



Impact of Actiwave Biostimulant on Physiological Traits, Nutrient Content and Yield of Tomato (*Solanum lycopersicum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Nowadays, seaweeds are more and more being utilized as plant biostimulants for the improvement of plant performance. Actiwave is a biostimulant product extracted from seaweed *Ascophyllum nodosum* and three main components of Actiwave are betaines, alginic acid and caidine. Hence, the objective of the present study is to evaluate the impact of Actiwave on the growth, physiology, nutrient uptake and yield of tomato under open field conditions. The field experiments were carried out at Eastern Block Farm, Tamil Nadu Agricultural university, Coimbatore during December 2021 – April 2022 (Season I) and June 2022 – October 2022 (Season II). The Actiwave biostimulant applied in three doses at the rate of 5.0 L ha⁻¹ (Act 1), 10.0 L ha⁻¹ (Act 2), 20.0 L ha⁻¹ (Act 3) into the soil at 45 and 60 days after transplanting. The Actiwave treatments showed a positive influence on plant performance, however, the most significant results were observed in

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Actiwave 10.0 L ha^{-1} (Act 2), which enhanced the SPAD value, photosynthetic rate, total dry matter production, leaf and stem nutrient content (N, P, K), average fruit weight, fruit yield per plant and yield per hectare of tomato. Hence, the use of Actiwave on tomato could be an effective way to boost production sustainably.

Keywords: Actiwave; *A. nodosum*; tomato; growth; nutrient content; yield.

1. Introduction

Biostimulants are substances and/or microorganisms that contain various bioactive components viz., amino acids, vitamins, humic acids, phytohormones, protein hydrolysates, yeast and seaweed extracts, etc.; applied to the crop as foliar spray or soil drenching. These biostimulants stimulate the nutrient uptake from the soil, nutrient use efficiency of crops and enhance the growth and metabolic activities of plants to improve crop quality and yield; it also gives a greater degree of tolerance to plants to a variety of abiotic stresses (Du Jardin et al., 2015; Roupheal et al., 2018).

Actiwave a biostimulant, which improves nutrient uptake by roots and it imparts tolerance against salt and drought stress (Spinelli et al., 2010). The Actiwave biostimulant was extracted from brown seaweed algae *Ascophyllum nodosum*; seaweeds are multi cellular macroscopic organisms, rich in organic osmolytes, nutrients, vitamins, peptides, polysaccharides, growth hormones and amino acids, etc. (Spinelli et al., 2006; Meng et al., 2023; Shukla et al., 2019). In addition to seaweed, Actiwave also contains betaine, alginic acid and caidine, which supports the formulation's efficacy (Vernieri et al., 2006). Betaine is a compatible solute act as an osmoprotectant, helps to maintain better plant water relations, overcomes the ionic and osmotic effect of salt and drought stress (Huang et al., 2000); betaines also exhibit cytokinin like activity in plants (Blunden et al., 1996).

The alginic acid present in Actiwave works as a soil conditioner by combining with metallic radicals it produces cross-linked polymers, which can improve the water holding capacity of rhizosphere; this increased moisture content in soil facilitates a better environment for the growth of microorganisms that could results in more mineral nutrient uptake (Chen et al., 2003). Caidrine, a derivative of vitamin K1, stimulates the secretion of H^+ ions in apoplast that results in lower pH in the rhizosphere zone; acidification of the rhizosphere reduces Fe^{3+} into Fe^{2+} and increases ion uptake by roots (Luthje and Bottger, 1995).

The Actiwave was applied to many crops including strawberry (Spinelli et al., 2010), grapes, apple and pear (Spinelli et al., 2010), carrot (Taha et al., 2015), leafy vegetable rocket (Vernieri et al., 2005), lettuce (Karapouloutidou & Gasparatos, 2019) to improve growth, yield, nutrient uptake and crop quality. Tomato is a commercially important vegetable crop, plays a crucial role in human diet by providing antioxidants, minerals, and vitamins A, and C (Farooq et al., 2020). Protected cultivation of vegetable crops requires more quantity of pesticides and fertilizers, which degrades soil health. In this way, biostimulants are emerging agronomic tools that may a good option for sustainable agriculture to improve the growth and productivity of crops without disturbing the soil and environment (Yakhin et al., 2017). By keeping this in view we fixed our objective to analyse the biostimulation effects of *A. nodosum* containing biostimulant Actiwave on growth, physiology, nutrient content and yield of tomato crops.

2. Material and Methods

2.1 Plant Material and Experimental Design

Semi determinate hybrid tomato seeds (Shivam) were sowed in portrays containing 1: 3 ratios of vermicompost and coir pith media for growth. The 25 days old seedlings were transplanted into the experimental field with a spacing of 60 x 45 cm. The field experiments were conducted at Eastern Block Farm, Department of Agronomy, Tamil Agricultural University, Coimbatore during December 2021 – April 2022 (Season I) and June 2022 – October 2022 (Season II). The field is situated at 110° N latitude and 770° E longitude with an elevation of 426.7 m above mean sea level. The field trials were conducted with six replications and four treatments in Randomized Block Design (RBD). Physio-chemical properties of the experimental field soil were recorded as Clay loam; pH of 7.57; electrical conductivity 0.66 (EC, ds m⁻¹), 0.69 % soil organic carbon (SOC), 275 kg available Nitrogen ha⁻¹; 38 kg of available phosphorous ha⁻¹; 975 kg available potassium ha⁻¹. The plot size is

15 m² and approximately 50 plants were maintained in each replication. The crop was irrigated at every 7 days interval based on its requirement and the basal fertilizer dose 50:250:100 kg ha⁻¹ of NPK, 50 kg ha⁻¹ of zinc sulphate were given. Top dressing of N and K each @ 50 kg ha⁻¹ was applied at 30, 45, and 60 days after transplanting (DAT).

2.2 Biostimulant Treatment

The Actiwave biostimulant was obtained from M/s. Valagro Bio Sciences Ltd., Hyderabad. The Actiwave is extracted from seaweed *Ascophyllum nodosum* and its major components are betaine, alginic acid, and caidine, a derivative of vitamin K1. The treatments control (CT), Actiwave 5.0 L ha⁻¹ (Act 1), Actiwave 10.0 L ha⁻¹ (Act 2), Actiwave 20.0 L ha⁻¹ (Act 3), were given as two applications i.e., 45 and 60 days after transplanting (DAT). The biostimulant solution was prepared by mixing 7.50 mL (Act 1), 15.00 mL (Act 2) and 30.00 mL (Act 3) of Actiwave in 5 L of water and 100 mL of prepared solution was given to each plant as soil drenching.

2.3 Sampling and Analysis

The physiological parameters were observed at two stages i.e., 10 days after first application (DAFA) and 10 days after second application (10 DASA) of Actiwave. The SPAD (Soil Plant Analysis Development) value and photosynthetic rate were measured from three plants (Three leaves from each plant) in each replication. The SPAD value was determined by using portable chlorophyll meter (soil plant analytical development) SPAD Model 5020 Minolta (Konica Minolta, INC, Tokyo, Japan). The photosynthetic rate was measured by using a portable photosynthesis system (PPS; LI-6400 XT, Licor Inc., Lincoln, NE, USA). The readings were taken on a clear sunny day between 10:00 a.m. to 12:00 noon and it was expressed as $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The plant samples from each replication were collected and dried in shade for 2 days then in a hot air oven for 48 h at 80°C to measure dry matter production and it was expressed in g plant⁻¹.

The nutrient contents (N, P, K) in tomato plants were analysed at harvest stage. The total nitrogen (N; %) was analysed by micro kjeldahl digestion method by using diacid mixture (5:2 ratio of Sulphuric acid and perchloric acid) following the procedure of Jackson, (1973). The total Phosphorous (P; %) content was estimated by calorimetric method by using the procedure given by Jackson, (1973). Briefly, 0.5 g of dried sample was digested by using a triple acid mixture (Nitric acid, sulphuric acid and perchloric acid; 9:2:1 v/v) and used for analysis. The total potassium (K; %) content was analysed by using flame photometer following the principle of Jackson, 1973.

The yield traits were recorded by manual harvesting of uniformly ripened tomato fruits and the fruits were harvested weekly twice. The average fruit weight (g), and fruit weight per plant (kg) were recorded at each harvest and presented the average value, and the cumulative data were used to calculate the yield per hectare (tonnes).

2.4 Statistical Analysis

The experimental data for two seasons and each stage were separately analysed by using SPSS software (version 16.0), analysis of variance (One- way ANOVA) was performed for all the traits and the results were presented as mean with standard error. By using Duncan's multiple range test (DMRT), the mean values were ranked at p = 0.05 level. The data visualization by graphs was prepared by using GraphPad Prism (version 8.2.0).

3. Results and Discussion

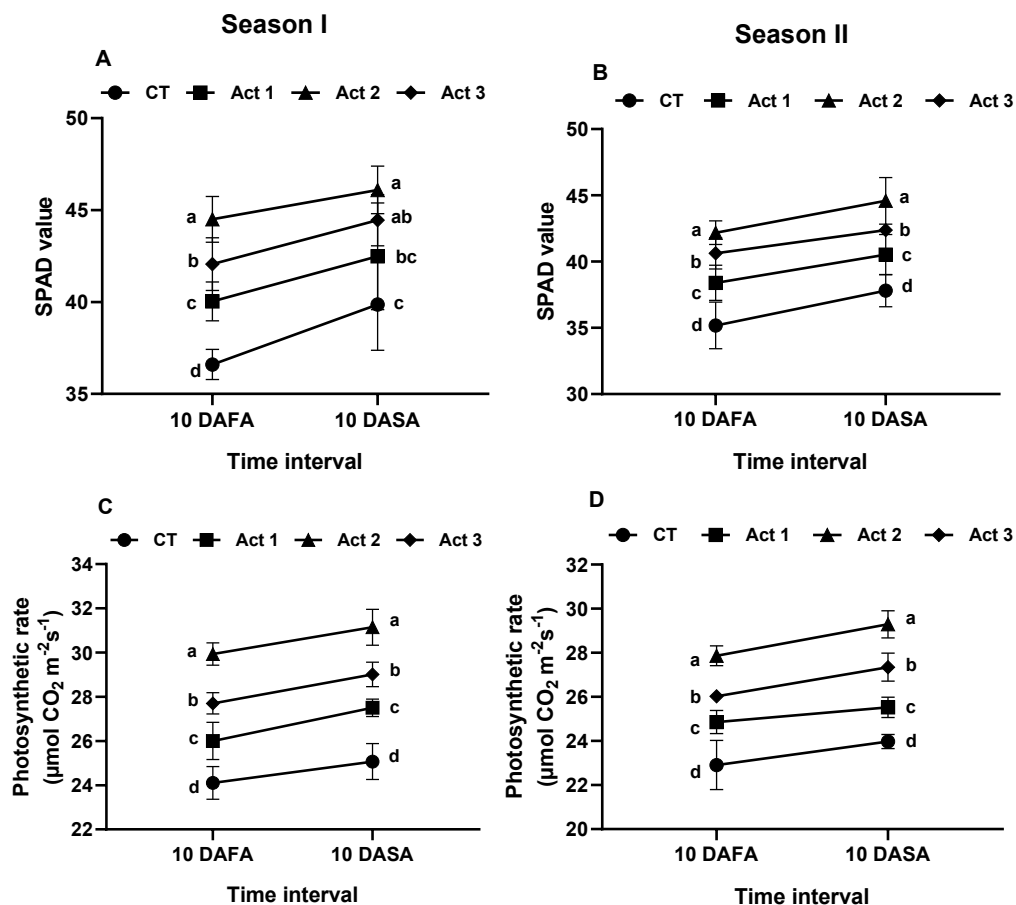
3.1 Effect of Actiwave on SPAD Value, Photosynthetic Rate and Total Dry Matter Production

The soil application of Actiwave at different doses significantly enhanced the SPAD value of tomato compared to the control across both seasons. Among the treatments, Actiwave at 10.0 L ha⁻¹ (Act 2) consistently recorded the highest SPAD values, indicating improved chlorophyll content. These findings are in close agreement with previous studies in strawberry (Spinelli et al., 2010) and leafy vegetables (Vernieri et al., 2005), where *Ascophyllum nodosum*-based biostimulants increased chlorophyll concentration. The enhancement in SPAD value observed in the present study may be attributed to improved iron uptake, which plays a critical role in chlorophyll biosynthesis, as well as the presence of betaines that facilitate nutrient assimilation (Blunden et al.,

1996). Similar improvements in chlorophyll content and SPAD values in tomato have been reported by Hussain et al., (2021), Della Lucia et al., (2022) and Subramaniyan et al., (2023), suggesting that seaweed extracts can delay chlorophyll degradation, regulate hormonal balance, and reduce leaf senescence.

The photosynthetic rate was also significantly influenced by Actiwave application, with the highest values recorded in Act 2 across both seasons. This increase in photosynthetic efficiency is consistent with earlier reports in tomato (Subramaniyan et al., 2023), broccoli (Kaluzewicz et al., 2017), and spinach (Castronuovo et al., 2023), where *A. nodosum* extracts enhanced carbon assimilation. The improved photosynthetic performance in the present study may be linked to increased stomatal conductance, better maintenance of cell turgor, and enhanced water relations mediated by betaines. Moreover, Santaniello et al., (2017) reported that seaweed biostimulants improve photosynthesis by protecting chloroplast membranes through antioxidant activity, thereby reducing photodamage and improving energy utilization. Compared to previous studies, the higher photosynthetic rates observed under field conditions in the present investigation further validate the effectiveness of Actiwave under practical agricultural environments.

Similarly, total dry matter production (TDMP) was significantly increased by Actiwave treatments, with the maximum values recorded under Act 2. These results corroborate earlier findings in strawberry (Spinelli et al., 2010), lettuce (Karapouloutidou & Gasparatos, 2019), and carrot (Taha et al., 2015), where seaweed-based biostimulants enhanced biomass accumulation. The increased dry matter production observed in this study can be attributed to improved nutrient uptake, enhanced photosynthetic activity, and better translocation of photoassimilates. Additionally, bioactive compounds present in *A. nodosum*, such as plant growth regulators, polysaccharides, and alginic acid, are known to stimulate root development, cell division, and metabolic activity (Khan et al., 2009; Shukla et al., 2019). Notably, the superior performance of the intermediate dose (10.0 L ha⁻¹) indicates that optimal concentration is critical for maximizing plant response, which aligns with previous reports suggesting that excessive biostimulant application may not proportionally enhance growth.



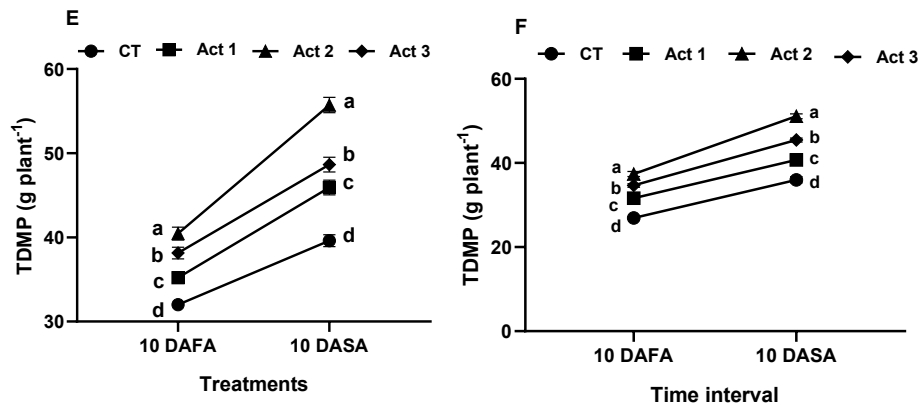


Fig. 1. Effect of Actiwave on SPAD value, photosynthetic rate and TDMP of tomato at 10 days after first and second application. A, B - SPAD value at season I and II; C, D - Photosynthetic rate at season I and II; E, F- TDMP (Total dry matter production); DAFA - Days after first application, DASA- Days after second application

3.2 Effect of Actiwave on Plant Nutrient Content (N, P and K) at Harvest Stage

The application of Actiwave significantly enhanced the plant nutrient content (N, P, and K) of tomato compared to the control across both seasons (Fig. 2). Among the treatments, Actiwave 10.0 L ha⁻¹ (Act 2) recorded the highest nutrient concentrations, followed by Act 3 and Act 1, indicating the superior efficiency of the intermediate dose in promoting nutrient acquisition and accumulation.

These findings are in close agreement with earlier studies in strawberry (Spinelli et al., 2010), lettuce (Lucini et al., 2015; Karapouloutidou & Gasparatos, 2019), carrot (Taha et al., 2015), and tomato (Dell'Aversana et al., 2021), where *Ascophyllum nodosum*-based biostimulants significantly enhanced plant mineral composition. The improved nutrient status observed in the present study can be attributed to enhanced root system architecture, including increased root length and surface area, which facilitates greater nutrient interception and absorption. In addition, biostimulants are known to improve nutrient solubilization in the rhizosphere and enhance nutrient use efficiency through better assimilation and translocation within the plant.

The presence of bioactive compounds such as betaines, alginic acid, and caidine in Actiwave plays a crucial role in regulating nutrient uptake processes. Betaines function as osmoprotectants that stabilize cell membranes and improve nutrient transport under varying environmental conditions. Alginic acid contributes to improved soil structure and moisture retention, thereby enhancing nutrient availability. Caidine has been reported to stimulate plasma membrane H⁺-ATPase activity, resulting in increased proton extrusion into the rhizosphere. This process creates an electrochemical gradient that facilitates the uptake of essential cations and enhances ion exchange capacity (Vernieri et al., 2006; Spinelli et al., 2010;).

Furthermore, Actiwave may positively influence rhizosphere microbial communities, which play a key role in nutrient cycling, mineralization, and solubilization of otherwise unavailable nutrients (Urashima et al., 2005; Colla et al., 2015). The synergistic interaction between improved root activity and microbial dynamics ultimately leads to enhanced nutrient uptake and accumulation in plants.

3.3 Effect of Actiwave on Yield Traits of Tomato

The application of Actiwave biostimulant significantly enhanced the yield attributes and overall productivity of tomato compared to the control across both seasons (Fig. 3). Among the treatments, Actiwave at 10.0 L ha⁻¹ (Act 2) recorded the highest average fruit weight (165.72 g and 158.57 g), fruit yield per plant (4.47 kg and 4.06 kg), and yield per hectare (29.98 and 24.26 t ha⁻¹) during Season I and Season II, respectively. This was followed by Actiwave 20.0 L ha⁻¹ (Act 3) and Actiwave 5.0 L ha⁻¹ (Act 1), indicating that the intermediate dose was more effective than both lower and higher concentrations.

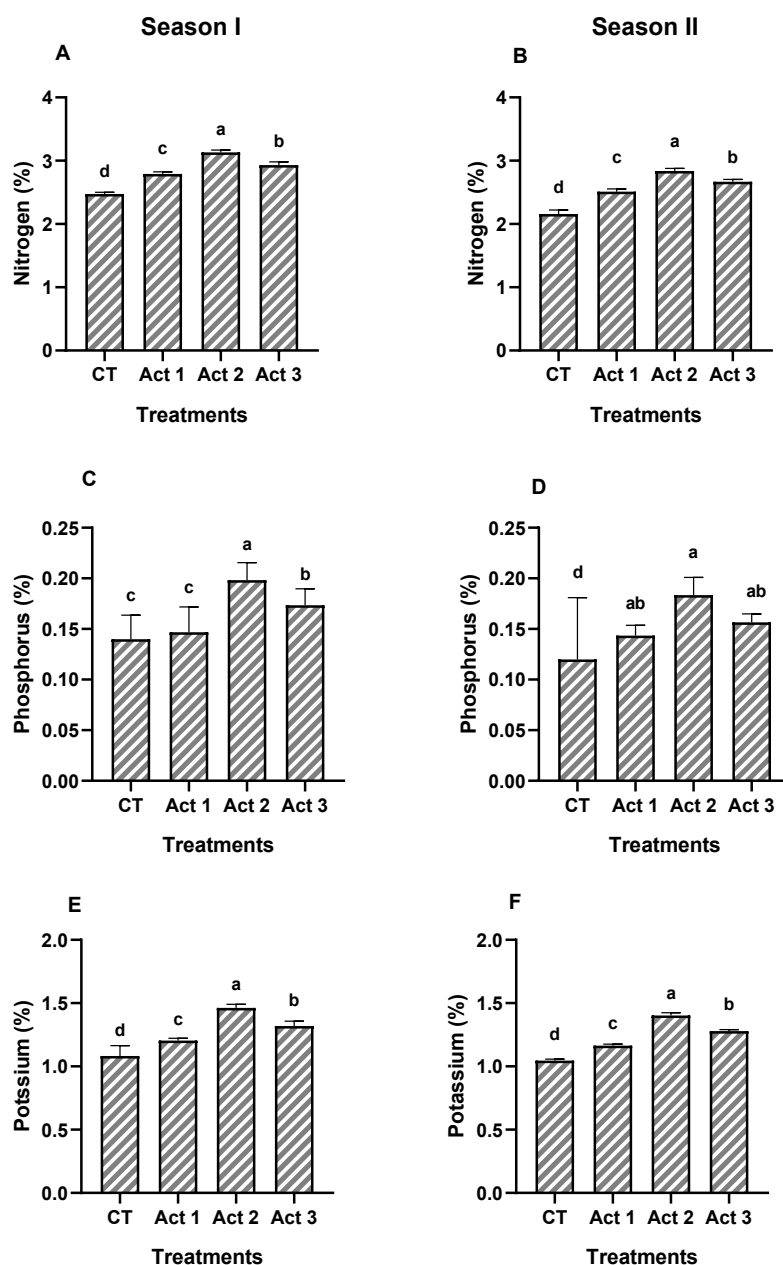


Fig. 2. Effect of Actiwave on plant nutrient content (N, P, K) of tomato. A, B - Nitrogen (N) content at season I and II; C, D - Phosphorus (P) content at season I and II ; E, F- Potassium (K) content at season I and II

The observed increase in yield and yield attributes is in agreement with earlier findings in strawberry (Spinelli et al., 2010), carrot (Taha et al., 2015), and rocket (Vernieri et al., 2005), where seaweed-based biostimulants significantly improved crop productivity. In tomato, several studies have reported similar enhancements in fruit weight and yield following the application of *Ascophyllum nodosum*-based products (Ali et al., 2016; Colla et al., 2017; Hussain et al., 2021; Subramaniyan et al., 2023; Di Mola et al., 2023). Compared to these reports, the present study provides robust field-level evidence across two seasons, further confirming the consistency and effectiveness of Actiwave under open field conditions.

The increase in yield observed in the present investigation can be attributed to improved physiological efficiency, enhanced nutrient uptake, and greater assimilate production, as reflected in higher SPAD values, photosynthetic rate, and total dry matter accumulation. In addition, seaweed-based biostimulants are known to

enhance nitrogen metabolism and nutrient use efficiency, thereby contributing to improved yield performance (Dell'Aversana et al., 2021). The presence of bioactive compounds such as polysaccharides, betaines, and alginic acid in Actiwave may stimulate endogenous plant hormones, particularly auxins and cytokinins, which promote cell division, fruit development, and sink strength (Ali et al., 2016; Colla et al., 2017; Kolečka et al., 2017).

Furthermore, seaweed extracts have been reported to improve rhizosphere microbial activity, facilitating better nutrient solubilization and uptake, which ultimately enhances plant growth and productivity (Sani et al., 2020; Gitau et al., 2022). The superior performance of the intermediate dose (10.0 L ha⁻¹) suggests that optimal application levels are critical for maximizing yield, as excessive doses may not proportionally enhance plant response. Overall, the present findings align well with previous literature and highlight the potential of Actiwave as an effective and sustainable strategy to improve tomato yield under field conditions.

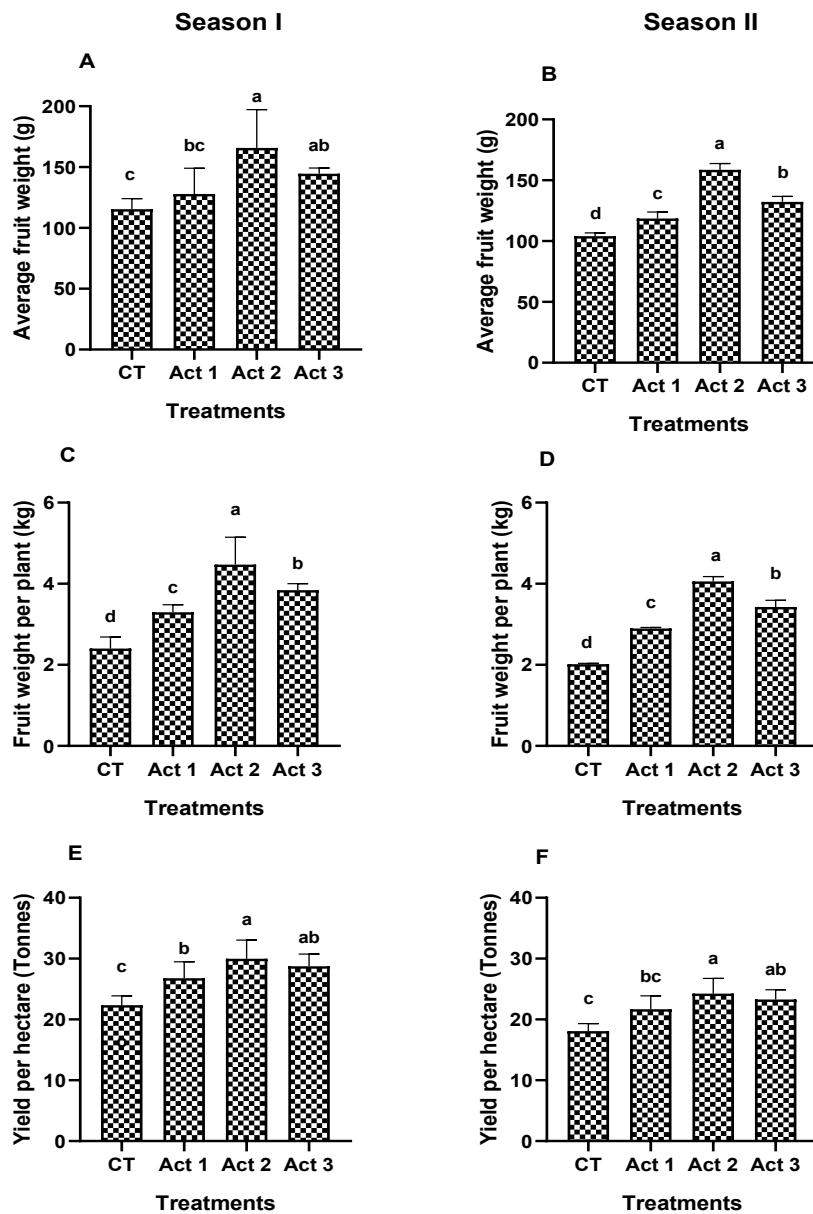


Fig. 3. Effect of Actiwave on yield and yield traits of tomato. A, B - Average fruit weight at season I and II; C, D- Fruit yield per plant at season I and II; E, F - Yield per hectare at season I and II

4. Conclusion

The present study demonstrated that soil application of Actiwave, a biostimulant enriched with seaweed extract, betaine, alginic acid, and caidine, markedly improved physiological attributes, increased plant nutrient uptake (N, P, K), and enhanced the yield characteristics of tomato. All three Actiwave treatments (Act 1, Act 2 and Act 3) improved the plant growth and yield compared to control but the treatment Act 2 (Actiwave 10.0 L ha⁻¹) performed better than the other two treatments in both season I and season II. However further research is required to know the molecular mechanism behind the nutrient uptake in Actiwave treatments, i.e., nutrient transporter genes in tomatoes. Hence, our study confirmed that the use of Actiwave biostimulant could be the best way to improve tomato production sustainably without polluting the environment.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Competing Interests

Authors have declared that no competing interests exist.

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