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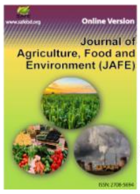

Research Article

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Foliar Application of Humic Acid and Ascorbic Acid Alleviates Salt Stress in Sweet Pepper (*Capsicum annuum* L.)

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A B S T R A C T

Salinity limits sweet pepper (*Capsicum annuum* L.) productivity in coastal Bangladesh. This study evaluated the comparative effectiveness of foliar-applied ascorbic acid (150 ppm) and humic acid (1200 ppm) in mitigating salinity stress on growth, physiology, and yield of sweet pepper cv. BARI Misty Morich-1. Plants were grown in pots under shade-net conditions and subjected to three salinity levels: non-saline control (0.7 dS m^{-1} EC, native soil), moderate salinity (4 dS m^{-1}), and high salinity (8 dS m^{-1}), in a two-factor completely randomized design with three replications. Foliar sprays were applied at 15, 30, and 45 days after transplanting. Salinity reduced growth, physiology, and yield ($P \leq 0.05$). Both biostimulants alleviated these salt-induced reductions, but humic acid consistently outperformed ascorbic acid. Under severe salinity (8 dS m^{-1}), humic acid produced a substantially greater yield increase over untreated plants than ascorbic acid did. The interaction of non-saline conditions with humic acid (S_0C_2) produced the maximum fruit yield (1170.08 g plant^{-1}), while severe salinity without treatment (S_2C_0) yielded the minimum (311.40 g plant^{-1}). Ascorbic acid was notably more effective in enhancing fruit vitamin C content. Foliar humic acid (1200 ppm) effectively alleviates salinity stress in sweet pepper and offers a practical mitigation strategy for salt-affected regions, though field validation across diverse saline environments is warranted.

INTRODUCTION

Salinity is one of the most devastating abiotic stresses limiting global agricultural productivity, affecting approximately 20% of irrigated land worldwide and reducing crop yields through osmotic stress, ion toxicity, and oxidative damage (Hasegawa *et al*., 2000; Munns and Tester, 2008). In Bangladesh, soil salinity poses a particularly acute threat to food security and farmer livelihoods. Coastal districts already account for over 1.0 million hectares of salt-affected land, and sea-level rise coupled with reduced upstream freshwater flow continues to expand saline intrusion into previously productive agricultural zones (SRDI, 2010; Hossen *et al*., 2022). Vegetable crops, which are generally more salt-sensitive than cereals, suffer disproportionate yield losses under these conditions, constraining dietary diversity and income generation for smallholder farmers (Miah *et al*., 2020).

Sweet pepper (*Capsicum annuum* L.) is a high-value vegetable crop with significant nutritional and economic importance in Bangladesh. The cultivar BARI Misty Morich-1, developed by the Bangladesh Agricultural Research Institute, is widely cultivated due to its adaptability to local conditions and stable yield performance (BARI, 2017). However, as a glycophyte, sweet pepper exhibits marked sensitivity to salinity, with yield declines reported at EC levels as low as 2–3 dS m^{-1} (Navarro *et al*., 2010; Salinas *et al*., 2023). The expansion of saline soils into Bangladesh's vegetable-growing regions therefore threatens the viability of sweet pepper production, necessitating cost-effective, farmer-accessible mitigation strategies.

Exogenous application of plant biostimulants has emerged as a promising approach to alleviate salinity-induced damage without the environmental and economic costs associated with soil reclamation or desalination (Akladious and Mohamed, 2018). Among biostimulants, ascorbic acid functions as a key non-enzymatic antioxidant that scavenges reactive oxygen species and stabilizes cellular structures under stress (Akram *et al*., 2017; El-Beltagi *et al*., 2022). Humic acid, a major component of soil organic matter, enhances nutrient uptake, stimulates root development, and regulates physiological processes that improve stress tolerance (Hayati *et al*., 2022; Saidimoradi *et al*., 2019). Both agents have demonstrated individual efficacy in various vegetable crops under salinity stress, but their comparative performance on Bangladesh-adapted sweet pepper cultivars remains unexplored.

Previous studies have applied ascorbic acid at concentrations ranging from 1.0–1.5 mM (approximately 150–250 ppm) and humic acid at 500–1500 ppm, with beneficial effects on growth, antioxidant status, and yield (Athar *et al*., 2009; Fadala *et al*., 2021; Sani, 2014). However, these studies differed in crop species, salinity regimes, application methods, and environmental conditions, making direct comparison impossible. Crucially, no study has simultaneously evaluated these two biostimulants under identical salinity conditions on a Bangladeshi sweet pepper cultivar grown in pot culture, a system that allows controlled stress imposition and precise measurement of physiological responses.

We hypothesized that humic acid would outperform ascorbic acid under salinity stress due to its broader effects on nutrient acquisition, root growth, and hormonal regulation, whereas ascorbic acid would show more targeted benefits on fruit antioxidant content. Accordingly, this study was designed to: (i) quantify the growth, physiological, and yield responses of sweet pepper cv. BARI Misty Morich-1 to three salinity levels; (ii) compare the relative effectiveness of foliar-applied ascorbic acid (150 ppm) and humic acid (1200 ppm) in mitigating salinity damage; and (iii) identify the most promising biostimulant strategy for sustaining sweet pepper productivity in salt-affected regions of Bangladesh.

MATERIALS AND METHODS

Plant materials, growing conditions, treatments

The experiment was conducted under pot culture in a shade-net house at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh (23.77° N, 90.38° E; 9 m altitude) during the Rabi season (October 2022–March 2023). Mean daytime and night temperatures were 23 ± 2°C and 13 ± 2°C, respectively; relative humidity was 65–70%; and natural photoperiod was ~11 h. Surface soil (0–30 cm) was air-dried, sieved, and analyzed at SRDI, Dhaka. The loam–clay loam soil (27% sand, 43% silt, 30% clay) had pH 5.6, organic carbon 0.45%, total N 0.03%, available P 20 ppm, exchangeable K 0.10 me 100 g^{-1}, and available S 45 ppm. Plastic pots (35 cm height × 30 cm diameter) were filled with 12 kg soil plus recommended fertilizers (BARI, 2021) and bottom-drilled for drainage. Seeds of sweet pepper (*Capsicum annuum* L.) cv. BARI Misty Morich-1 were raised under standard nursery conditions. Uniform 30-day-old seedlings (5–6 true leaves) were transplanted at one plant per pot.

The experiment followed a two-factor CRD with three replications. Factor A: salinity levels — control (native soil, 0.7 dS m^{-1} EC), moderate (4 dS m^{-1}), and high (8 dS m^{-1}). Factor B: foliar treatments — distilled water (control), ascorbic acid (150 ppm), and humic acid (1200 ppm). Nine treatment combinations × 3 replications × 3 pots per unit = 81 pots total; pot means within each replicate were averaged to form the experimental unit. Salinity was imposed using NaCl solution, increased gradually over 5–7 days post-establishment to avoid osmotic shock. Target EC levels were maintained throughout; irrigation was applied when soil moisture fell below 10%. Ascorbic acid (analytical grade) and humic acid (commercial preparation; source: Department of Horticulture, SAU) were dissolved in distilled water. Foliar sprays were applied at 15, 30, and 45 DAT until runoff (~20–30 mL $plant^{-1}$) in late afternoon. Control plants received distilled water. All intercultural operations were uniform.

Growth, yield, and physiological parameters were recorded from all plants per unit. For biochemical analyses, three fully expanded upper leaves were collected per unit in the morning.

Dry matter content of fruit (%)

Dry matter content of fruit (%) was determined after harvest. Fruits were harvested at regular intervals at physiological maturity, and a total of 5 harvests were conducted during the crop growth period. Fruits were collected at the mature green to fully ripe stage to ensure uniform marketable maturity. A randomly selected 100 g fruit sample was sliced into very thin pieces, placed into a paper envelope, and oven-dried at 80°C for 72 hours. After drying, samples were transferred to a desiccator and cooled to room temperature. The final dry weight was recorded. Dry matter content was calculated using the standard formula:

$$Dry\ matter\ content(\%) = \frac{Dry\ weight\ of\ fruit(g) \times 100}{Fresh\ weight\ of\ fruit(g)}$$

Chlorophyll Content

Chlorophyll content was measured using a portable SPAD-502 Plus meter. Four readings per leaf were averaged. Measurements were taken before harvest on salt-stressed plants and expressed as SPAD units.

Relative water content (RWC)

Relative water content (RWC) was measured from three fully expanded leaves per replicate. Leaves were weighed to obtain the fresh weight. Then, the leaves were floated in water under light until the weight remained constant to attain full turgidity, after which the turgid weight was recorded. The leaves were then kept in a hot air oven at 80°C for 48 hours and the dry weight was recorded. Finally, the leaf RWC was calculated using the formula below:

$$RWC(\%) = \frac{(FW - DW) \times 100}{(TW - DW)}$$

Vitamin C content (mg 100 g^{-1} FW)

Vitamin C content was determined following the method of AOAC International (2005) with slight modifications using 2,6-dichlorophenol indophenol (DCPIP) dye titration. A 5% oxalic acid solution was prepared by dissolving oxalic acid in distilled water and used as the extracting medium. Standard L-ascorbic acid solution was prepared in 5% oxalic acid and used for dye standardization. The DCPIP dye solution was prepared by dissolving the sodium salt of 2,6-dichlorophenol indophenol along with sodium bicarbonate in hot distilled water.

Fresh fruit samples were homogenized without adding water, filtered and the extract volume was adjusted with 5% oxalic acid. An aliquot of the sample extract was titrated against the standardized DCPIP dye until the endpoint was reached, indicated by a persistent light pink color. Vitamin C content was calculated from the volume of dye consumed and expressed as mg 100 g^{-1} fresh weight (FW).

$$unknown\ solution = \frac{0.5 \times y \times 100}{x \times 5}$$

X= Amount of dye for standard L-ascorbic

y = Amount of dye for sweet pepper solution

Electrolyte Leakage

Electrolyte leakage was measured to assess membrane permeability under salt stress. A 1 cm² leaf sample per treatment was washed, placed in 10 ml distilled water and incubated at 25 °C with shaking (100 rpm) for 24 h. Initial electric conductivity (EC_1) was recorded and then samples were autoclaved (120 °C; 20 min) cooled and final conductivity (EC_2) measured. Electrolyte leakage was calculated using the following formula:

$$Electrolyteleakage(EL\%) = \frac{EC_1}{EC_2} \times 100$$

Total Soluble Solids (TSS)

TSS was measured using a Brix refractometer (Model RHB 32 ATC). Fruit samples were ground and a drop of juice was placed on the glass and covered by upper glass and finally the TSS percentage was read directly**.**

Statistical analysis

Data were analyzed using two-way ANOVA for a factorial completely randomized design, with salinity level, foliar treatment, and their interaction as fixed factors. Treatment means were separated using LSD at $P \leq 0.05$ when ANOVA indicated significant effects. The replicate mean, obtained from three pots per replicate, was used as the experimental unit. Graphical representations were prepared using Microsoft Excel.

RESULTS

Plant height

Plant height of sweet pepper was significantly influenced by salinity levels, salinity-mitigating chemicals and their interaction effects (Figures 1–3). Plant height was measured at 100 days after transplanting (DAT). Plant height declined significantly with increasing salinity stress and the lowest value (25.60 cm) was recorded under severe salinity treatment (S_2) (Figure 1). Mitigating chemicals also had a significant effect where humic acid (C_2) produced the highest mean plant height (35.00 cm), followed by ascorbic acid (Figure 2). The interaction effect was significant, with the maximum plant height (43.33 cm) observed in S_0C_2 and the minimum (25.00 cm) in S_2C_0 (Figure 3). Under moderate and severe salinity conditions, both humic acid and ascorbic acid improved plant height compared with the untreated control, while humic acid was more effective in alleviating salinity stress.

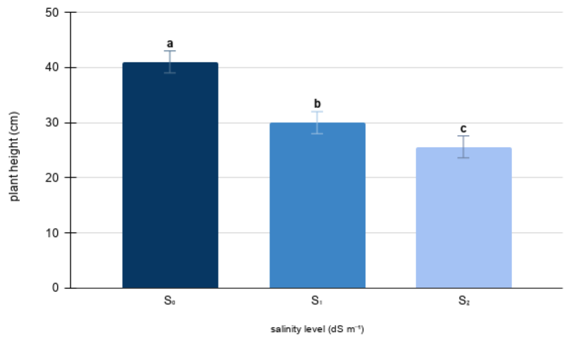

Figure 1: Effect of salinity levels on plant height of sweet pepper measured at 100 days after transplanting (DAT). Error bars represent mean ± SE (n = 3). Means followed by different lowercase letters are significantly different at $P \leq 0.05$ according to the LSD test. Salinity treatments were S_0 = 0.7 dS m^{-1}, S_1 = 4 dS m^{-1}, and S_2 = 8 dS m^{-1}.

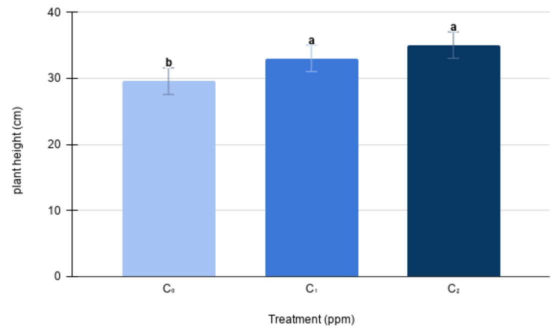

Figure 2: Effect of salinity-mitigating chemicals on plant height of sweet pepper measured at 100 days after transplanting (DAT). Error bars represent mean ± SE (n = 3). Means followed by different lowercase letters are significantly different at $P \leq 0.05$ according to the LSD test. Treatments were C_0 = control, C_1 = ascorbic acid (150 ppm), and C_2 = humic acid (1200 ppm).

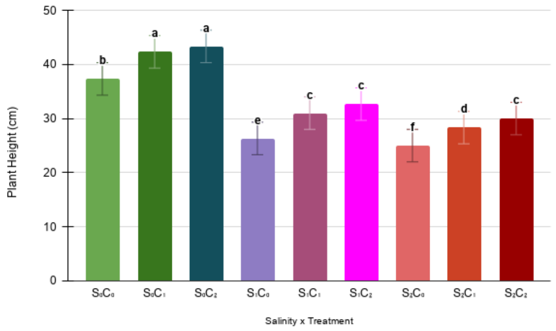

Figure 3: Interaction effects of salinity levels and salinity-mitigating chemicals on plant height of sweet pepper measured at 100 days after transplanting (DAT). Error bars represent mean ± SE (n = 3). Means followed by different lowercase letters are significantly different at $P \leq 0.05$ according to the LSD test. Salinity treatments were S_0 = 0.7 dS m^{-1}, S_1 = 4 dS m^{-1}, and S_2 = 8 dS m^{-1}; chemical treatments were C_0 = control, C_1 = ascorbic acid (150 ppm), and C_2 = humic acid (1200 ppm).

Number of flowers plant^{-1}

Salinity stress significantly reduced flower production in sweet pepper, while mitigating chemicals improved flowering performance (Tables 1–3). The greatest number of flowers per plant (31.33) was obtained under non-saline conditions (S_0), whereas severe salinity (S_2) produced the fewest flowers (22.33) (Table 1). Foliar application of humic acid (C_2) resulted in the highest flower number (29.77 plant^{-1}) (Table 2). Under combined treatments, S_0C_2 produced the maximum number of flowers (34.67 plant^{-1}), while S_2C_0 recorded the minimum value (18.00 plant^{-1}) (Table 3).

Number of fruits plant^{-1}

Fruit number per plant was markedly influenced by salinity and chemical treatments (Tables 1–3). Increasing salinity caused a progressive decline in fruit production, with plants under S_2 producing the lowest (5.60 plant^{-1}) (Table 1). Among the mitigating chemicals, humic acid (C_2) gave the highest fruit number (8.30 plant^{-1}), followed by ascorbic acid (7.77 plant^{-1}), whereas untreated plants produced only 5.56 fruits plant^{-1} (Table 2). The interaction treatment S_0C_2 produced the highest fruit number (10.33 plant^{-1}), which was statistically similar to S_0C_1(9.33 plant^{-1}), while S_2C_0 produced the fewest fruits (4.00 plant^{-1}) (Table 3). Application of both chemicals alleviated salinity-induced reductions in fruit production, with humic acid showing comparatively better performance.

Dry matter content

The dry matter content of sweet pepper was significantly affected by the various treatments (Table 1-3). Treatment containing 8 dS m^{-1} EC salinity (S_2), resulted in the lowest dry matter content (17.67%). Among chemical treatments, humic acid (C_2; 27.91%) showed the highest dry matter content. The most notable result was the significant increase in dry matter content observed in plants receiving the combination treatment. In the interaction effect, the maximum dry matter was observed in S_0C_2 (32.06%), whereas the minimum was found in S_2C_0 (9.33%) (Table 3), indicating strong mitigation of salinity stress by humic acid.

Table 1: Main effect of salinity levels on growth and yield-contributing characters of sweet pepper

Salinity level	Number of flowers plant^{-1}	Number of fruits plant^{-1}	Fruit dry matter (%)	Chlorophyll content (SPAD)
S_0	31.33 a	9.00 a	25.46 a	59.67 a
S_1	26.00 b	7.00 b	21.44 b	47.00 b
S_2	22.33 c	5.60 c	17.67 c	44.67 b
LSD (0.05)	1.80	0.72	1.29	2.70
CV (%)	6.86	10.62	6.02	5.47
Level of significance	**	**	**	**

Means followed by the different letter(s) within a column are significantly different at the 5% or 1% level of probability. [Here, S_0 = non-saline control (0.7 dS m^{-1}), S_1 = moderate salinity (4 dS m^{-1}), and S_2 = high salinity (8 dS m^{-1})]

Table 2: Main effect of salinity-mitigating chemicals on growth and yield-contributing characters of sweet pepper

Treatment	Number of flowers plant^{-1}	Number of fruits plant^{-1}	Fruit dry matter (%)	Chlorophyll content (SPAD)
C_0	21.77 b	5.56 b	12.77 c	42.33 c
C_1	28.11 a	7.77 a	23.89 b	51.33 b
C_2	29.77 a	8.30 a	27.91a	57.67 a
LSD (0.05)	1.80	0.72	1.29	2.70
CV (%)	6.86	10.62	6.02	5.47
Level of significance	**	**	**	**

Means followed by the different letter(s) within a column are significantly different at the 5% or 1% level of probability. [Here, C_0 = water spray (control), C_1 = ascorbic acid @ 150 ppm, and C_2 = humic acid @ 1200 ppm]

Table 3: Combined effect of salinity levels and salinity-mitigating chemicals on growth and yield-contributing characters of sweet pepper

Treatment combination	Number of flowers plant^{-1}	Number of fruits plant^{-1}	Fruit dry matter (%)	Chlorophyll content (SPAD)
S_0C_0	27.00 c	7.33 b	17.00 f	50.00 c
S_0C_1	32.33 a	9.33 a	27.30 b	63.33 a
S_0C_2	34.67 a	10.33 a	32.06 a	65.67 a
S_1C_0	20.33 f	5.30 e	12.00 g	39.67 f
S_1C_1	28.33 c	7.67 b	24.00 d	46.00 de
S_1C_2	29.33 b	8.00 b	28.33 b	55.33 b
S_2C_0	18.00 f	4.00 f	9.33 h	37.33 f
S_2C_1	23.67 e	6.30 d	20.33 e	44.67 e
S_2C_2	25.33 d	6.67 cd	23.33 d	52.00 bc
LSD (0.05)	3.15	1.25	2.24	4.70
CV (%)	6.86	10.62	6.02	5.47
Level of significance	**	**	**	**

Means followed by the different letter(s) within a column are significantly different at the 5% or 1% level of probability. [Here, S_0 = non-saline control (0.7 dS m^{-1}), S_1 = moderate salinity (4 dS m^{-1}), and S_2 = high salinity (8 dS m^{-1}), C_0 = water spray, C_1 = ascorbic acid @ 150 ppm, C_2 = humic acid @ 1200 ppm]

Chlorophyll content

The analysis of chlorophyll content revealed a significant influence of the various treatments (tables 1-3). Chlorophyll content was significantly affected by salinity levels. Plants exposed to only salinity stress exhibited the lowest chlorophyll content in case of (S_2, SPAD value 44.67), although S_1 and S_2 were statistically similar (Table 1). Mitigating treatments significantly improved chlorophyll content, with the highest value (57.67) recorded under humic acid (C_2, Table 2). The most favorable outcome for chlorophyll content under salinity stress emerged with the application of humic acid. The interaction showed the highest chlorophyll in S_0C_2 (65.67 SPAD), followed by ascorbic acid (63.33 SPAD) and the lowest in S_2C_0 (37.33 SPAD, Table 3).

Relative water content

Relative water content (RWC) was significantly reduced with increasing salinity stress, declining from 83.38% under non-saline conditions (S_0) to 51.56% under severe salinity (S_2, Table 4). Among chemicals, humic acid (C_2;75.16%) performed best followed by ascorbic acid (C_1;69.90%) (Table 5). These findings suggest a trend: salinity stress reduced RWC (S_2), while humic acid (C_2) and ascorbic acid (C_1) improved it even without stress. Furthermore, the combined treatment revealed maximum RWC in S_0C_2 (93.30%) indicating improved water status due to humic acid application (Table 6).

Vitamin C content

Salinity stress and salinity mitigating treatments exerted a significant influence on the vitamin C content of sweet pepper (Tables 4–6). Vitamin C content gradually declined with increasing salinity levels, and the lowest value (47 mg 100 g^{-1} FW) was obtained under the S_2 treatment, reflecting the inhibitory effect of salt stress on vitamin C accumulation (Table 4). Application of salinity alleviating compounds improved vitamin C content compared with the untreated control. Among the treatments, ascorbic acid (C_1) resulted in the highest vitamin C content (71 mg 100 g^{-1} FW), whereas the control treatment (C_0) produced the lowest value (55 mg 100 g^{-1} FW) (Table 5). In the case of interaction effects, S_0C_1 recorded the maximum vitamin C content (82 mg 100 g^{-1} FW), while the minimum value (32 mg 100 g^{-1} FW) was observed in S_2C_0 (Table 6). Although humic acid performed better for most parameters, ascorbic acid was more effective in improving vitamin C content in sweet pepper fruits.

Membrane permeability

The treatments had a significant effect on membrane permeability of sweet pepper under salinity stress (Tables 4–6). The highest membrane permeability was recorded under high salinity (S_2), with a value of 44.18%, which was significantly different from all other treatments, indicating greater cellular damage under severe stress (Table 4). When only salinity-mitigating chemicals were considered, the control treatment (C_0) showed the highest membrane permeability (48.18%), whereas the lowest value was observed in the humic acid treatment (C_2; 32.00%), demonstrating its protective effect on cell membranes (Table 5). In the interaction effect, the lowest membrane permeability was found in the combined treatment of non-saline condition with humic acid (S_0C_2), recording a value of 29.40%, suggesting improved membrane stability under optimal conditions with humic acid application (Table 6).

Table 4: Main effect of salinity levels on physio-chemical attributes of sweet pepper

Salinity level	Relative water content (%)	Vitamin C (mg 100 g^{-1} FW)	Membrane permeability (%)	TSS (%)
S_0	83.38 a	77.00 a	36.04 c	4.66 a
S_1	61.60 b	68.00 b	40.08 b	4.21 b
S_2	51.56 c	47.00 c	44.18 a	3.07 c
LSD (0.05)	1.44	1.84	1.60	0.11
CV (%)	2.2	2.93	3.60	2.99
Level of significance	**	**	**	**

Means followed by the different letter(s) within a column are significantly different at the 5% or 1% level of probability. [Here, S_0 = non-saline control (0.7 dS m^{-1}), S_1 = moderate salinity (4 dS m^{-1}), and S_2 = high salinity (8 dS m^{-1})]

Table 5: Main effect of salinity-mitigating chemicals on physio-chemical attributes of sweet pepper

Treatment	Relative water content (%)	Vitamin C (mg 100 g^{-1} FW)	Membrane permeability (%)	TSS (%)
C_0	51.52 c	55.00 c	48.18 a	3.43 b
C_1	69.90 b	71.00 a	40.32 b	4.22 a
C_2	75.16 a	66.00 b	32.00 c	4.28 a
LSD (0.05)	1.44	1.84	1.60	0.11
CV (%)	2.20	2.93	3.60	2.99
Level of significance	**	**	**	**

Means followed by the different letter(s) within a column are significantly different at the 5% or 1% level of probability. [Here, C_0 = water spray (control), C_1 = ascorbic acid @ 150 ppm, and C_2 = humic acid @ 1200 ppm]

Table 6: Combined effect of salinity levels and salinity-mitigating chemicals on physio-chemical attributes of sweet pepper

Treatment combination	Relative water content (%)	Vitamin C (mg 100 g^{-1} FW)	Membrane permeability (%)	TSS (%)
S_0C_0	66.40 d	69.00 d	41.33 cd	4.03 c
S_0C_1	90.40 b	82.00 a	37.40 e	4.83 b
S_0C_2	93.30 a	79.00 b	29.40 g	5.10 a
S_1C_0	48.30 g	64.00 e	48.00 b	3.57 d
S_1C_1	66.60 d	72.00c	40.25d	4.03 c
S_1C_2	70.50 c	69.00 d	32.00 f	5.03 a
S_2C_0	39.80 h	32.00 h	55.23 a	2.70 f
S_2C_1	53.30 f	59.00 f	43.33 c	3.53 d
S_2C_2	61.60 e	49.00 g	34.00 f	2.97 e
LSD (0.05)	2.49	3.00	2.80	0.20
CV (%)	2.20	2.93	3.60	2.99
Level of significance	**	**	**	**

Means followed by the different letter(s) within a column are significantly different at the 5% or 1% level of probability. [Here, S_0 = non-saline control (0.7 dS m^{-1}), S_1 = moderate salinity (4 dS m^{-1}), and S_2 = high salinity (8 dS m^{-1}), C_0 = water spray, C_1 = ascorbic acid @ 150 ppm, C_2 = humic acid @ 1200 ppm]

Total soluble solids (TSS)

TSS decreased with increasing salinity, from 4.66% (S_0) to 3.07% (S_2) (Table 4). Among chemicals, C_2 (4.28%) and C_1 (4.22%) improved TSS compared to control (3.43%) (Table 5). In the interaction, the highest TSS was recorded in S_0C_2, (5.10%), while the lowest was recorded in S_2C_0 (2.70%) (Table 6).

Fruit yield per plant

Fruit yield per plant was significantly influenced by salinity levels, salinity-mitigating chemicals, and their interaction effects (Figures 4–6). Yield decreased with increasing salinity stress, with the highest mean yield (932.94 g plant^{-1}) recorded under S_0 and the lowest (532.40 g plant^{-1}) under S_2 (Figure 4).

Application of mitigating chemicals significantly increased fruit yield compared with the untreated control. Humic acid (C_2) produced the highest mean yield (919.68 g plant^{-1}), followed by ascorbic acid (C_1; 755.57 g plant^{-1}), whereas the lowest yield (470.00 g plant^{-1}) was observed in C_0 (Figure 5).

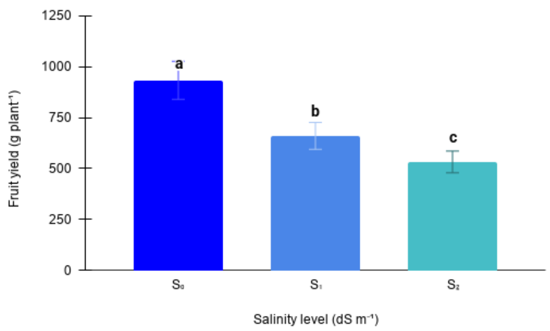

Figure 4: Effect of salinity levels on fruit yield per plant of sweet pepper. Error bars represent mean ± SE (n = 3). Means followed by different lowercase letters are significantly different at P ≤ 0.05 according to the LSD test. Salinity treatments were S_0 = 0.7 dS m^{-1}, S_1 = 4 dS m^{-1}, and S_2 = 8 dS m^{-1}.

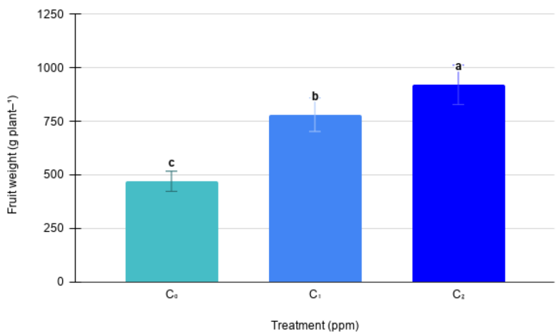

Figure 5: Effect of salinity-mitigating chemicals on fruit yield per plant of sweet pepper. Error bars represent mean ± SE (n = 3). Means followed by different lowercase letters are significantly different at P ≤ 0.05 according to the LSD test. Treatments were C_0 = control, C_1 = ascorbic acid (150 ppm), and C_2 = humic acid (1200 ppm).

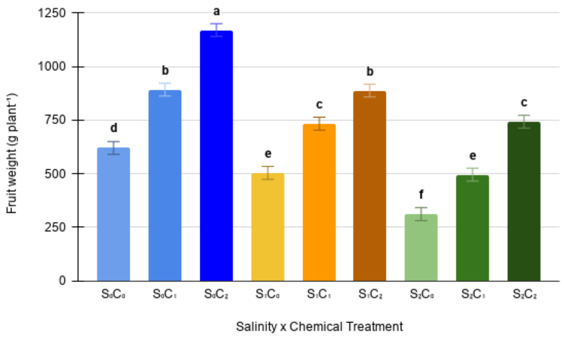

Figure 6: Interaction effects of salinity levels and salinity-mitigating chemicals on fruit yield per plant of sweet pepper. Error bars represent mean ± SE (n = 3). Means followed by different lowercase letters are significantly different at P ≤ 0.05 according to the LSD test. Salinity treatments were S_0 = 0.7 dS m^{-1}, S_1 = 4 dS m^{-1}, and S_2 = 8 dS m^{-1}; chemical treatments were C_0 = control, C_1 = ascorbic acid (150 ppm), and C_2 = humic acid (1200 ppm).

The interaction effect was also significant, where S_0C_2 produced the maximum yield (1170.08 g plant^{-1}), while S_2C_0 resulted in the minimum yield (311.40 g plant^{-1}) (Figure 6). Under saline conditions, both humic acid and ascorbic acid improved fruit yield, with humic acid showing comparatively greater effectiveness.

DISCUSSION

Salinity stress markedly reduced the growth and yield of sweet pepper in the present study. Morphological traits such as plant height, number of flowers, and number of fruits declined progressively with increasing salinity levels. These reductions likely reflect the combined effects of osmotic stress, ion toxicity, and restricted uptake of water and essential nutrients under saline conditions, which ultimately limited cell expansion and shoot development (Al-Karaki, 2000; Munns and Tester, 2008). Similar reductions in plant height under salinity were reported in tomato by Naeem *et al.* (2020).

When salinity increases, it forces plants into chronic stress which reduces total biomass production and ultimately dry matter content (Puvanitha and Mahendran, 2017). The whole plant is affected when roots grow in salt-stressed conditions, as altered root morphology changes ion and water uptake and affects the signals sent to shoots for growth (Tejera *et al.*, 2006). Reduced flowering and fruit formation under salt stress may further result from impaired assimilate partitioning and metabolic disturbances during reproductive development (Estaji *et al.*, 2019). Fruit yield progressively declined with increasing salinity levels, consistent with the linear relationship between NaCl concentration and total yield reduction reported in pepper (Navarro *et al.*, 2010). Salinas *et al.* (2023) also reported substantial and statistically significant reductions in total and marketable yield, dry matter production, and average fruit weight with increasing salinity.

Physiological and biochemical attributes were also adversely affected by salinity. Chlorophyll content and relative water content declined significantly with increasing salt concentration. Salinity-induced chlorophyll loss may be associated with stomatal limitation, ionic imbalance, and degradation of photosynthetic pigments, leading to lower photosynthetic efficiency. Similar findings were reported by Butt *et al*. (2021) in chili pepper under saline conditions. Membrane permeability increased under salinity stress, suggesting loss of membrane integrity. Vitamin C and total soluble solids also declined under higher salinity, reflecting deterioration in fruit quality and metabolic activity. Salt stress adversely affects antioxidant components and fruit biochemical quality (Julien *et al*., 2021).

Foliar application of ascorbic acid significantly alleviated the adverse effects of salinity and improved growth, physiological traits, and yield components compared with untreated plants. The beneficial effects observed are consistent with previous reports demonstrating that exogenous ascorbic acid enhances morphological and physiological performance of plants under salinity stress, including plant height, leaf production, leaf area, fruit yield components, and chlorophyll content (El-Beltagi *et al*., 2022). Ascorbic acid is known to function as a non-enzymatic antioxidant that can stabilize cellular structures and protect the photosynthetic apparatus under stress conditions (Akram *et al*., 2017). Improved physiological stability under ascorbic acid treatment may support sustained metabolic activity and assimilate production, resulting in better growth and yield formation under saline conditions (Munns and Tester, 2008; Niu *et al*., 2013). The increase in vitamin C content under this treatment may reflect enhanced ascorbate metabolism and reduced oxidative degradation of the vitamin in fruits (El-Beltagi *et al*., 2022). However, the present study did not directly measure reactive oxygen species, antioxidant enzyme activities, or lipid peroxidation; therefore, specific inferences regarding antioxidant-mediated protection remain speculative.

Among the mitigating treatments, humic acid proved more effective than ascorbic acid in improving plant performance under both saline and non-saline conditions. Humic acid application resulted in the highest plant height, indicating strong growth-promoting potential. Similar enhancement in plant height has been reported in canola, where foliar application of humic acid significantly increased shoot growth compared with the control (Sani, 2014). This growth stimulation may be attributed to improved nutrient uptake efficiency and enhanced root system development induced by humic substances, though the underlying hormonal or signaling mechanisms were not directly investigated in this study.

Humic acid also significantly improved yield-related traits, including fruit number and overall productivity under salinity stress. The increase in fruit number and yield may be associated with improved nutrient balance and enhanced source-sink relationships, which together support better reproductive development under stress conditions (Amerian *et al*., 2024). In addition, humic acid is known to function as a biostimulant that can enhance salinity tolerance by improving assimilate production and fruit set, ultimately leading to higher yield per plant, as reported in tomato (Rahman, 2018).

Physiological improvements were also evident. Humic acid application reduced membrane permeability, indicating enhanced membrane stability under salinity stress. This protective effect has been linked to the ability of humic substances to maintain cellular integrity under stress conditions (Saidimoradi *et al*., 2019), though the specific biochemical basis was not examined here. Humic acid also improved relative water content, suggesting better water status and osmotic adjustment under saline conditions (Kaya *et al*., 2018).

Fruit quality attributes were improved by humic acid application. Total soluble solids and vitamin C content were enhanced under humic acid treatment, indicating improved carbohydrate accumulation and fruit nutritional quality (Fadala *et al*., 2021). This improvement may be linked to better assimilate partitioning towards developing fruits. Fruit yield was significantly reduced by increasing salinity, whereas both mitigating chemicals substantially improved yield under stress conditions. The highest fruit yield was obtained from the combined treatment of non-saline conditions with humic acid (S_0C_2), while the lowest yield was recorded under severe salinity without mitigation (S_2C_0). Even under moderate and severe salinity, humic acid maintained higher yield than ascorbic acid and the untreated control. These results indicate that humic acid was highly effective in alleviating salinity-induced yield losses and sustaining productivity of sweet pepper under salt-affected conditions. The greater effectiveness of humic acid relative to ascorbic acid may reflect its broader influence on nutrient uptake, water retention, and growth regulation, which helped maintain better plant growth and fruit development under salinity stress (Kaya *et al*., 2018).

The consistent outperformance of humic acid over ascorbic acid likely reflects multiple biostimulant activities of humic substances, including micronutrient chelation, hormone-like growth modulation, and rhizosphere microbial interactions (Hayati *et al*., 2022; Amerian *et al*., 2024), though the present study did not discriminate among these mechanisms. The selected concentration of 1200 ppm was literature-based (Fadala *et al*., 2021; Sani, 2014), but without a dose-response curve, optimality remains undetermined and potential phytotoxicity at higher doses cannot be excluded (Akladious and Mohamed, 2018). Additionally, the acidic soil pH (5.6) may have reduced humic acid bioavailability through protonation and aggregation, an interaction not examined here (Kaya *et al*., 2018). Finally, microbial biostimulants and integrated approaches combining humic substances with PGPR or mineral amendments offer promising synergies that were beyond this study's scope.

This study was conducted under pot-culture shade-net conditions using NaCl-induced salinity; therefore, the results may not fully represent field conditions or naturally saline coastal soils where mixed salts, soil heterogeneity, and fluctuating environmental factors occur. Additionally, the study did not measure direct indicators of oxidative stress (e.g., hydrogen peroxide, malondialdehyde, antioxidant enzyme activities) or tissue ion concentrations (Na^+, K^+, Cl^-); consequently, interpretations regarding specific stress-alleviation mechanisms remain indirect. Further field trials across seasons, dose-response studies, and mechanistic investigations are needed before broad recommendation.

CONCLUSIONS

Salinity progressively impaired growth, physiology, and yield of sweet pepper, with maximum damage at 8 dS m^{-1} EC. Foliar application of both ascorbic acid (150 ppm) and humic acid (1200 ppm) significantly alleviated salt stress, though humic acid consistently outperformed ascorbic acid across most parameters. The optimal combination, non-saline conditions with humic acid (S_0C_2), achieved 1170.08 g plant^{-1}, a 3.76-fold yield advantage over the most stressed untreated plants (S_2C_0: 311.40 g plant^{-1}). Humic acid's superiority likely reflects broader growth-regulatory and nutrient-enhancement effects beyond direct antioxidant supplementation. These results support foliar humic acid as a practical, low-cost biostimulant for sustaining sweet pepper productivity in salt-affected areas of Bangladesh. Multi-location field trials and dose-optimization studies are warranted to validate these pot-culture findings under natural saline field conditions.

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethics statement

This study was conducted in accordance with all relevant ethical guidelines and regulations.

Authors' contributions

M.H.K. designed and supervised the experiment and final edited the manuscript, F.T.Z conducted the research and wrote the original draft. S.A. and M.S analyzed the data and performed the statistical analysis. M.S, R.M.A.A., D.O., H.I.N. and I.N. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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