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Efficacy of humic acid and seaweed extracts on the growth, yield and biochemical properties of chilli

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The authors declare no conflict of interests.

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Abstract: Chilli is a significant horticultural crop in Bangladesh, contributing to spice production and the rural economy. This investigation aimed to evaluate the effect of humic acid and seaweed extracts as bio-stimulants on plant growth, yield, and biochemical properties of chilli (var. Hira 1701 Supreme). There were four treatments in this research: T_0 (control), T_1 (recommended fertilizer + humic acid), T_2 (recommended fertilizer + seaweed extract), and T_3 (recommended fertilizer + both seaweed extract and humic acid). The results indicated that T_2 possessed the highest values in the most studied parameters, including plant height (60.13 cm), number of leaves (98.75), number of branches (13.25), number of flowers (37.63), fruit length (7.92 cm), seeds per fruit (79.75), yield per plot (1156.5 g), yield per hectare (7.23 ton), plant fresh weight (187.50 g^{-1} Plant), plant dry weight (41.00 g^{-1} Plant), vitamin C (177.80 mg 100 g^{-1} FW), total phenolic content (164.87 mg 100 g^{-1} FW), total flavonoid content (35.95 mg 100 g^{-1} FW), crude protein content (8.63%), and total soluble solids content (5.80 °Brix). While T_1 and T_3 also performed better than the control (T_0), T_2 consistently outperformed all other treatments. Correlation analysis revealed strong positive associations among yield, plant physiology, and key biochemical traits, indicating their interdependence. Moreover, PCA-based biplot analysis clearly separated the treatments and confirmed that T_2 showed a strong correlation with enhanced agronomic performance and superior nutritional quality. These findings show that seaweed extract foliar spray with the recommended fertilizer is superior to humic acid alone for enhancing chilli growth, productivity, and nutritional quality.

1. Introduction

The chilli pepper (*Capsicum annuum* L.) is an herbaceous perennial spice crop belonging to the Solanaceae family, commonly grown in tropical regions, and is believed to have originated in Central America (Idrees *et al*., 2020; Adom *et al*., 2024). Chilli is a significant vegetable and spice crop consumed worldwide, both domestically and commercially. It is an important cash crop and spice in many nations worldwide. To give food a strong, hot flavor, chillies are frequently used in a variety of dishes (Al-Imran *et al*., 2025; Kim *et al.,* 2025). Chilli is widely used in the production of curry paste, curry powder, and various pickles, as well as in the preparation of soups and salads. In the human diet, it is regarded as a significant source of β-carotene, total soluble solids, phenolic compounds, flavonoid compounds, vitamin A, vitamin C, and other phytochemicals that are essential antioxidants (Lahbib *et al*., 2021; Azlan *et al*., 2022). Chilli's pungency is caused by the alkaloid «capsaicin,» which also has a strong physiological effect and is utilized in a variety of pharmaceutical preparations, including ointments for chest congestion, colds, and sore throats (Rezazadeh *et al*., 2023). Chilli's pungency is caused by the presence of capsaicin, a crystalline, acrid, volatile alkaloid found in the placenta and pericarp of the fruit. Capsaicin has numerous preventive and therapeutic benefits in allopathic and ayurvedic medicine (Aimol *et al*., 2023). Modern agriculture is seeking new technologies to reduce the use of chemical inputs without compromising crop production or farm income worldwide. In agriculture and horticulture, seaweed and humic acid are used as nutritional supplements, bio-stimulants, or bio-fertilizers to promote plant growth and yield (Pavani *et al.,* 2022). Seaweed extracts are eco-friendly alternatives to chemical fertilizers, as they are nontoxic and do not pollute the soil or the environment. Rich with growth-promoting substances such as auxins, cytokinins, kinetin, zeatin, and gibberellins, seaweeds positively influence germination, crop establishment, and yield while also increasing tolerance to biotic and abiotic stresses (Mughunth *et al*., 2024; Sobuj *et al*., 2024).

Chilli, a widely consumed and economically significant crop, is an ideal subject for examining the impact of bio-stimulants such as seaweed and humic acid. A previous study indicated that the use of SLFs increases germination, growth, and yield of chilli plants (Vijayakumar *et al*., 2019). Auxins and cytokinins, two naturally occurring plant growth regulators (PGRs) found in seaweed extracts, regulate plant growth and structural development. Seaweed contains very low levels of PGRs, measured in parts per million. However, buds and cytokinins promote plant growth, while the indole compounds in the seaweed extract aid root development. It revitalizes leaves and promotes photosynthesis when applied to foliage. Consequently, a higher yield was obtained when the seaweed extract was used in the formulation. Additionally, humic acids encourage plants to produce antioxidants, which help reduce free radicals caused by stressors such as heat, UV light, and dehydration. Because they are potent oxidants, these radicals damage the DNA, proteins, and lipids in plant cells. Enzymes and metabolites known as antioxidants hunt down free radical molecules and shield plants from harm. They consist of water-soluble substances, such as vitamin C, and lipid-soluble substances, such as beta-carotene and vitamin E (Mukherjee and Patel, 2020; Nanda *et al*., 2022). Positive ions are drawn to humic acid, which chelates with micronutrients and releases them gradually when plants need them. By acting as a chelating agent, it stops micronutrients in the soil from precipitating, fixing, leaching, and oxidizing. Furthermore, humic compounds exhibit auxin-like activity, triggering hormonal responses that increase nutrient absorption, cell permeability, and catalytic activity, thereby enhancing dry matter production. Humic acids have great potential for foliar application in sustainable agricultural production as nutrient transporters (Eshwar *et al*., 2017). This study explores environmentally friendly alternatives, specifically seaweed extract and humic acid, to enhance chilli crop morphology and yield, and improve biochemical attributes. Chilli was selected for its susceptibility to various agronomic challenges. The effects of these bio-stimulants on growth, yield, and biochemical parameters were evaluated via a scientific experimental design. The primary objective of this study was to generate evidence-based recommendations for sustainable chilli cultivation. Ultimately, this study aims to advance agricultural knowledge of the effective use of bio-stimulants in crop production.

2. Materials and Methods

Experiment material and growing condition

The experiment was conducted from April to August 2023 at the IUBAT Agricultural Research Station, Rajendrapur, Gazipur, Bangladesh, located in AEZ 28 (Greater Dhaka). The location of Rajendrapur, Gazipur is 23.8734°N, 90.438° E, and it is approximately 9-10 meters above sea level (Quddus, 2009). The soil, a sandy loam, was prepared following the BARC Fertilizer Recommendation Guide (BARC, 2012), with cow dung (5 t/ha), TSP (330 kg/ha), gypsum (110 kg/ha), borax (1.5 kg/ha), zinc sulfate (3.91 kg/ha), and half of urea (210 kg/ha) and MoP (200 kg/ha) applied during final land preparation, while the remaining urea and MoP were added 30 days post transplantation. Humic acid (Shakti Humic Acid; Ispahani Agro Limited) was used as a powder at 1.5 g L^{-1}, as per the manufacturer's recommendations and those of Alizadeh *et al*. (2022). The *Sargassum wightii* specimens were harvested from the coast of Bangladesh, cleaned and dried in either sunlight or shade, and then pulverized. Seaweed extract (2 g/L) was prepared by grinding 2 g of seaweed powder in 1 L of distilled water for 24 h at room temperature, then filtering the extract and storing it in airtight, opaque containers at <25°C, as described by Al-Hasany *et al.* (2019).

A locally bred high-yielding chilli (*Capsicum annuum* L.) hybrid, 'Hira 1701 Supreme' (Fig. 1), popularly grown in Bangladesh, was used as a planting material. Medium-long, glossy red, very hot fruit are borne on this productive variety known for its versatility and tolerance to common field pressures. Thirty-day-old seedlings were transplanted at 60 cm × 40 cm spacing in a RCBD with four replications, comprising 16 plots (1.6 m × 1.0 m each). The treatments consisted of T_0 (RDF only), T_1 (RDF + humic acid 1.5 g/L), T_2 (RDF + seaweed extract 2 g/L) and T_3 (RDF + humic acid 1.5 g/L + seaweed extract 2g/L). Humic acid and seaweed extract were first sprayed 15 days after transplanting, then once a week during evening hours for a total of 4 applications. Irrigation and field management, as well as other standard agronomic and intercultural practices, were uniformly followed. Growth and yield characters of plants were noted at regular intervals. Fruit was harvested several times at commercial maturity (visually mature green stage) for yield, and pooled representative samples from each plot were transported to the laboratory for biochemical analysis.

Assessment of bio-chemical properties

Assessment of vitamin c (ascorbic acid). Vitamin C from fresh chilli was measured according to a modified method of Salkic *et al.* (2009). A 1 g sample was mixed with 0.056 M sodium oxalate for 2 minutes in a 10 mL solution, then allowed to stand for 5 minutes to obtain a suitable result before filtration. The filtered extract (0.5 mL) was diluted to 5 mL with sodium oxalate. The absorbance was measured using a UV-Vis spectrophotometer (UV-1900i, Shimadzu, Japan) against a sodium oxalate reference. The final vitamin C concentration was determined using a standard curve.

Assessment of total phenolic content (TPC). TPC photochemical was determined in some fresh chilli using the modified method of Al Kafi *et al*. (2025). Six milliliters of 80% ethanol were mixed with 1.0 g of fresh chilli material, and the supernatant obtained after centrifugation at 10,000 RPM and 4°C for 25 minutes was kept for further analysis. For the determination of phenolic content of the extract, the extract (1.0) was combined with Folin-Ciocalteu reagent (0.75), 7.5% sodium carbonate solution (0.25), and distilled water (1.0). The solution turned blue after 90 min in a water bath at 30°C. Absorbance data at 765 nm were collected using a

Fig. 1 - (A) Experimental field, (B) Chili plant with fruits, and (C) Effects of different treatments on chili, where T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

spectrophotometer (UV-1900i, Shimadzu, Japan). Finally, the total phenolic content was determined using a gallic acid standard curve.

Assessment of total flavonoid content (TFC). The modified Wolfe *et al*. (2003) method was used to determine the phytochemical total flavonoid content of fresh chilli. After thoroughly mixing the sample (1.0 g) with 6 mL of chilled 80% ethanol, it was centrifuged for 25 min at 10,000 RPM and 4°C. The following reagents were combined with 0.4 mL of the extract for analysis: 2 mL of distilled water, 0.12 mL of 5% sodium nitrite (which was incubated for 5 minutes), 0.24 mL of 10% aluminum nitrate, 0.8 mL of 1 mol/L sodium hydroxide, and finally 0.44 mL of distilled water. A UV-Visible spectrophotometer (Model UV-1900i, Shimadzu, Japan) was used to measure absorbance at 420 nm. TFC, which is measured in milligrams of rutin equivalents per gram of fresh weight (mg RE/g FW), was computed using a rutin standard curve.

Assessment of β Carotene. After preparing a 4:6 acetone-hexane solution, 10 mL of the solution was mixed with 1.0 g of fresh chilli, and the mixture was centrifuged. Following the procedure outlined by Nagata and Yamashita (1992) and Sharmin *et al.* (2024), the optical density (OD) of the clear supernatant was measured at 663 nm, 645 nm, 505 nm, and 453 nm using a UV-Visible spectrophotometer (Model UV-1900i, Shimadzu, Japan). The amount β-carotene was calculated using the following formula:

$$\beta\text{carotene (mg/100 g)} = 0.216 \times OD_{663} + 0.452 \times OD_{453} - 1.22 \times OD_{645} - 0.304 \times OD_{505}.$$

Here, OD represents the optical density readings at the respective wavelengths.

Assessment of total antioxidant. The antioxidant ability of the methanol extract of the plant was determined using a DPPH radical scavenging assay based on the procedures of Brand-Williams *et al.* (1995) and Susanti *et al.* (2007). 4 mg of DPPH were dissolved in 100 mL of 95% methanol to create a 0.004% solution, which was then left in the dark. The chile extract was made at a concentration of 1 mg/mL by dissolving 5 mg of crude extract in 5 mL of methanol, whereas ascorbic acid was utilized as a control at 0.1 mg/mL. Three milliliters of DPPH solution were combined with one milliliter of the powder or sample, and the mixture was left in the dark for 30 minutes. Antioxidant activity (%) was measured only at 100 µg/mL. Absorbance was measured at 517 nm to determine the radical scavenging activity, and the calculation was done using the following equation:

$$\text{Percent inhibition} = [(\text{ADPPH} - \text{A sample})/\text{ADPPH}] \times 100$$

Where, ADPPH indicated Absorbance of Control DPPH and A sample indicated Absorbance of sample

Assessment of crude protein (%). The Kjeldahl method for protein measurement in crude form was used to determine nitrogen content, following a slightly modified version of the method described by Mortuza *et al.* (2009). In a Kjeldahl flask, 0.5 g of a chilli sample was digested at 200°C with 10 mL of concentrated H_2SO_4, 9 g of K_2SO_4, and 1 g of $CuSO_4$ until a clear green solution was seen. After cooling, the liquid was steam-distilled, and 150 milliliters of distilled water were added. The distilled ammonia was trapped in a mixed indicator containing 4% boric acid. Ammonia was liberated by adding a 40% NaOH solution, and total nitrogen was determined by titrating the distillate with 0.2N HCl. Nitrogen content (N_2%) was calculated, and crude protein (CP) was determined using the formula:

$$CP\ (\%) = N_2\ (\%) \times 6.25$$

Assessment of total soluble solids (TSS). Total soluble solids (TSS) were determined using a digital refractometer, which expressed as °Brix (°B). First of all, chilli pulp was blended and filtered through filter paper. Finally, a drop of the filtered juice was given on the refractometer to check the reading and determine the result (Kafi *et al*., 2025).

Data collection and statistical analysis

Data on morphological, yield traits and biochemical properties including plant height, branches, leaves, flowering, fruit characteristics, root traits, yield, Vitamin C, TPC, TFC, β Carotene, Antioxidants, TSS etc. were collected from cultivated chilli among the treatments. Statistical analysis was performed using STATISTIX-10, R software and treatment means were compared using the LSD test at a 5% significance level.

The following formula was used to compute the increased yield percentage compared to the control:

$$\text{Increased yield (\%)} = (\text{Yield with treatment} - \text{Yield of control})/\text{Yield of control} \times 100.$$

3. Results

Morphological characteristics

The results demonstrated that chilli plant height was significantly influenced by humic acid and seaweed levels. After 90 days of transplanting, treatment T_2 (60.13 cm) showed the highest plant height, while T_0 (48.38 cm) had the lowest. Similar trends were observed at 45, 60, and 70 days of measurement. The results indicated that the different treatments significantly influenced the number of chilli leaves. The highest leaf count at 90 days after planting was observed in T_2 (98.75), while the lowest was recorded in the control treatment, T_0 (77.19). Similar trends were observed at 45, 60, and 70 days. The highest number of chilli branches was recorded at 70 days after planting in T_2 (13.25), while the control treatment T_0 showed the lowest number of branches (8.19). Similar trends were observed in the branch count at 90 days. The highest number of flowers was observed at 70 and 90 days after transplanting (DAT) in T_2, with counts of 37.63 and 32, respectively, in chilli. In contrast, the lowest number of flowers was recorded in T_0, with values of 20.88 and 8.69, respectively (Table 1).

Yield and yield attributes

The highest fruit length was observed in T_2 (7.92 cm), while the lowest fruit length was recorded in T_1 (5.69 cm) among the four treatments in chilli. Similarly, T_2 showed a larger fruit diameter (7.48 cm) than the control. These results also suggest that humic acid and seaweed did not significantly affect fruit diameter. The highest chilli seed count was observed in treatment T_2 (79.75), while the lowest was recorded in the control treatment T_0 (64.44). No significant differences in seed weight were observed, with values around 10.79 g across treatments, suggesting that humic acid and seaweed applications did not have a notable effect on seed weight. The results indicated that the highest chilli yield was achieved in treatment T_2 (1156.5 g per plot and 7.23 tons per hectare), while the lowest yield was recorded in the control treatment (T_0) at 801.75 g per plot and 5.01 tons per hectare (Table 2). The yield in T_2 showed the most significant increase over the control, with a 44.24% improvement compared to all other treatments. Both humic acid and seaweed treatments had a substantial effect on yield and yield-related attributes, with the seaweed treatment (T_2) performing best.

Table 1 - Morphological characteristics of Chili under different humic acid and seaweed treatments

Treatment	Plant height (cm)				Number of leaves				Number of branches		Number of flowers	
	45 days	60 days	70 days	90 days	45 days	60 days	70 days	90 days	70 days	90 days	70 days	90 days
T_0	26.56±2.20 b	28.25±2.25 b	31.44±3.66 b	48.38±3.84 c	48.94±5.13 ab	33.56±3.88 b	49.94±6.11 a	77.19±3.30 c	8.19±1.34 b	9.13±0.67 a	20.88±1.47 c	8.69±2.78 a
T_1	30.44±3.63 a	47±6.43 a	41.75±3.51 a	58.5±3.24 a	71.50±7.36 ab	52.38±2.87 a	84.88±7.03 a	96.19±4.67 ab	10.63±1.65 ab	11.56±0.84 a	29.75±2.92 b	26.06±4.45 a
T_2	28.44±2.72 ab	46.50±8.32 a	44.69±2.80 a	60.13±3.21 a	104.75±8.91 a	38.48±6.07 ab	84.06±4.19 a	98.75±7.89 a	13.25±1.21 a	10.38±0.80 a	37.63±2.03 a	32±3.34 a
T_3	29.56±2.20 ab	39.69±2.33 ab	40.88±3.14 a	54.19±3.12 b	33.13±6.92 b	46.94±6.63 ab	75.19±6.18 a	80.31±3.70 bc	12.75±0.83 a	9.31±0.06 a	22.93±2.05 c	29.81±5.79 a
CV	7.69	22.90	8.83	4.01	54.20	27.21	38.34	12.92	20.88	15.14	18.74	69.18
LSD	3.54	14.78	5.60	3.55	56.38	18.64	45.02	18.25	3.74	2.44	2.57	26.72
Level of significance	NS	NS	**	***	NS	NS	NS	*	*	NS	***	NS

*** = Significant at a 0.1% probability level; ** = Significant at a 1% probability level; * = Significant at a 5% probability level; NS = Not significant;
LSD = Least significant difference; CV = Coefficient of variation; Letters a, b, and c represent statistically significant differences among treatments and same letters did not show any difference; Data were represented as mean ± standard error for four replications; T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

Table 2 - Yield and yield attributes of chili under different humic acid and seaweed treatments

Treatment	Fruit length (cm)	Fruit diameter (cm)	Seed per fruit	Seed weight per 1000 seed (g)	Yield per plot (g)	Yield ton per hectare	Increased yield over control (%)
T0	5.99±0.31 ab	5.56±0.47 b	64.44±3.57 c	10.64±1.15 a	801.75±30.06 d	5.01±0.19 d	0
T1	5.69±0.45 b	7.40±0.12 a	73.66±1.62 bc	10.52±0.58 a	939.50±19.77 c	5.87±0.12 c	17.8
T2	7.92±0.72 a	7.48±0.36 a	79.75±5.47 ab	10.75±1.15 a	1156.50±66.40 a	7.23±0.41 a	44.24
T3	6.62±1.22 ab	7.34±0.11 a	88.63±4.31 a	10.79±1.00 a	892.50±56.04 b	5.58±0.35 b	11.31
CV	19.74	8.11	11.61	16.89	1.67	1.67	
LSD	2.07	0.90	14.19	3.60	31.61	0.86	
Level of significance	NS	**	*	NS	***	***	

*** = Significant at a 0.1% probability level; ** = Significant at a 1% probability level; * = Significant at a 5% probability level; NS = Not significant; LSD = Least significant difference; CV = Coefficient of variation; Letters a, b, c, and d represent statistically significant differences among treatments and same letters did not show any difference; Data were represented as mean ± standard error for four replications; T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

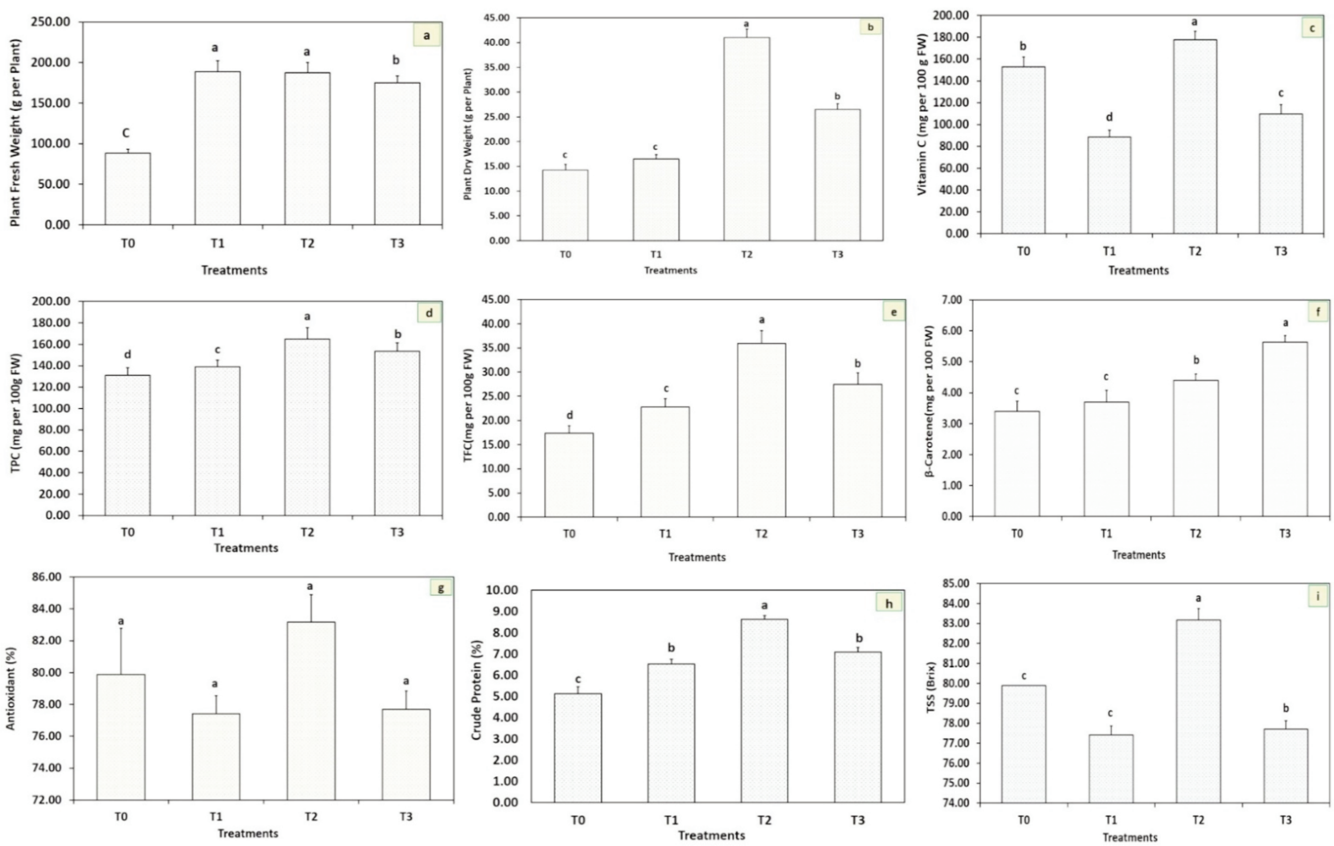

Fig. 2 - Plant physiological and biochemical properties of chili under different humic acid and seaweed treatments. Here, (a)=Plant Fresh Weight (g plant-1); (b)=Plant Dry Weight (g plant-1); (c)=Vitamin C (mg 100 g^{-1} FW); (d)=TPC (mg 100 g^{-1} FW); (e)=TFC (mg 100 g^{-1} FW); (f)=β Carotene (mg 100 g^{-1} FW); (g)=Antioxidant (%); (h)=Crude Protein (%); (i)=TSS; Letters a, b, c, and d represent statistically significant differences among treatments and same letters did not show any difference; The figure also indicates the standard error bar; T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

Plant physiological and biochemical properties of chilli

The highest and lowest weights were recorded for both fresh and dry chilli plants (Fig. 2a and Fig. 2b). The relatively maximum fresh weight was observed in T_2 (187.50 g), while the lowest fresh weight was found in T_0 (88.25 g). In terms of dry weight, the highest value was recorded in T_2 (41 g), while the lowest was in T_0 (14.25 g). The results revealed significant variation in ascorbic acid (vitamin C) content among treatments in chilli fruits. Treatment T_2 exhibited the highest vitamin C content (177.80 mg 100 g^{-1} FW). In comparison, the lowest level was observed in T_1 (88.39 mg 100 g^{-1} FW) among all treatments (Fig. 2c). The application of bio-stimulants significantly influenced the total phenolic and flavonoid content in chilli fruits (Fig. 2d). The highest TPC was recorded in the seaweed-treated plants (T_2) with 164.87 mg 100 g^{-1} FW. The lowest TPC value, 131.07 mg 100 g^{-1} FW, was observed in the control (T_0). Bio-stimulant treatments enhanced the total flavonoid content of chilli. T_2 showed the highest flavonoid concentration, 35.95 mg 100 g^{-1} FW. At the same time, the lowest TFC was recorded in the control, 17.37 mg 100 g^{-1} FW (Fig. 2e). In chilli, the combined treatment (T_3) resulted in the highest accumulation of β-carotene, 5.63 mg 100 g^{-1} FW. In contrast, the control T_0 exhibited the lowest value, 3.40 mg 100 g^{-1} FW (Fig. 2f). These findings suggest that seaweed and humic acid, particularly when applied individually or in combination, effectively enhance the phytochemical composition of chilli fruits. The results indicated that all four treatments did not influence the total antioxidant percentage in chilli fruits. No significant differences were observed in Total antioxidant percentage, which ranged from 77.41% to 83.16%, and T_2 showed the best result. However, it was not statistically higher than the other treatments (Fig. 2g). The application of biostimulants significantly increased crude protein content in chilli fruits. The highest crude protein percentage was observed in plants treated with seaweed extract (T_2), recording 8.63%. Humic acid alone (T_1) resulted in a moderate protein content of 6.53%. In comparison, the lowest value was recorded in the control treatment (T_0) at 5.13% (Fig. 2h). The results demonstrated a significant difference in the total soluble solids (TSS) content across the treatments in chilli fruits. Treatment T_2 recorded the highest TSS value (5.8 °Brix), while the lowest was observed in the control treatment (T_0) at 4 °Brix (Fig. 2i).

Comparative evaluation of different traits among the treatments

A Pearson correlation heatmap was generated to elucidate the associations among the studied morphological, yield-contributing, and biochemical traits (Fig. 3). The results revealed several significant positive correlations, underscoring strong interdependence among growth, productivity, and nutritional attributes. Out of the 171 trait pairs analyzed, 18 traits exhibited statistically significant positive correlations, indicating a focused set of interrelated parameters with potential breeding value. Yield per plot (YP) exhibited a highly significant positive correlation with increased yield over control (IYC; r = 1, ***p < 0.001), plant fresh weight (PFW; r = 1, **p < 0.01), number of flowers at 70 days (NF.70D; r = 0.97, *p < 0.05), plant dry weight (PDW; r = 0.93, *p < 0.05), and fruit length (FL; r = 0.98, *p < 0.05). These findings suggest that biomass accumulation and floral characteristics are key determinants of yield performance. Strong inter-correlations were also observed among biochemical traits. Crude protein (CP) exhibited highly significant positive correlations with total phenolic content (TPC; r =

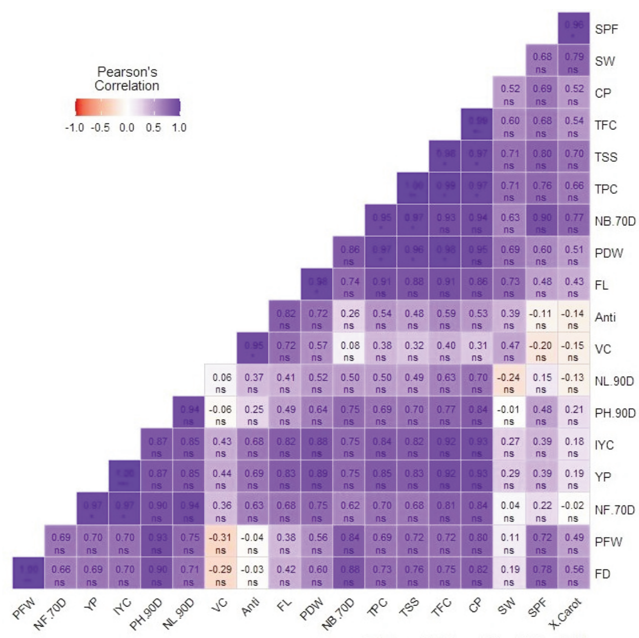

Fig. 3 - Correlation heatmap of yield and biochemical traits of chili under different humic acid and seaweed treatments. Here, PH 90D-Plant height at 90 days; NL 90D-Number of leaves at 90 days ; NB 70D-Number of branches at 70 days; NF 70D-Number of flowers at 70 days; FL- Fruit length; FD-Fruit diameter; SPF- Seed per fruit; SW-Seed weight per 1000 seed; YP- Yield per plot; mIYC-Increased yield over control; PFW-Plants fresh weight ; PDW- Plants dry weight ; VC- Vitamin C; TPC-Total phenolic content; TFC- Total flavonoids content; XCarot-βCarotene; Anti-Total antioxidants; CP-Crude protein; TSS-Total soluble solids.

0.99, *$p < 0.05$), total flavonoid content (TFC; r = 0.99, **$p < 0.01$), and total soluble solids (TSS; r = 0.98, *$p < 0.05$), indicating their potential co-regulation and contribution to fruit quality. Furthermore, antioxidant activity (Anti) showed a strong positive correlation with vitamin C (VC; r = 0.95, *$p < 0.05$), suggesting their synergistic role in enhancing nutraceutical value. In contrast, certain traits exhibited weak or negative associations. Notably, some negative non-significant correlations were found. Overall, the correlation analysis highlights meaningful trait interrelationships, emphasizing the importance of simultaneously considering both agronomic and biochemical characteristics in breeding strategies aimed at developing high-yielding, nutritionally enhanced cultivars.

Principal Component Analysis (PCA) explained 84.18% of the total variability, with PC_1 accounting for 66.89% and PC_2 for 17.29% (Fig. 4). The biplot clearly separated the treatments, with T_2 and T_3 showing strong positive associations with key agronomic and nutritional traits. Yield per plot (YP), increased yield over control (IYC), plant dry weight (PDW), total phenolic content (TPC), total flavonoid content (TFC), and crude protein (CP) were the dominant contributors along PC_1. Traits such as antioxidant activity (Anti) and vitamin C (VC) were mainly associated with PC_2. T_0 and T_1 were negatively associated with most traits, indicating lower performance. Traits such as β-carotene (βCarot), seed per fruit (SPF), and plant fresh weight (PFW) contributed moderately. Close grouping of YP, IYC, PDW, and TFC vectors indicated strong positive inter correlations. The biplot effectively illustrated the interactions between traits and treatments, with T_2 identified as the most effective treatment for enhancing yield and improving biochemical quality.

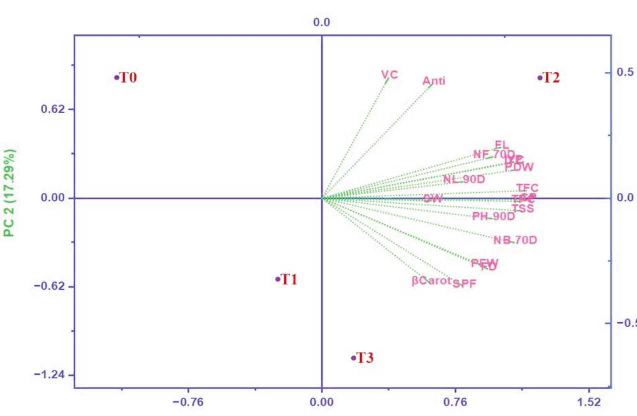

Fig. 4 - Biplot of yield and biochemical traits of chili under different humic acid and seaweed treatments. Here, PH 90D-Plant height at 90 days; NL 90D-Number of leaves at 90 days ; NB 70D-Number of branches at 70 days; NF 70D-Number of flowers at 70 days; FL- Fruit length; FD-Fruit diameter; SPF- Seed per fruit; SW-Seed weight per 1000 seed; YP- Yield per plot; IYC-Increased yield over control; PFW-Plants fresh weight ; PDW-Plants dry weight ; VC-Vitamin C; TPC-Total phenolic content; TFC- Total flavonoids content; βCarot-βCarotene; Anti- Total antioxidants; CP-Crude protein; TSS-Total soluble solids; T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

4. Discussion and Conclusions

Bio-stimulants such as seaweed extract and humic acid are increasingly recognized as sustainable inputs that enhance plant growth, yield, and biochemical composition by modulating key physiological and metabolic processes. In this study, seaweed extract promoted chilli vegetative growth and fruit quality more effectively than humic acid under regular fertilizer application. This finding is in agreement with Alaway and Hasan (2023) and Shahen *et al.* (2019), who reported pronounced improvements in vegetative development in Capsicum following seaweed treatments. Similar observations were made by Jan *et al*. (2020) regarding branching enhancement after seaweed application, while humic acid has also been shown to stimulate branch formation, indicating that both bio-stimulants can improve plant architecture, albeit to different extents and depending on crop-specific responses. The positive impact of seaweed extract on reproductive performance is also consistent with previous studies. Jayasinghe *et al.* (2016) reported increased flower production in seaweed-treated chilli plants, supporting the reproductive improvement observed here. The enhanced fruit characteristics reported in this study align with the findings of Segmen and Ozdamar (2023) and Dutta *et al*. (2019), who documented improvements in fruit size and seed parameters following seaweed application. From a yield and quality perspective, our results align with Ashour *et al*. (2021) and Ali *et al.* (2023), who demonstrated that seaweed-based treatments significantly improved yield components and the biochemical composition of *Capsicum annuum*. Similarly, Mohamed *et al.* (2021) observed substantial increases in pepper biomass following seaweed extract application, reinforcing its positive effect on vegetative accumulation.

The application of seaweed to chilli plants increased the chilli's biochemical properties, including vitamin C levels, as reported by Segmen and Ozdamar (2023). Increasing vitamin C content and other biochemical properties, as reported here, have been linked to enhanced nutrient availability and uptake, photosynthetic efficiency, and stress tolerance in response to various biostimulants (Khan *et al*., 2022; Mohamed and Hassan, 2025). The total phenolic content (TPC) was also significantly enhanced by both seaweed and humic acid in our study, in agreement with Musolo *et al.* (2020) and Jalali *et al.* (2022). Humic acid-treated peppers showed 36.4% and 31.8% increases in TPC relative to the controls, consistent with this study's findings and those reported by Zamljen *et al.* (2025). Similarly, Ashour *et al.* (2021) reported that the most excellent TFC value was recorded from seaweed-treated plants and associated this response with the presence of phytohormones, polyphenols, and flavonoids in the biostimulant. Mohamed *et al.* (2021) and Zohaib *et al.* (2023) demonstrated that combined seaweed and humic acid applications increased the β-carotene content in chilli fruits, suggesting a synergistic effect on pigment biosynthesis and overall nutritional potential. The differences between studies on the impact of biostimulants have been widely described and linked to differences in environmental conditions, crop species, and their physiological status, as stressed by both Roleda and Hurd (2019). These response context-dependencies need to be taken into account when extrapolating our findings to larger agro-ecological scales. In terms of protein, Yilmaz *et al.* (2018) and Veliz *et al.* (2023) demonstrated that seaweed and humic acid improve nitrogen assimilation and metabolic processes, resulting in higher crude protein accumulation in the biomass. Comparini *et al.* (2021) also reported significant responses in hot chilli peppers, including variable morphological traits, field yield, and protein content, following biostimulant application. Similarly, applying biostimulants or seaweed to chilli plants increases TSS content (Mohamed *et al*., 2021). All these combined lines of evidence strongly support the higher efficiency of seaweed extract compared with humic acid in improving chilli growth, yield, and biochemical quality. Seaweed extract, when added to regular fertilizer schedules, appears to be a good, sustainable option for enhancing chilli production and nutritional quality.

This study explored the application of seaweed and humic acid as biostimulants on growth, yield attributes, and biochemical constituents in chilli. The idea was to investigate alternative, eco-friendly soil amendments to increase chilli yield and improve fruit nutritional quality. Among the treatments, the highest response in plant growth, yield, and biochemical contents was observed with seaweed extract spray (T_2-RDF + Seaweed extract 2 g/L), followed by the suggested level of fertilizers. The treatment T_2 had the highest yield parameters, as well as elevated nutritional value of chilli fruits in terms of vitamin C, phenolic compounds, flavonoids, β-carotene, and crude protein. The correlation and PCA analyses also supported the conclusion that T_2 was strongly associated with superior agronomic traits and biochemical characters, suggesting it as an efficient bio-stimulant for chilli production. In conclusion, this study's results show that seaweed extract could be a promising, environmentally friendly input for sustainable chilli cultivation systems. Given their ability to increase crop yields without causing additional environmental stress, seaweed-based biostimulants could be a pragmatic solution for sustainable agriculture. In the future, more studies are required to refine application rates, evaluate performance in multi-season field trials, and assess the cross-crop relevance of seaweed-based bio-stimulants. Such a development can contribute to broader agricultural advancement, enabling the sector to create sustainable, high-quality crop production systems that are profitable and beneficial to the environment.

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