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Effects of Nanocarbon-Fortified Fish Hydrolysate on the Morphophysiological Responses of Chilli (*Capsicum annuum* L.) under Drought Stress

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ABSTRACT

Chilli (*Capsicum annuum* L.) is an economically important seasonal spice crop, and its productivity and harvest quality are highly constrained by drought stress under many cultivation conditions. This study aimed to evaluate the effectiveness of nanocarbon-enriched fish hydrolysate in enhancing drought tolerance in chilli plants. The MI2 chilli variety was cultivated in pots using a Randomized Complete Block Design with seven treatments and six replicates per treatment. Treatments included a commercial Albert nutrient solution (positive control I), an inorganic solid fertilizer (positive control II), fish hydrolysate alone at 2.5% (v/v), and enriched with carbon nanoparticles at concentrations of 25, 50, and 75 mg/L, and water as a negative control. Following transplanting, four foliar spray applications were applied at five-day intervals. After the fertilization phase, drought stress was imposed by withholding irrigation for seven consecutive days. Growth parameters, including plant height, leaf area, root length, root volume, and root dry weight, and physiological parameters such as chlorophyll content and leaf relative water content were recorded. Although positive control I exhibited the highest plant height (16.42 cm) and leaf area (10.92 cm^2), nanocarbon-enriched fish hydrolysate treatments at 50 mg/L and 75 mg/L displayed higher root length, root volume, root dry weight, chlorophyll content, and relative water content compared to both positive controls and fish hydrolysate alone, demonstrating that nanocarbon incorporation improves the efficacy of fish hydrolysate. Therefore, nanocarbon-enriched fish hydrolysate could be recommended as a cost-effective, environmentally friendly, and sustainable strategy under limited water availability in chilli cultivation.

Keywords: Carbon nanoparticles, Chilli, Drought stress, Drought tolerance, Fish hydrolysate

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INTRODUCTION

Chilli (*Capsicum annuum* L.) is a widely cultivated spice and vegetable crop of significant economic importance belonging to the family Solanaceae. It is valued for its rich content of vitamins and bioactive compounds, which confer antioxidant, anti-inflammatory, and antimicrobial properties (Bal *et al*., 2022). Despite its importance, global agricultural productivity is increasingly constrained by abiotic stresses, with drought being one of the most critical factors limiting crop growth and yield worldwide (Begna, 2020). Chilli is particularly susceptible to drought stress during its vegetative and flowering stages, resulting in reductions in leaf relative water content, chlorophyll fluorescence, stomatal conductance, photosynthetic rate, transpiration, and various growth parameters such as root and shoot length, stem thickness, and leaf area (Lestari *et al*., 2023; Molla *et al*., 2023).

Several strategies have been employed to mitigate the adverse effects of drought stress, including conventional breeding approaches (Momo *et al*., 2022) and the application of synthetic fertilizers (Ichwan *et al*., 2021). However, sustainable, cost-effective, and farmer-friendly alternatives are increasingly sought. Among these, foliar application of fish protein hydrolysate has been shown to enhance growth and productivity in various crops under stress conditions. For instance, Al-Malieky and Jerry (2019) reported that fish hydrolysate significantly improved lettuce growth under salinity stress, while Nuzhyna *et al*. (2024) demonstrated its effectiveness in enhancing germination and growth and mitigating yield losses in bean and corn under low-temperature stress.

Nanotechnology also offers promising solutions for improving plant stress tolerance. Application of soluble carbon nanoparticles has been reported to enhance seed germination, lateral root development, and chlorophyll content under salinity stress in lettuce (Baz *et al*., 2020). Similarly, foliar treatment with carbon nanoparticles reduces drought-induced damage in bell pepper roots and improves fruit production at intermediate concentrations (Ahmadi *et al*., 2025), while carbon nanotubes enhance growth, photosynthesis, nutrient uptake, and yield in peanut under drought conditions (Bakry *et al*., 2024).

Despite these advances, information on the combined use of carbon nanoparticles and fish hydrolysate in chilli under drought stress remains limited. Therefore, this study aimed to investigate the synergistic effect of carbon nanoparticles and fish hydrolysate on improving drought tolerance in chilli, compared to the individual application of fish hydrolysate and conventional inorganic fertilizers.

MATERIALS AND METHODS

Experimental Site

The study was conducted under a poly-tunnel of the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila, from February to June 2025.

Synthesis of Nanocarbon

Carbon nanoparticles were synthesized using the combustion method, a simple, efficient, and cost-effective technique. Coconut oil was used as the carbon source and placed in an oil lamp fitted with a combustible wick. The lamp was allowed to stand for several minutes to ensure adequate absorption of oil by the wick before ignition. The wick was then ignited and allowed to burn steadily, and an inverted ceramic plate was positioned above the flame to collect the carbon soot generated during combustion, following the method described by Muhsan *et al*. (2019). The synthesis process was conducted under open-air laboratory conditions. The deposited carbon soot derived from coconut oil was carefully scraped from the plate and initially washed with tap water, followed by repeated washing with distilled water to remove impurities and unwanted residues. The collected carbon particles were subsequently oven-dried at approximately 120°C for more than 12 hours to obtain dry carbon nanoparticles (Jayaprakasan *et al*., 2013).

Preparation of Nano Solution

Carbon nanoparticles were dispersed in methanol using a sonicator bath to obtain a homogenous suspension (Ahmadi *et al*., 2025). Nanocarbon-methanol mixture was added to 200 mL of distilled water, and methanol was removed using a rotary evaporator. The resulting nanocarbon solution was used as the stock solution for foliar fertilizer formulation.

Determination of Particle Size Distribution

The particle size distribution of nanocarbon particles in the prepared nanocarbon solution was determined using a particle size analyser (ANALYSETTE 22 NanoTec plus).

Nursery Establishment

MI 2 chilli seeds treated with fungicide were sown in a seed tray filled with sand and compost in a 1:1 ratio. The tray nursery was maintained regularly until the seedlings were transplanted.

Experimental Layout

The experiment was laid out in a Randomized Complete Block Design (RCBD), applying seven treatments with six replicates for each treatment. All seven treatments are outlined in Table 1.

Crop Establishment

Twenty-one-day-old healthy seedlings were transplanted into pots containing soil: sand: compost in the ratio of 1:1:1.

Table 1. Tested Fertilizer Formulations

Treatment (T)	Fertilizer Combinations and Concentrations
T1	25 mg/L Nanocarbon + 2.5% Fish Hydrolysate (Foliar Application)
T2	50 mg/L Nanocarbon + 2.5% Fish Hydrolysate (Foliar Application)
T3	75 mg/L Nanocarbon + 2.5% Fish Hydrolysate (Foliar Application)
T4	2.5% Fish Hydrolysate (Foliar Application)
T5	DOA Recommended Dosage of Inorganic Fertilizers - Positive control II (Soil Application)
T6	Recommended Dosage of Albert – Positive Control I (Foliar Application)
T7	Control (Without Fertilizer, only water)

DOA – Department of Agriculture

Application of Fertilizer Treatments

Preparation of Nanocarbon-Based Foliar Fertilizer

Three nanocarbon-based foliar fertilizer treatments were formulated using fish hydrolysate and varying concentrations of nanocarbon. For Treatment 1 (T1), 25 mL of fish hydrolysate and 25 mL of nanocarbon stock solution (containing 25 mg nanocarbon) were added to a beaker and diluted to a final volume of 1 L with distilled water. For Treatment 2 (T2), 25 mL of fish hydrolysate and 50 mL of nanocarbon stock solution (containing 50 mg nanocarbon) were diluted to 1 L. For Treatment 3 (T3), 25 mL of fish hydrolysate and 75 mL of nanocarbon stock solution (containing 75 mg nanocarbon) were diluted to 1 L.

The foliar fertilizers prepared for T1, T2, and T3 were applied only to plants assigned to their respective treatments. Applications were carried out four times at five-day intervals following transplanting. A volume of 5 mL of foliar fertilizer solution was applied per plant during each application.

Preparation and Application of Fish Hydrolysate Treatment

Fish hydrolysate was purchased from Mihilak Fertilizer Company, Western Province, Sri Lanka. Based on the manufacturer's product specifications, the fertilizer contained nitrogen (>1000 mg 100 mL^{-1}), phosphorus (>1000 mg 100 mL^{-1}), and potassium (>3000 mg 100 mL^{-1}).

For Treatment 4 (T4), a 2.5% fish hydrolysate solution was prepared by adding 25 mL of fish hydrolysate to a beaker and making the volume up to 1 L with distilled water. The solution was applied only to plants assigned to T4. Similar to the nanocarbon-based foliar treatments, 5 mL of solution per plant was applied four times at five-day intervals following transplanting.

Application of Department of Agriculture Recommended Inorganic Fertilizer

For Treatment 5 (T5), inorganic fertilizers were applied according to the recommendations of the Department of Agriculture (DOA), Sri Lanka. Urea, triple superphosphate (TSP), and muriate of potash (MOP) were used as fertilizer sources.

As a basal application, 0.0636 g of TSP (equivalent to 100 kg ha^{-1}) and 0.0318 g of MOP (equivalent to 50 kg ha^{-1}) were applied to each pot, supplying 0.0293 g of phosphorus (P) and 0.0191 g of potassium (K), respectively.

Nitrogen was supplied through split applications of urea. At two weeks after crop establishment, 0.0636 g of urea (equivalent to 100 kg ha^{-1}) was applied to each pot, supplying 0.0293 g of nitrogen (N). At four weeks after establishment, 0.0795 g of urea (equivalent to 125 kg ha^{-1}) was applied, providing 0.0366 g N per pot. At eight weeks after establishment, 0.0795 g of urea and 0.0318 g of MOP were applied, supplying 0.0366 g N and 0.0191 g K per pot. Additional applications of 0.0795 g of urea were made at 12 and 16 weeks after crop establishment, each supplying 0.0366 g N per pot.

Preparation and Application of Albert's Solution

The Albert's fertilizer solution was prepared according to the manufacturer's recommendation by dissolving 2.2 g of Albert's fertilizer (manufactured by Grow More Inc., USA) in 1 L of water.

For Treatment 6 (T6), the prepared Albert's solution was applied only to plants assigned to this treatment. A volume of 5 mL per plant was applied four times at five-day intervals following transplanting. Each application supplied approximately 0.011 g of Albert's fertilizer per plant.

Control Treatment

Treatment 7 (T7) served as the control treatment. No fertilizer was applied to plants in this treatment. Plants were supplied only with water according to their growth requirements.

After completion of all foliar treatments, irrigation was withheld for seven consecutive days to induce drought stress.

Data Recording

Data were recorded between each foliar application and after the drought stress period.

Growth Parameters

Five growth parameters were assessed, namely leaf area, plant height, root length, root volume, and root dry weight.

Physiological Parameters

Chlorophyll content was measured both during the fertilization period and after the imposition of drought stress. Leaf relative water content (RWC) was determined during the drought stress period following the method described by Chakma *et al*. (2021).

Statistical Analysis

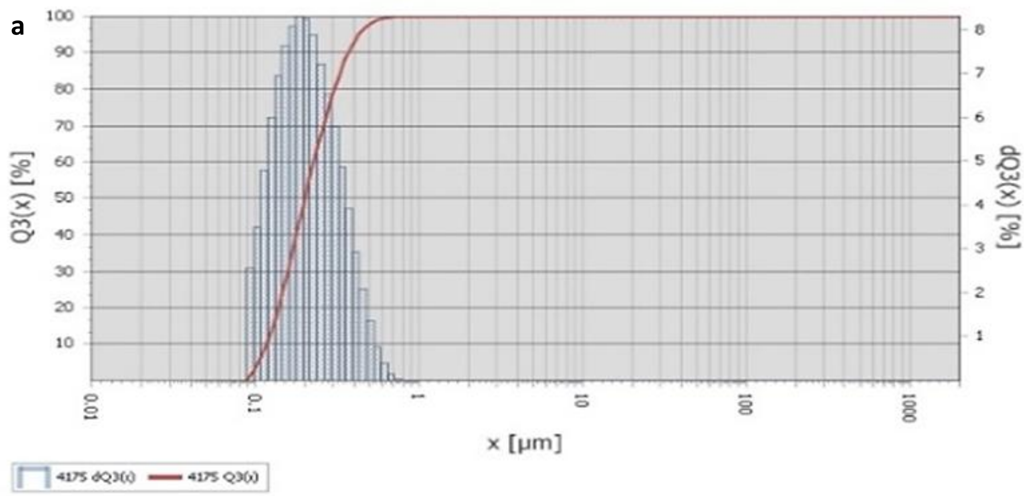
Data were analysed using the Analysis of Variance (ANOVA) and group means were compared by the Least Significant Test using R statistical software (version 4.2.1).

RESULTS AND DISCUSSION

Particle Size Distribution

Sizes of particles in the nano solution ranged from below 100 nm to around 400 nm. The majority of particles were distributed between 100 nm and 300 nm. The volume-based frequency distribution ($Dq_3(x)$) was peaked at 0.19 µm (Figure 1).

a



Mode 0.19 µm Span (d90-d10)/d50 1.25 D[4,3] 0.3 µm

b

Q3(x) [%]	x [µm]
10	0.1
20	0.1
30	0.2
40	0.2
50	0.2
60	0.2
70	0.3
80	0.3
90	0.4

Figure 1. Particle size distribution of prepared nanocarbon solution: a: Graphical representation of particle size distribution, b: Particle size statistics

Effect of Fertilizer Treatments on Morphological Growth Parameters

Plant Height

The results indicated that plant height differed significantly ($p < 0.05$) among treatments (Table 2). The greatest mean plant height was recorded in T6 (Albert foliar fertilizer), with a value of 16.42 cm. However, treatments T2 and T3, which received fish hydrolysate enriched with nanocarbon at 50 mg/L and 75 mg/L, respectively, also exhibited comparatively high plant heights (14.58 cm and 14.32 cm, respectively), showing no significant difference from several other treated groups. These results indicate that, although Albert fertilizer promoted the highest plant height, nanocarbon-incorporated fish hydrolysate treatments were able to maintain plant height at levels comparable to the commercial fertilizer under drought stress conditions. No significant differences were observed among T1, T2, T3, T4, and T5, further suggesting the effectiveness of nutrient supplementation in supporting vegetative growth.

Similar findings were reported by Perera *et al*. (2022), who observed significantly greater plant height in chilli plants treated with Albert fertilizer compared to compost tea from three to ten weeks after transplanting. In contrast, the lowest mean plant height was recorded in T7, which received only water, highlighting the importance of external nutrient inputs for sustaining plant growth under drought stress. Overall, the results demonstrate that nanocarbon-enriched fish hydrolysate has considerable potential to support maintaining the height in chilli plants, approaching the effectiveness of conventional foliar fertilizers during water-limited conditions.

Leaf Area

The mean leaf area differed significantly ($p < 0.05$) among treatments (Table 2). T6 (Albert foliar fertilizer) exhibited the highest leaf area (10.92 cm^2), which was significantly greater than all other treatments. Among the nanocarbon-enriched fish hydrolysate treatments, T2 (50 mg/L nanocarbon) produced the next highest leaf area (9.01 cm^2), which was significantly greater than fish hydrolysate alone but not statistically different from T1 (8.14 cm^2) and T3 (7.85 cm^2). In contrast, T5 and T7 recorded the lowest leaf areas, indicating limited leaf development under drought stress.

The results suggest that, while conventional Albert fertilizer supports maximal leaf expansion, nanocarbon-incorporated fish hydrolysate can also maintain comparatively high leaf area under water-limited conditions. Maintaining a larger leaf area under drought stress is important for sustaining photosynthetic capacity, improving light interception, and potentially enhancing yield, as commonly reported in crops experiencing limited water availability (Zhang *et al*., 2025). Therefore, the application of nanocarbon-enriched fish hydrolysate not only mitigates the negative effects of drought on leaf growth but also supports physiological functions that are critical for crop productivity under stress conditions.

Root Length

Root length differed significantly ($p < 0.05$) among treatments (Table 2). The longest roots were observed in T2 (fish hydrolysate with 50 mg/L nanocarbon), indicating superior root development compared to all other treatments. T2 root length was significantly higher than T4, T5, and T7, and slightly higher, though not significantly different, from T1, T3, and T6 (Albert foliar fertilizer). All nanocarbon-enriched fish hydrolysate treatments (25, 50, and 75 mg/L), as well as the Albert fertilizer (T6), demonstrated enhanced root length relative to fish hydrolysate alone and the unfertilized control (T7), which exhibited the lowest mean root length (11.63 cm). Enhanced root development is critical under drought stress, as longer roots enable plants to access deeper soil water, maintain nutrient uptake, and sustain physiological functions, thereby contributing to drought tolerance (Molla *et al*., 2021).

The results indicate that incorporating nanocarbon into fish hydrolysate is particularly effective in promoting root elongation compared to fish hydrolysate alone, with the 50 mg/L concentration showing the most pronounced effect. This suggests that nanocarbon-enriched hydrolysate is a promising foliar fertilizer strategy for improving root system architecture and enhancing drought resilience in chilli cultivation.

Root Weight

Root dry weight differed significantly ($p < 0.05$) among treatments (Table 2). The highest root dry weights were observed in T2 (fish hydrolysate with 50 mg/L nanocarbon, 0.30 g), T3 (75 mg/L nanocarbon, 0.29 g), and T6 (Albert foliar fertilizer, 0.29 g), with no significant difference among these treatments. These results indicate that the 50% and 75% nanocarbon-enriched fish hydrolysate treatments produced root biomass comparable to the commercially available Albert fertilizer, demonstrating their potential to support root development under drought stress. In contrast, T1, T4, T5, and T7 exhibited significantly lower root dry weights. Among the nanocarbon treatments, T1 showed significantly lower root biomass than T2 and T3, highlighting the contribution of nanocarbon to enhanced root growth. During drought, increased root dry weight is a vital trait; greater biomass improves anchorage, water and nutrient uptake, and overall resilience, ensuring plant survival and continued physiological function (Wasaya *et al*., 2018).

These findings suggest that nanocarbon incorporation into fish hydrolysate not only improves root dry matter accumulation but also provides a practical strategy to enhance drought tolerance in chilli cultivation, with 50% and 75% concentrations showing performance close to conventional foliar fertilizers.

Root Volume

Root volume differed significantly ($p < 0.05$) among treatments (Table 2). The highest root volumes were observed in T3 (fish hydrolysate with 75 mg/L nanocarbon, 4.25 cm^3) and T2 (50 mg/L nanocarbon, 3.50 cm^3), which were significantly greater than those in T1, T4, T5, T6, and T7. The lowest root volume was recorded in T7 (unfertilized control). These results indicate that the application of nanocarbon-enriched fish hydrolysate, particularly at 50% and 75% concentrations, substantially enhanced root volume compared to fish hydrolysate alone and even outperformed the inorganic liquid fertilizer (T6), which is commonly recommended by the Department of Agriculture for root performance. Increased root volume contributes to drought tolerance, as seedlings with denser and more developed root systems can access deeper soil moisture, maintain cell turgidity, and sustain physiological activity under water-limited conditions (Molla et al., 2021).

Table 2. Mean values of growth parameters

Trt	Plant Height (cm) 7DADI	Leaf Area (cm^2) 7DADI	Root Length (cm) 7DADI	Root Volume (cm^3) 7DADI	Root Dry Weight (g) 7DADI
T1	14.07 ± 0.28^{b}	8.14 ± 0.61^{bc}	18.00 ± 1.03^{ab}	2.42 ± 0.20^{c}	0.19 ± 0.01^{b}
T2	14.58 ± 0.42^{b}	9.01 ± 0.70^{b}	20.25 ± 0.31^{a}	3.50 ± 0.22^{ab}	0.30 ± 0.03^{a}
T3	14.32 ± 0.46^{b}	7.85 ± 0.55^{bc}	18.33 ± 0.84^{ab}	4.25 ± 0.36^{a}	0.29 ± 0.02^{a}
T4	13.83 ± 0.63^{b}	7.00 ± 0.44^{c}	17.10 ± 0.38^{b}	3.33 ± 0.33^{b}	0.23 ± 0.01^{b}
T5	13.60 ± 0.34^{b}	4.84 ± 0.45^{d}	16.12 ± 0.78^{b}	1.92 ± 0.08^{cd}	0.12 ± 0.01^{c}
T6	16.42 ± 0.43^{a}	10.92 ± 0.51^{a}	18.12 ± 1.10^{ab}	3.33 ± 0.33^{b}	0.29 ± 0.01^{a}
T7	12.17 ± 0.36^{c}	4.05 ± 0.30^{d}	11.63 ± 1.43^{c}	1.33 ± 0.21^{d}	0.11 ± 0.01^{c}

DADI- Days After Drought Induction. Within columns, mean values followed by different superscript letters are significantly different ($p < 0.05$)

Effect of Fertilizer Treatments on Physiological Parameters

Chlorophyll Content (SPAD)

The mean chlorophyll content of chilli leaves, expressed as SPAD values, ranged from 29.57 to 33.78 across treatments. Although the differences were not statistically significant ($p > 0.05$), the highest chlorophyll content was observed in T3 (fish hydrolysate with 75 mg/L nanocarbon, 33.78) and T2 (50 mg/L nanocarbon, 33.43), followed by T6 (Albert foliar fertilizer, 31.90). The lowest chlorophyll content was recorded in the unfertilized control (T7, 29.57) (Figure 2). Maintenance of higher chlorophyll content under drought stress is critical, as it reflects sustained photosynthetic capacity, efficient light capture, and continued production of assimilates necessary for growth and yield under water-limited conditions (Wang *et al*., 2025). The results indicate that nanocarbon-enriched fish hydrolysate, particularly at 50% and 75% concentrations, effectively preserves chlorophyll levels under drought stress, performing comparably or better than conventional commercial fertilizers, and thus may contribute to improved physiological resilience in chilli plants.

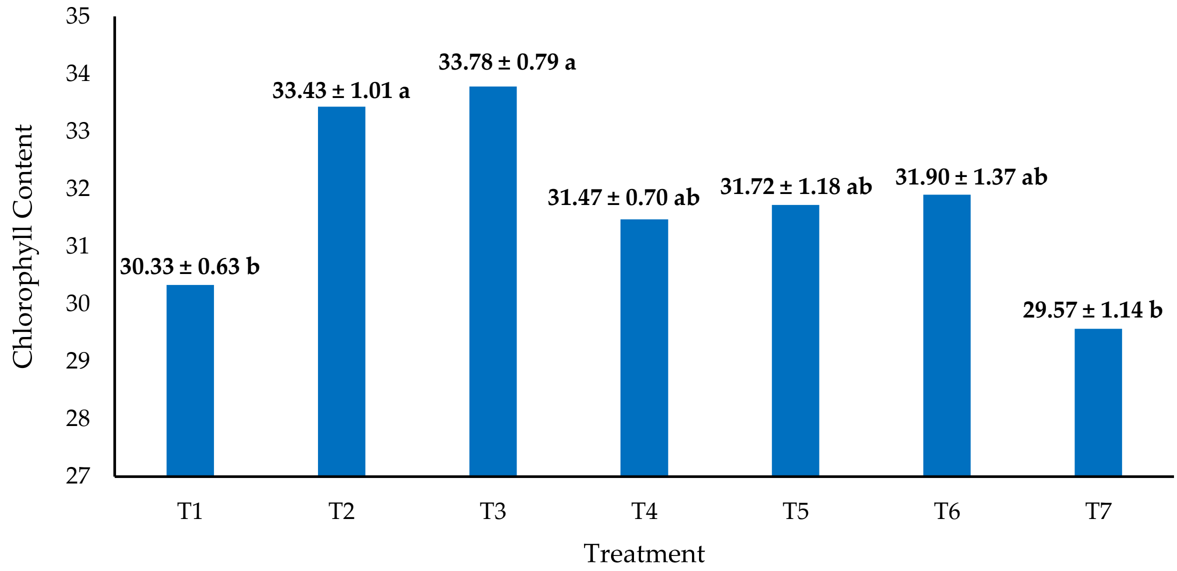


Figure 2. Average chlorophyll content 7 days after drought induction

Leaf Relative Water Content Percentage

Analysis of leaf relative water content (RWC) revealed a significant difference ($p < 0.05$) among treatments. The highest RWC values were recorded in T3 (fish hydrolysate with 75 mg/L nanocarbon, 54.23%), T2 (50 mg/L nanocarbon, 50.47%), and T6 (Albert foliar fertilizer, 49.50%), with no significant difference among these treatments (Figure 3). In contrast, T5 and T7 exhibited significantly lower RWC values compared to the other treatments.

Leaf RWC is a critical physiological indicator under drought stress, as it reflects the water status of the plant, turgor maintenance, and the ability to sustain metabolic and photosynthetic activities under limited water availability (Priya *et al*., 2023). The results indicate that nanocarbon-enriched fish hydrolysate at 50% and 75% concentrations effectively preserved leaf water content, comparable to the commercial Albert fertilizer, thereby enhancing drought tolerance in chilli plants.

These findings suggest that maintaining higher RWC through nanocarbon incorporation into foliar fertilizer formulations can mitigate the adverse effects of water deficit and support plant growth and productivity under drought conditions.

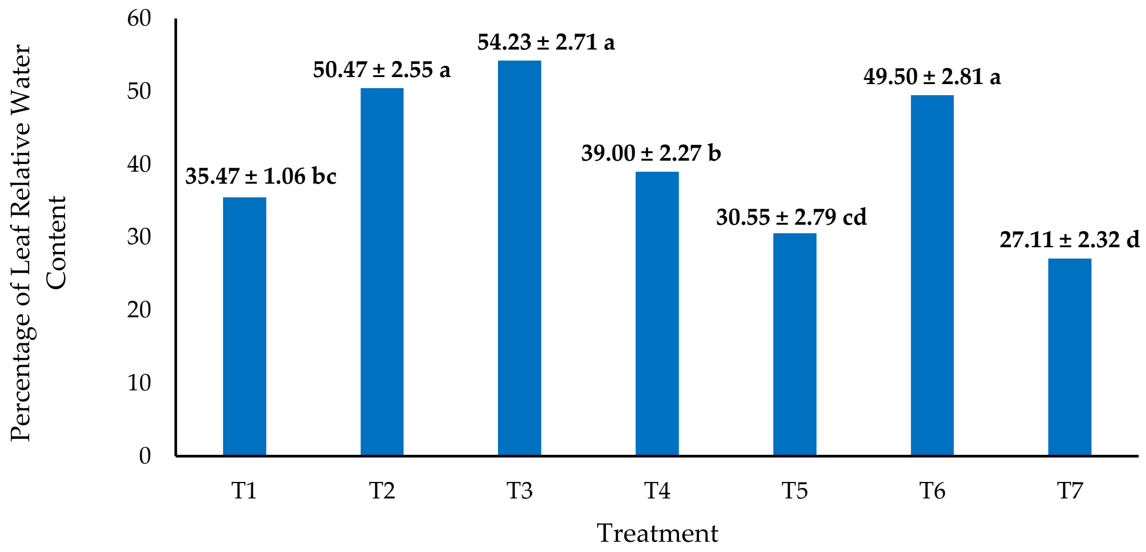


Figure 3. Average leaf relative water content percentage 7 days after drought induction

CONCLUSION

The findings of the present study clearly show that both nanocarbon-incorporated hydrolysate treatments and Albert fertilizer significantly enhance the growth and physiological performance of chilli plants compared to the other treatments tested. Notably, the fertilizers enriched with nanocarbon demonstrated high effectiveness over the use of fish hydrolysate alone.

Among the various nano-hydrolysate formulations, concentrations of 50 mg/L and 75 mg/L nanocarbon-combined fish hydrolysate proved particularly beneficial, especially in enhancing the plants' ability to withstand drought conditions. This may be due to the fact that fish hydrolysate itself contains amino acids, peptides, micronutrients, and organic compounds that act as biostimulants. The addition of nanocarbon particles may further enhance nutrient absorption, water-use efficiency, and stress signalling, helping plants tolerate drought more effectively.

Based on these results, the study recommends the use of nanocarbon-incorporated fish hydrolysate fertilizers as a promising approach to reduce the negative impacts of drought stress in crop production. This type of fertilizer offers an eco-friendly, cost-effective, and sustainable alternative that can support healthier plant growth while contributing to more resilient agricultural systems.

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