SVU-International Journal of Agricultural Sciences
Volume 7 Issue (1) pp: 31-40, 2025
Print ISSN 2636-3801 | Online ISSN 2636-381X
Doi: 10.21608/svuijas.2025.346517.1425

RESEARCH ARTICLE

Impact of bio stimulants on the growth performance of *Capsicum annuum* L.

Shimaa T.H. Oraby *, A.M.A. Rashwan and A.A.H. El-Shaieny

Horticulture Department, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt.

Abstract

This study evaluated the influence of foliar applications of seaweed extract, fulvic acid, and chitosan on the vegetative growth characteristics of F_1 hybrid pepper *Capsicum annuum* L. "Biskra" over two growing seasons. The experimental design consisted of eleven treatments, including individual applications of each substance and combinations thereof. Treatments T1 and T2 utilized seaweed extract, T3 and T4 used fulvic acid, and T5 and T6 employed chitosan at concentrations of 0.3% and 0.4%, respectively. Additionally, T7 through T10 involved combinations of these substances. T7 (T1+ T3+ T6), T8 (T1+ T4 + T6), T9 (T2 + T3 + T5); T10 (T2 + T4 + T6). Tap water served as the control treatment. The results showed that the combination of 0.4% concentration for each of seaweed, fulvic acid, and chitosan extract (T10) enhanced ($P<0.05$) vegetative growth parameters, such as plant height, number of branches per plant, and both fresh and dry biomass, leaves number, chlorophyll content and the index of leaf area. Notably, T10, along with treatments T3 (0.3% fulvic acid) and T4 (0.4% fulvic acid), produced the tallest plants and the highest number of branches per plant, with plant heights approaching 47 cm. Treatments with chitosan (0.3%) and seaweed (0.4%) also resulted in greater plant height and number of branchs with increment by 9.1 and 6.2% for chitosan as well as 5.6 and 14.1% for seaweed, respectively compared to the control. In terms of biomass, T10 exhibited superior performance, achieving dry weights of approximately 27 g and fresh weights of around 63 g, while the control treatment consistently demonstrated the lowest growth metrics. In conclusion, this study revealed that combining seaweed extract, fulvic acid, and chitosan (T10) as foliar sprays significantly boosted pepper plant growth, *i.e.*, increasing height, branching, and biomass compared to other treatments.

Keywords: Algae; Chitosan; Fulvic acid; Growth characteristics; Hot pepper.

1. Introduction

The pepper (*Capsicum annuum* L.) is one of most important vegetable crops in the world and has important economic value. Worldwide, the total cultivated area in 2017 was approximately 2 million hectares, leading to the production of approximately 36 million tons (FAO, 2018). In Egypt, it covers a production area of old lands 43702 feddans that yielded 411116 tons and production area in new lands 48625 feddans that yielded 459027 tons, according to Ministry of Agriculture Statistics in 2019 (Eldewini *et al.*, 2023). Its fruits are high in thiamine, beta-carotene, folic acid, and vitamins A, C, and E. They are also utilized for a variety of dishes, including pickles, sauces, greens, and spices.

Natural plant extracts are emerging as environmentally friendly alternatives to traditional chemicals for plant improvement, offering benefits for both human safety and plant growth (Peter, 1999; Matysiak *et al.*, 2010; Noha *et al.,* 2018). These extracts are rich in volatile components, proteins, lipids, tannins, minerals, antioxidants, vitamins, and sulfur compounds, making them valuable sources of nutrients. Their antioxidative properties help mitigate reactive oxygen species, promoting

.***Corresponding author: Shimaa T.H. Oraby**
Email: a.elsamman12@gmail.com
Received: December 23, 2024; Accepted: January 22, 2025; Published online: February 7, 2025.

overall plant health (Kirtikar and Basu, 1984; Botelho *et al.,* 2007; Bhanu *et al.,* 2013).

Key substances like fulvic acid, chitosan, and seaweed extracts enhance growth by increasing levels of amino acids, sulfur-containing compounds, and plant hormones such as gibberellic acid (GA3) and indoles, while reducing total phenols and abscisic acid (ABA) (Kubota *et al.,* 1999; Kubota *et al.,* 2000). Additionally, the antioxidants and organic compounds in these extracts contribute to improved growth and fruiting in various crops (Osawa, 1994; Reddy *et al.,* 2000; Bruneton, 2001; Prakash and Majeed, 2003; Pons, 2003; Chowdhury *et al.,* 2007; Bhadwaj *et al.,* 2010; Hanafy *et al.,* 2012; Akl *et al.,* 2017; and Li *et al.,* 2021).

Natural, inexpensive, and low-toxic, chitosan is an environmentally benign, biodegradable substance with a number of uses in agriculture. It has a great deal of untapped potential that could contribute to the realization of sustainable agriculture. In contrast to untreated plants, Ghoname *et al*., (2010) and Sharifa (2013) found that foliar sprays of chitosan on *Phaseolus vulgaris* plants improved physiological components in plant shoots in stressed or non-stressed conditions as well as plant growth, yield, and quality. Additionally, it was shown that applying chitosan to pepper increased vegetative growth and enhanced the quality of its fruit (Farouk *et al.,* 2011). Other researchs has also employed chitosan to promote grape development and fruiting (El-Kenawy, 2017; Refaai and Silem, 2021), and this could be due its low molecular weight and capability to easily link minerals and elements into its molecular structure, leading them to dissociate and become activated fulvic complexity, therefore, fulvic acid is highly active. Typically, its molecular structures contain at least 70 mineral and trace elements (Aiken *et al.,* 1985).

Because they can promote the uptake of components from plant exteriors into plant tissues and aid in absorption to plant parts, Fulvic acids carry trace minerals straight to plant cell metabolic sites after being applied to the leaves. Therefore, the main method for increasing a plant's potential for production is to apply foliar sprays with mineral chelated chemicals during particular periods of plant growth (Chen *et al.,* 2004).

In studies of Khang, (2011), Elattar, (2012), Suh *et al.* (2014a&b), El-Borai *et al.* (2015), Abou El Hassan and Husein (2016), El-Hassanin *et al.* (2016), and El-Kenawy (2017) on radish, palm, tomato, potato, grapevine, tomato, sugar beet and grapevine, respectively reported that fulvic acid topically or as a soil treatment improved plant growth, chemical composition, yield, and quality.

Seaweeds are recognized as effective biofertilizers and biostimulants due to their rich content of trace elements and plant growth regulators like auxins, gibberellins, and cytokinins. They are commonly used to enhance the growth and yield of various crops globally. For instance, seaweed extract is often applied foliar to improve potato productivity. Prajapati *et al.* (2016) demonstrated that using seaweed extract alongside recommended fertilizer doses significantly improved growth characteristics of potato plants, including height, number of stems, and tubers, compared to untreated controls. This approach also helps reduce dependence on chemical fertilizers.

Applying biostimulants to pepper plants will result in a significant increase in plant growth compared to untreated plants. This study was performed to evaluate the response of pepper plants subjected to various treatments with individual biostimulants and their mixtures, and to determine a possible mechanism of plant performance improvement by applying such biostimulants.

2. Materials and methods

The study was conducted during winter seasons of 2021–2022 and 2022–2023 at a private farm in Nag Hammadi, Qena Governorate, Egypt, to examine the effects of using several biostimulants foliar sprays on pepper vegetative growth. On August 15, 2021, and 2022, hot pepper seeds (cv. Biskra F_1, Sun Seed Company, USA) were planted in the Gauze cloth shaded nursery.

In a randomized complete block design with three replicates, transplants were placed in the field at the 3–4 leaf stage 50 days after the seeds were sown.

Table 1: Chemical composition and physical properties of experimental soil.

Soil properties	Value	Soil properties	Value
	Physical and Chemical		
Clay %	10.82	$CaCo_3$	6.25
Silt %	2.00	EC (dsm^{-1} at 25°)	1.25
Sand %	87.12	Total N	0.30
Texture	Loamy Sand	Available P ppm	30
Organic matter	2.05	pH (1:2.5)	8.25
	Soluble anions (meq/L)		
Cl	3.89	SO_4	5.36
HCO_3	3.45		
	Soluble cations (meq/L)		
Ca^{++}	4.25	Na^{+}	1.37
Mg^{++}	1.75	K^{+}	4.45

Each plot measured 10.5 m² and had three rows, each measuring 3.0 m in width and 3.5 m in length. Plants were spaced 30 cm apart. As recommended by the Egyptian Ministry of Agriculture and Land Reclamation, common cultural activities related to pepper production, including surface irrigation, fertilization, weed control, and insect management, were carried out as needed.

2.1. Experimental Treatments and Application

Eleven treatments were applied during the experiment as the following T1: Seaweed extract (SW_1 0.3%); T2. Seaweed extract (SW_2 0.4%); T3. Fulvic acid (FA_1 0.3%), T4. Fulvic acid (FA_2 0.4%), T5. Chitosan (CH_1 0.3%), T6. Chitosan (CH_2 0.4%), T7. Combined (SW_1+ FA_1+ CH_2), T8. Combined (SW_1+ FA_2 + CH_2), T9. Combined (SW_2 + FA_1 + CH_1); T10 combined (SW_2 + FA_2 + CH_2). In addition to the aforementioned 10 treatments, tap water was sprayed in the control treatment. However, the green macroalga *Halimeda opuntia*, generously, provided by Plant and Microbiology Department, Faculty of Science, South Valley University, was used to prepare the seaweed ethanolic extracts. Both the seaweed extracts and the solutions of fulvic acid and chitosan were applied to the plants via foliar sprays.

2.2. Application Procedure

All pepper plants were sprayed thoroughly from bottom to top using a fine mist until runoff, ensuring complete coverage of all plant organs. This application method adhered to standardized protocols. Each treatment was applied three times. The initial foliar spraying commenced 15 days after seedling transplantation, followed by subsequent applications at 15-day intervals throughout the growing season of the hot pepper plants.

The soil type used at the experimental site was loamy sand (Table 1). The physical and chemical properties of the soil were determined using the methods outlined by Jackson (1973).

2.3. Data recoded

Plant height (PH, cm), number of branches/plant (NB), number of leaves/plant (NL), leaf area/plant (LAI, cm^2), fresh (FW, g) and dry

(DW, g) weight of plant (roots, stem, leaves), and SPAD units were measured in a random sample of five plants from each plot.

2.4. Statistical Analysis

Data obtained in this study were analyzed using the Statistix 8.1 statistical software package (Statistix 8.1.,2003), The collected data were statistically analyzed using analysis of variance (ANOVA) as described by Snedecor and Cochran (1980), and the least significant difference (L.S.D.) at 5% was employed to compare the means.

3. Results

The results presented in Figs. 1-4 show that vegetative growth characteristics (plant height, number of branches, fresh and dry weight of plant, number of leaves, chlorophyll and leaf area) were strongly influenced by foliar spraying with some elements. Combined (SW_2 + FA_2 + CH_2) treatment (T10) foliar spray surpassed the other treatments in all the studied traits during the two seasons of study.

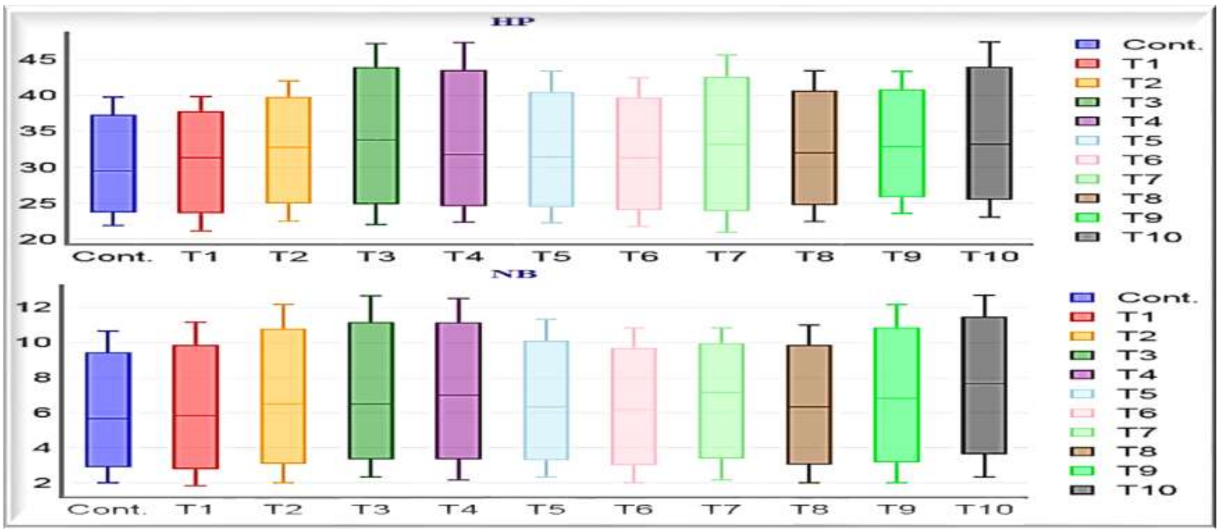

Figure 1. Pox-plot for plant height (PH) and number of branches (NB)
T1: Seaweed extract (0.3%); T2: Seaweed extract: (0.4%); T3: Fulvic acid (0.3%), T4: Fulvic acid (0.4%), T5: Chitosan (0.3%), T6: Chitosan (0.4%), T7: Combined (T_1+ T_3+ T_6), T8: Combined (T_1+ T_4 + T_6), T9. Combined (T_2 + T_3 + T_5); T10 combined (T_2 + T_4 + T_6).

The best treatments in respect of plant height and number of branches were T10, T3 (FA_1) and T4 (FA_2) with no significant differences between them (Figs. 1, 2). They recorded 47.44, 47.175 and 47.34 cm for plant height and 12.7, 12.665 and 12.495 for number of branches, respectively. Chitosan (CH_1, T5) and seaweed (SW_2, T2) also produced significantly longer plants (43.41 and 42.02, respectively) and numerous branches (11.33 and 12.165, respectively) than the control, with no differences between them. However, control treatment recorded the lowest values of PH and NB in both seasons. The increment percentages (Fig.2) associated with the previous prevalent treatments were 19.23%, 18.56%, 18.97%, 9.1% and 5.6% for plant height and 19.08%, 18.75%, 17.16%, 6.24% and 14.06% for number of branches, over the control, respectively. The presented data also revealed that the 2nd season of study was significantly higher than the 1st one for plant height.

As for dry and fresh plant weights, the results presented in Figs. 3, 4 exhibited that FW and DW of plant were strongly influenced by foliar spraying with some elements. Combined (SW_2 + FA_2 + CH_2) treatment (T10) foliar spray significantly surpassed the other treatments in both traits through both seasons of study. The most effective treatments in this respect were T10, T4 (FA_2) and T5 (CH_1) with no significant differences between T4 and T5 for DW.

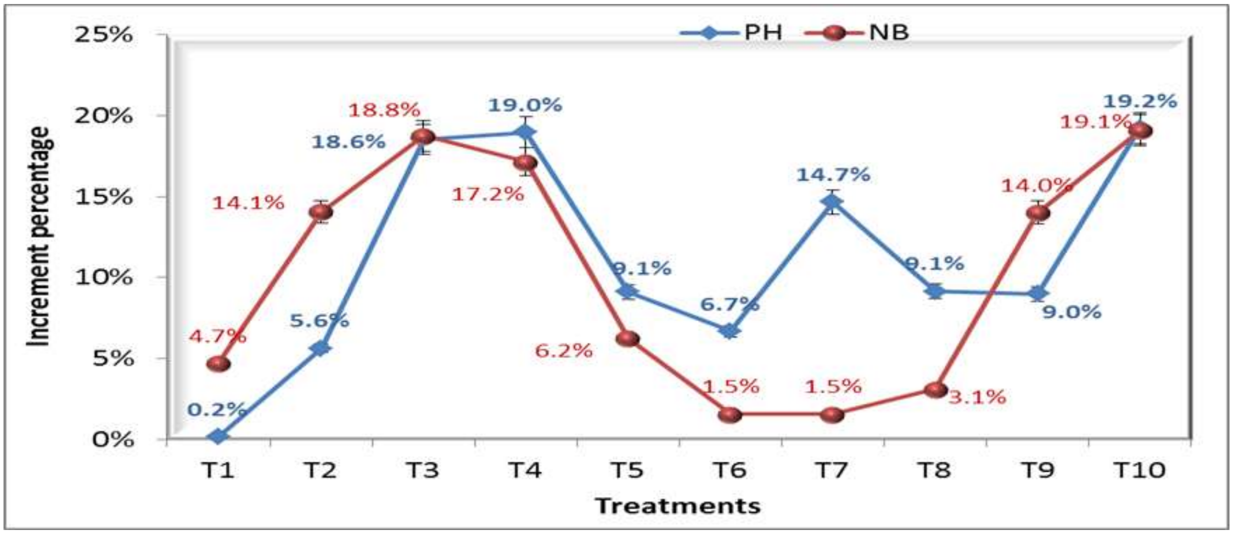

Figure 2. Increment percentage of plant height (PH) and number of branches (NB)
T1: Seaweed extract (0.3%); T2: Seaweed extract: (0.4%); T3: Fulvic acid (0.3%), T4: Fulvic acid (0.4%), T5: Chitosan (0.3%), T6: Chitosan (0.4%), T7: Combined (T_1+ T_3+ T_6), T8: Combined (T_1+ T_4 + T_6), T9. Combined (T_2 + T_3 + T_5); T10 combined (T_2 + T_4 + T_6).

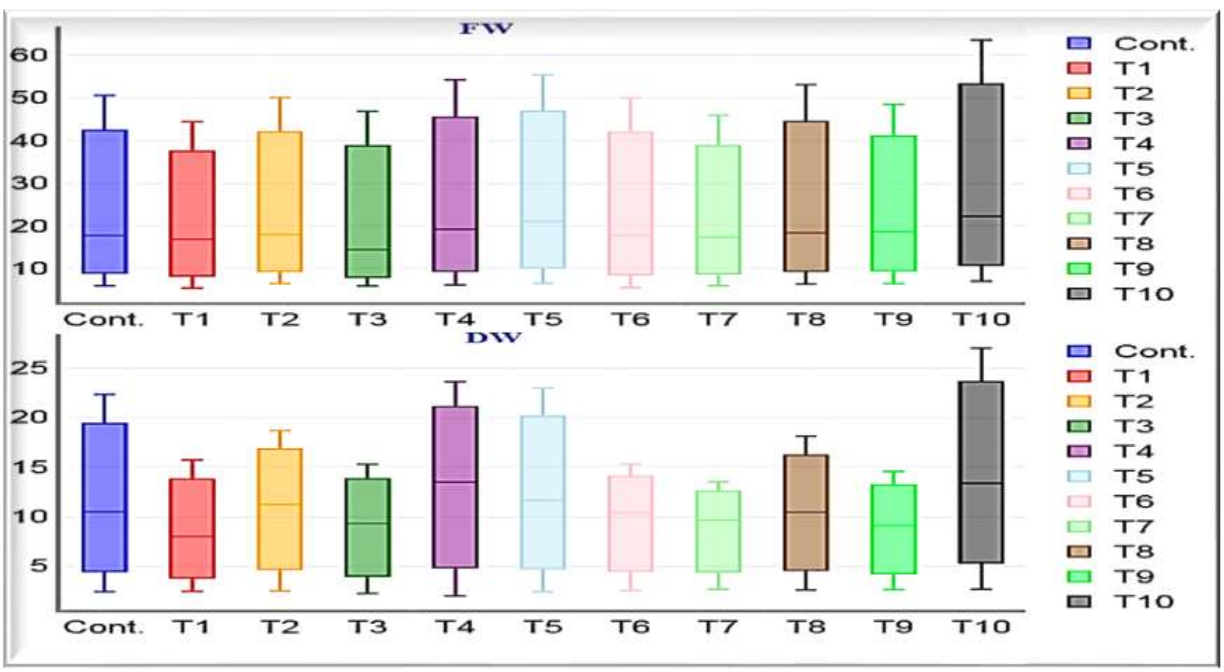

Figure 3. Pox-plot for plant fresh weight (FW) and dry weight (DW)
T1: Seaweed extract (0.3%); T2: Seaweed extract: (0.4%); T3: Fulvic acid (0.3%), T4: Fulvic acid (0.4%), T5: Chitosan (0.3%), T6: Chitosan (0.4%), T7: Combined (T_1+ T_3+ T_6), T8: Combined (T_1+ T_4 + T_6), T9. Combined (T_2 + T_3 + T_5); T10 combined (T_2 + T_4 + T_6).

They recorded 27.025, 23.63 and 22.965 g for dry plant weight (DW) and 63.55, 54.24 and 55.395 g for fresh plant weight (FW), respectively.

T8 (SW_1+ FA_2 + CH_2), also produced significantly heaviest fresh plants (53.065 g) than the control with no significant differences between both Chitosan (CH_2, T6) and seaweed (SW_2, T_2) and with control.

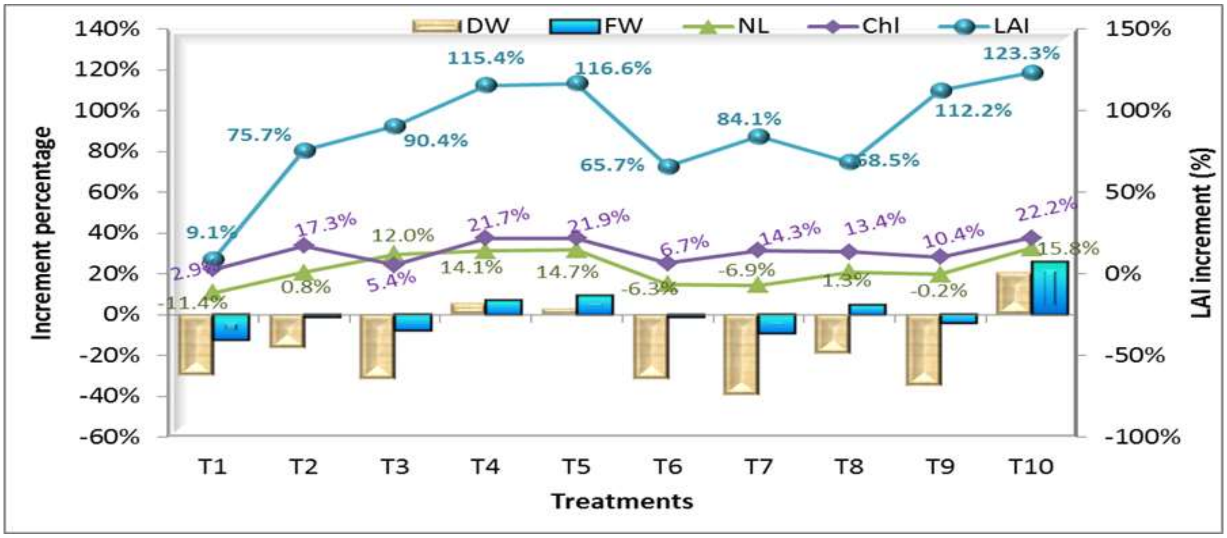

Figure 4. Increment percentage of five pepper traits
T1: Seaweed extract (0.3%); T2: Seaweed extract: (0.4%); T3: Fulvic acid (0.3%), T4: Fulvic acid (0.4%), T5: Chitosan (0.3%), T6: Chitosan (0.4%), T7: Combined (T_1+ T_3+ T_6), T8: Combined (T_1+ T_4 + T_6), T9. Combined (T_2 + T_3 + T_5); T10 combined (T_2 + T_4 + T_6).

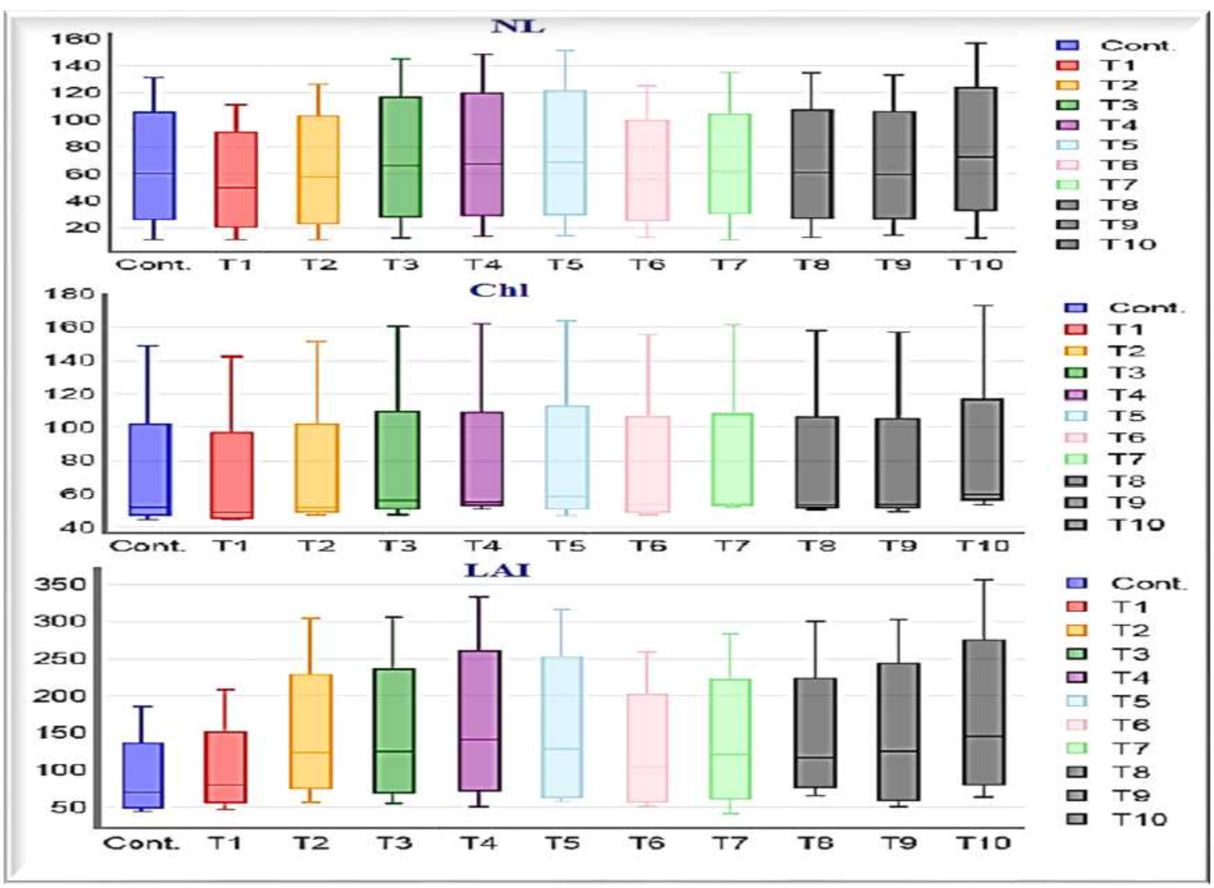

Figure 5. Pox-plot for number of leaves (NL), Chlorophyll (Chl) and leaf area index (LAI)
T1: Seaweed extract (0.3%); T2: Seaweed extract: (0.4%); T3: Fulvic acid (0.3%), T4: Fulvic acid (0.4%), T5:

Chitosan (0.3%), T6: Chitosan (0.4%), T7: Combined (T_1+ T_3+ T_6), T8: Combined (T_1+ T_4 + T_6), T9. Combined (T_2 + T_3 + T_5); T10 combined (T_2 + T_4 + T_6).

The increment percentages (Fig.4) associated with the previous prevalent treatments were 20.97% (T10), 5.77% (T4) and 2.80% (T5) for dry weight and 25.69% (T10), 7.28% (T4) and 9.56% (T5) as well as 4.95% (T8), -1.10% (T2) and -1.17% (T6) for fresh weight over the control, respectively.

The results of NL, Chl and LAI presented in Figs. 4, 5 show that they were strongly influenced by foliar spraying with biostimulant. Combined (SW_2 + FA_2 + CH_2) treatment (T10) foliar spray significantly surpassed the other treatments in the three traits during through both seasons of study. The most effective treatments in this respect were T10, T4 (FA_2) and T5 (CH_1) with no differences between T4 and T5 for all the three traits. They recorded 91.665 (T10), 90.33 (T4) and 90.83 (T5) for leaves (NL), 54.67 (T10), 54.475 (T4) and 54.53 (T5) for chlorophyll (Chl) as well as 194.735, 187.805 and 188.895 cm^2 for LAI, respectively. All other treatments exhibited values that were significantly equal or higher than the control for all the three traits except for T1, T6 and T7 of number of leaves (NL) which were lower than the control. The increment percentages (Fig.4) associated with the previous prevalent treatments were 15.80% (T10), 14.11% (T4) and 14.74% (T5) for NL, and 22.18% (T10), 21.75% (T4) and 21.87% (T5) for Chl and 123.35% (T10), 115.40% (T4) and 116.65% (T5) for LAI over the control, respectively.

4. Discussion

Humic acid, which is created when microorganisms break down plant matter in a soil that has enough oxygen, is comparable to fulvic acid. According to Suh *et al.* (2014a & b); Abou El Hassan and Husein (2016); Mostafa *et al.* (2017) and Li *et al.* (2021), the application of fulvic acid results in favorable growth outcomes. Fulvic acid-bearing transport carries minerals straight to plant cell metabolic sites after being applied to leaves. The main method for increasing a plant's capacity for production is to apply foliar sprays with mineral chelated chemicals at particular periods of plant growth (Chen *et al.,* 2004). Additionally, pepper, radish, and cucumber plants grew larger and produced better-quality fruit when foliar chitosan was applied (Kazimi and Saxena, 2023). On the other hand, chitosan's positive impacts typically depend on its concentration, growth conditions, preparation method, and environmental factors (Pirbalouti *et al.*, 2017). This increase could be due to the synergistic role of chitosan in stimulating growth compared with untreated plants. The improvement of growth characteristics through chitosan foliar application is consistent with those observed on mung beans (Mondal *et al.*, 2012), radish (Farouk *et al.*, 2011), garlic (Fawzy *et al.*, 2012), sweet pepper (Ghoname *et al.,* 2010), squash (Sabreen *et al.,* 2015) and strawberry (Abdel-Mawgoud *et al.*, 2010). Finally, seaweed extracts are natural fertilizers containing various nutrients, vitamins, amino acids, and plant hormones. Seaweed extracts are used for enhancing growth and yield. Many investigators found that seaweed extract stimulated the vegetative growth (Taskos *et al.,* 2019; Pessenti *et al.*, 2022). Noteworthy, according to Calvo *et al.* (2014), the advantages of seaweed extracts are mostly ascribed to their ability to promote plant growth rather than to supply nutrients. Seaweed extracts could therefore operate as a link between crops and fertilizer, balancing the markets for agricultural inputs and providing financial gain.

5. Conclusion

The study found that foliar spraying pepper plants with biostimulant treatments, particularly a combination of seaweed extract, fulvic acid, and chitosan, significantly improved their growth. This was evident in increased plant height, branching, both fresh and dry weight, leaf number, chlorophyll content, and leaf area. The combined treatment consistently outperformed other treatments in both growing seasons. The second season generally showed better growth compared to the first, suggesting continued application could lead to further improvements. Overall, the results highlight the effectiveness of using specific biostimulants combinations to optimize pepper plant performance and productivity.

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