf dry weight ($F(2,12) = 199.439, p < 0.01, \eta^2 = 0.971$). In the change in leaf dry weight, the main effect of humic acid application was found to be significant ($F(1,12) = 24.188, p < 0.01, \eta^2 = 0.668$) and the interaction of salt and humic acid applications was also found to have a statistically significant effect ($F(2,12) = 7.949, p < 0.01, \eta^2 = 0.570$). The maximum leaf dry weight was determined in control and humic acid treatments and the lowest from the 150 mM NaCl concentration (Table 2). Increasing salt concentration led to a decline in leaf dry weight of purple basil. Accordingly, leaf dry weight decreased by 25% at 75 mM NaCl and 62.5% at 150 mM NaCl relative to control groups. Similar findings were reported by Arslan et al. (2018), who reported that salt stress negatively affected the leaf dry weight of *Allium schoenoprasum* plants. In another study, leaf dry weight of wheat exposed to 150 mM saltinity was significantly reduced relative to the control (Yassin et al., 2019). Reductions in leaf dry weight have been associated with severe restriction of photosynthesis through stomatal closure in salt-stressed plants (Silva et al., 2010). Humic acid application compared to salt application alone showed an increase of 16.7% at 75 mM NaCl and 66.7% at 150 mM NaCl. Earlier researches have indicated that humic acid applied to pepper seedlings under salt stress increased leaf dry weight relative to without stress plants (Yildiztekin et al., 2018). These results suggest that humic acid can mitigate the detrimental effects of salinity stress on plants and increase plant leaf dry weight. The constructive effect of humic acid on photosynthetic pigment levels with an increase in chlorophyll production and slowing its degradation may have led to increased photosynthesis and thus increased leaf dry weight (Sofi et al., 2018; Amerian et al., 2024).

Table 2. Salt stress, humic acid application ve their interactions effect on plant height, leaf length, leaf fresh and dry weight of purple basil

Parameters	Humic Acid Doses (mg L ⁻¹)	Salt Doses (mM NaCl)			Salt Doses Mean
		0	75	150	
Plant Height (cm)	0	54 ^a	38.6 ^d	28.6 ^f	40.4
	1000	51.6 ^b	41.3 ^c	31.3 ^e	41.4
Humic Acid Mean		52.8	39.95	29.95	
Length of Leaf (cm)	0	7.6 ^a	6.1 ^c	5.5 ^d	6.4
	1000	7.3 ^a	6.6 ^b	5.8 ^{cd}	6.6
Humic Acid Mean		7.5	6.4	5.7	
Leaf Fresh Weight (cm)	0	0.69 ^a	0.48 ^c	0.35 ^d	0.5
	1000	0.66 ^a	0.56 ^b	0.46 ^c	0.6
Humic Acid Mean		0.68	0.52	0.41	
Leaf Dry Weight (cm)	0	0.08 ^a	0.06 ^c	0.03 ^e	0.06
	1000	0.08 ^a	0.07 ^b	0.05 ^d	0.07
Humic Acid Mean		0.08	0.07	0.04	

Values represent means and according to Duncan's test results, different letters indicate differences between groups.

3.2. Electrolyte leakage (%)

The effects of salt treatment on electrolyte leakage in purple basil leaf tissue were found to be statistically significant ($F(2,12) = 4555.311, p < 0.01, \eta^2 = 0.999$). Humic acid application alone was significant ($F(1,12) = 20.508, p < 0.01, \eta^2 = 0.631$) and when humic acid and salt stress were evaluated together, a statistically significant interaction ($F(2,12) = 8.719, p < 0.01, \eta^2 = 0.592$) on electrolyte leakage was determined. The highest electrolyte leakage was obtained at 150 mM NaCl concentration, the lowest at control and humic acid treatment (Table 3). As the salt level increased, electrolyte leakage increased. Electrolyte leakage value increased by 132% at 75 mM NaCl concentration and 164.9% at 150 mM NaCl concentration in comparison with unstressed plants. Abu-Ria et al. (2023) reported that electrolyte release increased in rice leaves exposed to salt stress in their study. The increase in electrolyte leakage in saline environment is believed to be the result of disruption to cell membrane integrity and damage due to oxidative stress (Henschel et al., 2024). This investigation, the impact of humic acid treatment on electrolyte leakage under salt stress varied. When the effect of humic acid on salt stress was evaluated, to salt applications alone, 6.3% reduction was occurred at 75 mM NaCl while no statistical difference was observed at 150 mM NaCl. The reduction in electrolyte leakage by humic acid application was also observed in cucumber plants and can be explained by the fact that humic acid reduces oxidative stress by interacting with reactive oxygen species signalling, which can be explicated by the preservation of the cell membrane integrity and photosynthetic capacity (Amerian et al., 2024). However, the finding that humic acid was not effective against electrolyte leakage at high salt concentration (150 mM NaCl) is with the findings received by Bahjat et al. (2023) in soybean. Humic acid is known to be effective in maintaining ionic homeostasis and supporting the antioxidant defence system (Abu-Ria et al., 2023; Malik et al., 2023). However, when ionic imbalances or oxidative stress predominate in extreme or prolonged salt stress, the potential of humic acid to reduce electrolyte leakage may remain limited.

3.3. Chlorophyll fluorescence

The effect of salt stress on the chlorophyll fluorescence (Fv/Fm) value of purple basil seedlings was found to be statistically significant ($F(2,12) = 196.763, p < 0.01, \eta^2 = 0.970$). Similarly, the main effect of humic acid on the Fv/Fm value ($F(1,12) = 199.184, p < 0.01, \eta^2 = 0.943$) and its interaction with salt stress were also found to be statistically significant ($F(2,12) = 8.605, p < 0.01, \eta^2 = 0.589$). The smallest Fv/Fm worth was determined at 150 mM NaCl concentration and the greatest Fv/Fm worth was determined in the humic acid treatment (Table 3). The Fv/Fm ratio declined with an increase in salt concentration. In comparison with control plants, the Fv/Fm ratio decreased by 1.7% at 75 mM NaCl and by 2.3% at 150 mM NaCl. Zhao et al. (2019) reported that saline conditions resulted in reduction Fv/Fm content in *Ginkgo biloba*. The diminished Fv/Fm demonstrates that photosystem II

reaction centres are damaged by salinity stress, leading to photoinhibition (Akhter et al., 2021). However, humic acid application increased the Fv/Fm ratio by 1.4% at 75 mM NaCl and by 1.5% at 150 mM NaCl compared to the salt stress only groups. In a similar study, humic acid was reported to help minimize the unfavorable effects of salt stress conditions on chlorophyll fluorescence (Bano et al., 2022). This has been attributed to humic acid reducing chlorophyll degradation, reducing excess excitation energy in photosystem II and protecting photosystem II components such as D1 protein (Ozfidan-Konakci et al., 2018).

Table 3. Salt stress, humic acid application ve their interactions effect on electrolyte leakage, chlorophyll fluorescence and photosynthetic pigments of purple basil

Parameters	Humic Acid Doses (mg L ⁻¹)	Salt Doses (mM NaCl)			Salt Doses Mean
		0	75	150	
Electrolyte leakage (%)	0	35.3 ^d	81.9 ^b	93.5 ^a	70.2
	1000	35.3 ^d	76.7 ^c	91.7 ^a	67.9
Humic Acid Mean		35.3	79.3	92.6	
Chlorophyll Fluorescence (Fv/Fm)	0	0.843 ^{ab}	0.829 ^{cd}	0.824 ^d	0.832
	1000	0.849 ^a	0.841 ^{ab}	0.836 ^{bc}	0.842
Humic Acid Mean		0.846	0.835	0.83	
Chlorophyll a (mg g ⁻¹ FW)	0	0.244 ^a	0.206 ^b	0.142 ^c	0.197
	1000	0.216 ^b	0.240 ^a	0.115 ^d	0.190
Humic Acid Mean		0.23	0.223	0.129	
Chlorophyll b (mg g ⁻¹ FW)	0	0.061 ^a	0.056 ^a	0.033 ^b	0.05
	1000	0.056 ^a	0.057 ^a	0.019 ^c	0.044
Humic Acid Mean		0.059	0.057	0.026	
Total Chlorophyll (mg g ⁻¹ FW)	0	0.304 ^a	0.254 ^{bc}	0.175 ^d	0.244
	1000	0.271 ^b	0.297 ^{ab}	0.134 ^c	0.234
Humic Acid Mean		0.288	0.276	0.155	
Total Carotenoid (mg g ⁻¹ FW)	0	0.053 ^a	0.048 ^a	0.034 ^b	0.045
	1000	0.052 ^a	0.049 ^a	0.027 ^c	0.043
Humic Acid Mean		0.053	0.049	0.031	

Values represent means and according to Duncan's test results, different letters indicate differences between groups.

3.4. Photosynthetic Pigments

Salt concentrations have a significant effect on chlorophyll a ($F(2,12) = 208.732, p < 0.01, \eta^2 = 0.972$). However, while the effect of humic acid alone on chlorophyll a was statistically insignificant ($F(1,12) = 2.714, p > 0.05, \eta^2 = 0.184$), its interaction with salt was found to be significant ($F(2,12) = 20.362, p < 0.01, \eta^2 = 0.772$). Chlorophyll b content was significantly affected by salt stress ($F(2,12) = 39.177, p < 0.01, \eta^2 = 0.867$). However, the effect of humic acid application alone ($F(1,12) = 3.559, p > 0.05, \eta^2 = 0.229$) and the interaction of salt and humic acid on chlorophyll b was found to be statistically insignificant ($F(2,12) = 1.686, p > 0.05, \eta^2 = 0.219$). The effect of salt concentrations on total chlorophyll is statistically significant ($F(2,12) = 147.496, p < 0.01, \eta^2 = 0.961$). While the effect of humic acid alone on total chlorophyll content is insignificant ($F(1,12) = 2.468, p > 0.05, \eta^2 = 0.171$), it had a statistically significant effect in combination with salt stress ($F(2,12) = 14.458, p < 0.01, \eta^2 = 0.707$). The main effect of salt treatments on total carotenoid content is statistically significant ($F(2,12) = 102.060, p < 0.01, \eta^2 = 0.944$). However, the main effect of humic acid on total carotenoids ($F(1,12) = 2.407, p > 0.05, \eta^2 = 0.167$) and its interaction with salt stress are statistically insignificant ($F(2,12) = 3.807, p > 0.05, \eta^2 = 0.388$). Photosynthetic pigments were highest in the control group and lowest in the humic acid + 150 mM NaCl treatment (Table 3). Compared to the control, 75 mM NaCl application decreased chlorophyll a content by 15.6% and total chlorophyll content by 16.4%, while these decreases were 41.8% and 42.4% at 150 mM NaCl, respectively. While chlorophyll b and total carotenoids content showed no statistically significant difference at 75 mM NaCl relative to non-stressed plants, they respectively decreased by 45.9% and 35.8% at 150 mM NaCl. Similarly, it was found

that increasing salinity concentration (0, 10, 25, 50, 75 and 100%) decreased chlorophyll a, chlorophyll b, total chlorophyll and carotenoid ingredients in *Abelmoschus esculentus* L. Moench plant (Alabdallah and Alzahrani, 2020). Gerami et al. (2020) reported that saline conditions negatively affected the photosynthetic pigment contents (chlorophyll a, b, total chlorophyll and carotenoid) in *Stevia rebaudiana* Bertoni. The diminish in photosynthetic pigment level under salinity may be related to a decrease in chlorophyll biosynthesis, acceleration in chlorophyll degradation and disruption of chloroplast membranes (Ashraf and Harris, 2013; Kaya et al., 2018). In addition, decrease in chlorophyll content is linked to lipid peroxidation and an increase in reactive oxygen species in the chloroplast, and these processes lead to the dissociation of the phytol tail from the chlorophyll porphyrin ring (Ashraf and Harris, 2013; Amerian et al., 2024). In contrast, humic acid application raised chlorophyll a and total chlorophyll content by 16.5% and 16.9% at 75 mM NaCl and decreased by 19% and 23.4% at 150 mM NaCl, respectively, when contrasted with salt treatments alone. However, no significant impact of humic acid was observed effect on chlorophyll b and total carotenoids at 75 mM NaCl, whereas at 150 mM NaCl it caused a decrease of 42.4% and 20.6% respectively. This suggests that humic acid can optimise photosynthetic efficiency at a given level of stress, but that this effect may be limited at high salt concentrations. Gulmezoglu and Izci (2020) reported that chlorophyll content decreased with increasing salt dose in humic acid applications in beans, and these findings align with our study. Although humic acid is known to increase improvement of water and nutrient absorption Mindari et al. (2018) indicated that humic acid did not significantly mitigate salinity. This suggests that high salt concentrations may limit the functionality of humic acid and that reductions in chlorophyll content may have resulted from the severe stress conditions.

4. Conclusions

In conclusion, our study demonstrated that different concentrations of salt treatments had adverse effects on plant height, leaf fresh and dry weight, leaf length, electrolyte leakage, chlorophyll fluorescence and photosynthetic pigments (except 75 mM NaCl chlorophyll b and total carotenoids) of purple basil. Humic acid application reversed the detrimental effects of salinity on growth parameters and chlorophyll fluorescence at both salt concentrations (except leaf length at 150 mM NaCl). However, humic acid treatment increased chlorophyll a and total chlorophyll content only at 75 mM NaCl concentration. While the electrolyte leakage increased in parallel with increasing salt concentrations, humic acid treatment contributed to a reduction at 75 mM NaCl concentration. The data from the study showed that humic acid was more effective against salt damage at 75 mM NaCl. Based on these results, it is suggested that humic acid application may give more favourable results in plants under low concentration salt stress.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept and Design: Torun, H.; Data Collection or Processing: Yalcin, V.; Torun, H.; Statistical Analyses: Yalcin, V.; Literature Search: Yalcin, V.; Writing, Review and Editing: Yalcin, V.; Torun, H.

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