
Responses of drought-stressed hot pepper to seaweed extract application: Agronomic and physiological perspectives

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Abstract A significant interaction between field capacity (FC) and seaweed extract (LSE) was observed for total chlorophyll content, with the highest level (4.74 mg/g tissue) in 0% LSE under 75% FC and the lowest (1.73 mg/g tissue) in 3.75% LSE under the same FC. While FC significantly affected growth and physiological traits, it had minimal impact on yield. Fruit weight and length varied slightly across FC levels: 100% FC (2.78 g & 11.79 cm), 75% FC (3.12 g & 13.41 cm), 50% FC (2.63 g & 12.46 cm), and 25% FC (3.12 g & 13.24 cm). In contrast, yield variables based on LSE treatment—fruit weight per plant and number of fruits per plant—showed moderate differences, with 0% LSE producing 17.51 g and 6.37 fruits, 1.25% LSE 19.95 g and 6.61 fruits, 2.5% LSE 17.98 g and 6.61 fruits, 3.75% LSE 22.09 g and 7.11 fruits, and 5% LSE 17.37 g and 6.52 fruits.

Keywords: Agro-physiological, Biostimulant, Field capacity, Hot pepper, Seaweed extracts

Introduction

Drought stress, intensified by climate change, is among the most detrimental abiotic factors constraining agricultural productivity, leading to substantial yearly yield losses (Ahluwalia *et al.*, 2021; Zhang *et al.*, 2022). It is recognised as one of the most severe limitations to crop cultivation, affecting vast farming areas and altering key metabolic processes (Jekabsone *et al.*, 2022). Agricultural drought, intensified by water scarcity and rising food demand from population growth, places mounting strain on global farming systems (O’Connell *et al.*, 2017). Its adverse effects on plant growth and metabolism stem from interconnected physiological disruptions. Reduced soil moisture lowers turgor and structural stability, often causing wilting and growth inhibition (Haworth *et al.*, 2022). Stomatal closure conserves water but restricts photosynthesis by

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limiting CO₂ uptake (Ojuederie *et al.*, 2019), while reduced assimilation disrupts carbon balance and energy production critical for metabolism (Aroca, 2012).

Moreover, stomatal closure under drought conditions enhances the buildup of reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂) and superoxide (O₂⁻), which disrupt photosynthetic processes and further limit CO₂ assimilation in leaves (Oukaltouma *et al.*, 2022; Bondok *et al.*, 2022; Seleiman *et al.*, 2021). Plants mitigate drought stress by accumulating osmolytes—such as sugars, proline, and polyols—that preserve osmotic balance, protein stability, and membrane integrity. Concurrently, signalling of abscisic acid (ABA) regulates stress responses by promoting stomatal closure, activating defence genes, and modulating metabolism (Sachdev *et al.*, 2021; Kumar *et al.*, 2018). Drought-induced reductions in electron transport lower photosynthetic efficiency, leading to decreased growth, biomass, and resource uptake, which ultimately reduce crop productivity and water-use efficiency (Ouhaddou *et al.*, 2023; Farooq *et al.*, 2019; Mouradi *et al.*, 2018).

Various technologies and management strategies have been introduced to alleviate the adverse impacts of drought on plant growth and development. These include genetic modification, biochar application, plant growth regulators, beneficial microorganisms, fertilisers, seed priming techniques, and seaweed-derived extracts (Hussain *et al.*, 2018; Ali *et al.*, 2017). Liquid extracts from the brown seaweed *Sargassum polycystum* (LEsp) function as effective biostimulants representing over one-third of the global biostimulant market (El Boukhari *et al.*, 2020). *Ascophyllum nodosum* is this industry's most widely exploited species (Shukla *et al.*, 2019a). Their effectiveness is primarily linked to reducing oxidative stress, enhancing antioxidant activity, regulating abscisic acid, improving photosynthesis, and promoting osmolyte accumulation (El Boukhari *et al.*, 2021; Sharma *et al.*, 2019).

Hot pepper (*Capsicum annuum* L.), a key vegetable and spice worldwide, is valued for its capsaicinoids—alkaloids unique to *Capsicum*—with uses in food, pharmaceuticals, and pesticides (Pastor *et al.*, 2013; Ruiz *et al.*, 2011). However, its shallow root system makes it highly susceptible to drought, often leading to severe yield losses, particularly during germination and early growth (Bayoumi *et al.*, 2008; Sezen *et al.*, 2006). Water stress reduces tissue hydration and potential, triggering morphological, physiological, and biochemical alterations that compromise plant performance (Misra *et al.*, 2011). This study evaluated the role of liquid seaweed extracts (LSEsp) in enhancing drought tolerance of hot pepper by testing different concentrations under varying field capacity levels (FC) and assessing their effects on growth, yield, and physiology.

Materials and methods

Experiment site

The experiment was conducted in a screenhouse at the Agronomy Field Laboratory, Department of Crop Production, University of Bengkulu, Indonesia, from August to November 2024. Postharvest analyses of harvested fruits were subsequently conducted in the Agronomy Laboratory to evaluate selected yield and physiological traits.

Experimental treatments and design

The experiment was established using a completely randomised design (CRD) with three replications. Treatments comprised five concentrations of liquid seaweed extract (LSEsp) (0%, 1.25%, 2.5%, 3.75%, and 5%) combined with four soil moisture regimes set at 100%, 75%, 50%, and 25% of field capacity. Each pot was treated as an independent experimental unit representing a distinct treatment combination.

Crop husbandry

Dried hot pepper seeds were thoroughly rinsed with distilled water and surface-sterilised in 10% H₂O₂ solution following Mukherjee *et al.* (2023). Sterilised seeds were then sown in trays containing a growth medium of 90% rice husk charcoal and 10% compost. Seedlings were maintained in a propagation unit for 30 days, fertilised every three days using an NPK formulation, starting 14 days after emergence. Vigorous seedlings were subsequently transplanted into polybags (50 × 50 cm) filled with 10 kg of topsoil mixed with vermicompost at a 1:1 (v/v) ratio.

Growth and yield parameters

Observations were recorded for plant height (cm), stem diameter (mm), dichotomous height (cm), number of primary branches, harvest age (weeks after planting, WAP), fruit number per plant, individual fruit weight (g), fruit length (cm), and total fruit yield per plant (g plant⁻¹).

Proline analysis

Fresh leaf tissue (0.2–0.5 g) was homogenised in 10 mL 3% sulfosalicylic acid and centrifuged (3,000 rpm, 10 min). The supernatant (2 mL) was mixed with 2 mL acid ninhydrin reagent and 2 mL glacial acetic acid, then incubated at 100 °C for one hour. After cooling, 4 mL of toluene was added and vortexed, and the colored upper phase was collected. Absorbance was read at 520 nm against a toluene blank. A standard curve was prepared using L-proline, and proline concentration was expressed as $\mu\text{mol g}^{-1}$ fresh weight based on sample weight and extract volume.

Total chlorophyll content

Fresh leaf tissue (300 mg) was finely chopped and homogenised in 80% acetone. The homogenate was centrifuged at 3000 rpm, and the resulting supernatant was adjusted to a final volume of 25 ml with 80% acetone. Absorbance readings were recorded at 645 and 663 nm using a spectrophotometer. Chlorophyll concentration was then determined following the equations of Yoshida *et al.* (1971) and expressed as mg g^{-1} of fresh leaf weight.

$$\text{Chlorophyll a} = [12.7(A_{663}) - 2.69 (A_{645})] * V/1000 * W$$

$$\text{Chlorophyll b} = [22.9(A_{645}) - 4.68 (A_{663})] * V/1000 * W$$

$$\text{Total Chlorophyll} = [20.2 (A_{645}) + 8.02(A_{663})] * V/1000 * W$$

$$\text{Chlorophyll a b ratio} = \text{Chlorophyll a} / \text{Chlorophyll b}$$

Where:

A = Absorbance of specific wavelength

V = Final volume of Chlorophyll extract in 80% Acetone

W = Weight of leaf sample in gram.

Data analysis

Data were analyzed using analysis of variance (ANOVA) using the BNT test at a 5% of probability level. The treatment means were separated using Least Significance Different (LSD) 5%.

Results

Analysis of variance indicated a notable interaction between field capacity and seaweed extract treatments solely for total chlorophyll content, suggesting that the physiological response of chlorophyll accumulation in chilli plants is

sensitive to the combined effects of water availability and biostimulant application. In contrast, field capacity independently significantly influenced several vegetative growth parameters, including plant height, stem diameter, and dichotomous height, as well as key yield components such as the number of fruits per plant and fruit weight per plant. These results indicate that water availability remains a primary determinant of structural development and reproductive performance in chilli plants under drought stress conditions.

Conversely, the application of seaweed extract did not result in statistically significant differences in growth or yield traits, implying that, under the experimental conditions, the exogenous biostimulant primarily modulated physiological processes rather than directly enhancing vegetative or reproductive traits. The selective increase in chlorophyll content observed with seaweed extract treatment may be attributed to bioactive compounds, such as cytokinins and betaines, which enhance chloroplast biogenesis, delay leaf senescence, and protect photosynthetic machinery from oxidative stress. This targeted physiological response suggests that while seaweed extracts may not directly increase plant size or fruit yield under drought stress, they can improve photosynthetic efficiency and stress resilience, potentially supporting long-term productivity under suboptimal water conditions (Table 1).

Table 1. Analysis of variance for each observed variable

Variable	F-Value			Coefficient of diversity (%)
	Field Capacity (FC)	Liquid Seaweed Extract (LSEsp)	Interaction	
Total Chlorophyll Content	0,0138 *	0,0338 *	0,0331 *	14.22
Proline	0,00001 **	0,0030 **	0,6825 ns	37.48
Fruit weight	0,4684 ns	0,1113 ns	0,6551 ns	35.75
Fruit length	0,0690 ns	0,0644 ns	0,3367 ns	14.22
Fruit weight per plant	0,0011 **	0,4470 ns	0,3090 ns	37.89
Number of fruits per plant	0,0002 **	0,7647 ns	0,3557 ns	21.37
Harvest age	0,3228 ns	0,0801 ns	0,1523 ns	8.70
Plant height	0,00001 **	0,3516 ns	0,1294 ns	17.28
stem diameter	0,00001 **	0,2110 ns	0,0782 ns	18.25
Dichotomous height	0,0002 **	0,5868 ns	0,7059 ns	16.05

Noted: * = significant difference at 5 % significance level according to Least Significant Different (LSD).

Interaction between field capacity and seaweed extract on hot pepper

The present study revealed a significant interaction between field capacity and seaweed extract treatments, specifically for total chlorophyll content in chilli plants. The highest chlorophyll concentration (4.74 mg/g tissue) was recorded in plants subjected to 0% seaweed extract combined with 75% field capacity. In contrast, the lowest value (1.73 mg/g tissue) occurred with 3.75% seaweed extract under the same 75% field capacity. Interestingly, no significant difference was observed between plants treated with 5% seaweed extract and 0% seaweed extract under 25% field capacity, yielding 2.17 mg/g and 2.24 mg/g tissue, respectively. These findings indicate that, although a statistically significant interaction exists, the response of total chlorophyll content does not follow a consistent or predictable trend across the different treatment combinations. This suggests that the physiological response of chlorophyll accumulation in chilli plants is influenced by a complex interplay between water availability and exogenous biostimulant application, rather than solely dependent on either factor. Such interactions may reflect underlying stress adaptation mechanisms, including modulation of chloroplast biogenesis, osmotic adjustment, and photoprotection under varying drought conditions (Table 2).

Table 2. Interaction between field capacity and liquid seaweed extract concentration on total chlorophyll content of hot pepper

Field Capacity (%)	Concentration Liquid Seaweed Extract (%)				
	5 (S1)	3.75 (S2)	2.5 (S3)	1.25 (S4)	0 (S5)
100 (K1)	2,92 abcde	2,68 bcde	2,10 de	3,95 abcd	3,72 abcd
75 (K2)	3,04 abcde	1,73 e	2,08 de	4,08 abc	4,74 a
50 (K3)	4,49 ab	4,27 ab	3,69 abcd	2,9 abcde	3,85 abcd
25 (K4)	2,17 cde	3,05 abcde	2,12 de	3,51 abcde	2,24 cde

Notes: Numbers followed by the same letter in the same column are not significantly different in the BNT test at the 5% level.

Effect of field capacity on total morpho-physiological traits of hot pepper

Analysis of fruit and growth characteristics revealed that fruit weight and length were largely unaffected by variations in field capacity. Plants grown under 25%, 50%, and 75% field capacity produced similar fruit weights per plant of 7.19 g, 6.72 g, and 7.53 g, respectively, significantly higher than those recorded under 100% field capacity (5.13 g). Conversely, plant height was more responsive to field capacity: the tallest plants were observed at 75% field capacity (62.27 cm), followed by 50% (55.07 cm), 25% (52.43 cm), and the lowest under

100% field capacity (40.47 cm). This indicates an inverse relationship between excessive water availability and vegetative growth, where moderate water limitation promotes enhanced plant height and biomass accumulation.

Physiologically, sub-saturated conditions induce moderate drought stress, promoting osmolyte (proline, soluble sugars) accumulation, maintaining cell turgor, stabilizing proteins, and protecting membranes. Moderate stress also stimulates antioxidant defenses, scavenging reactive oxygen species (ROS) and sustaining photosynthesis. As a result, plants under 25–75% field capacity retained higher chlorophyll content and photosynthetic activity, supporting nutrient uptake and biomass production. In contrast, full saturation (100% field capacity) likely restricted root oxygenation, slowed metabolism, and reduced nutrient assimilation, limiting growth and fruit yield (Table 3).

Table 3. The effect of field capacity on total chlorophyll content, proline, growth and yield of hot pepper

Variable	Field Capacity (%)			
	100 (K1)	75 (K2)	50 (K3)	25 (K4)
Total Chlorophyll Content	3,08 b	3,14 ab	3,83 a	2,62 b
Proline	0,39 b	0.56 b	1,17 a	1,05 a
Fruit weight	2,78	3,12	2,63	3,12
Fruit length	11,79	13,41	12,46	13,24
Fruit weight per plant	13,28 c	24,84 a	18,24 bc	19,56 ab
Number of fruits per plant	5,13 b	7,53 a	6,72 a	7,19 a
Harvest age	88,47	83,47	86,87	85,53
Plant height	40,47 c	62,27 a	55,07 b	52,43 b
stem diameter	4,03 c	6,07 a	5,51 ab	5,2 b
Dichotomous height	27,8 b	36,97 a	34,93 a	33,17 a

Notes: Numbers followed by the same letter in the same column are not significantly different in the BNT test at the 5% level.

Effect of seaweed extract on total morpho-physiological traits of hot pepper

The application of seaweed extract (LSEsp) was not statistically significant affected on the primary growth and yield parameters of chili plants, including plant height, stem diameter, dichotomous height, fruit number, fruit weight, and fruit length. However, significant differences were observed for key physiological indicators, namely total chlorophyll and proline content, highlighting the role of seaweed extracts in modulating biochemical responses rather than directly influencing gross morphological traits. Specifically, total chlorophyll content in plants treated with 0% and 1.25% seaweed extract was

comparable, measuring 3.64 mg/g and 3.61 mg/g tissue, respectively, indicating that low concentrations of seaweed extract may not markedly enhance chlorophyll synthesis under the given experimental conditions. Similarly, the harvest age across treatments ranging from 0% to 5% seaweed extract did not differ significantly, varying between 83 and 89.25 days after planting, suggesting that seaweed application did not accelerate or delay crop maturation.

Regarding stem development, no significant differences were detected among treatments, although a trend was noted: plants receiving 5% seaweed extract exhibited the largest stem diameter (5.48 mm), while untreated plants (0% seaweed extract) showed the smallest diameter (4.68 mm). This pattern suggests a potential, albeit non-significant, effect of higher seaweed extract concentrations on stem thickening, possibly mediated by the bioactive compounds in the extract, such as cytokinins and betaines, which are known to influence cell division and elongation processes. These results indicated that while seaweed extract may not directly enhance visible growth or yield traits under the tested conditions. It was significantly modulated physiological parameters that contributed to plant stress tolerance and metabolic resilience (Table 4).

Table 4. The effect of liquid seaweed extract on total chlorophyll content, proline, growth and yield of hot pepper

Variable	Concentration Liquid Seaweed Extract (%)				
	5 (S4)	3,75 (S3)	2,5 (S2)	1,25 (S1)	0 (S5)
Total chlorophyll content	3,16 ab	2,93 ab	2,50 b	3,61 a	3,64 a
Proline	0,79 b	0,83 ab	0,58 b	0,67 b	1,07 a
Fruit weight	2.63	3.62	2.67	3.01	2.63
Fruit length	13.57	13.61	11.93	12.42	12.09
Fruit weight per plant	17.37	22.09	17.98	19.95	17.51
Number of fruits per plant	6.52	7.11	6.61	6.61	6.37
Harvest age	83.17	83	89.75	85.25	89.25
Plant height	56.42	52.5	49.79	54	50.08
stem diameter	5.48	5.43	5.04	5.38	4.68
Dichotomous height	33.38	33.33	31.08	34.63	33.67

Notes: Numbers followed by the same letter in the same column are not significantly different in the BNT test at the 5% level.

Discussion

Vegetable crops frequently encounter abiotic stresses, with water deficit significantly limiting global food production. Excessive soil water levels can

induce physiological drought, carbon limitation, impaired nitrogen metabolism, and restricted nutrient uptake (Joseph *et al.*, 2021; Bista *et al.*, 2018). Drought also stimulates the overproduction of reactive oxygen species (ROS), which, while serving as signalling molecules under normal conditions, cause oxidative damage at elevated levels (Cao *et al.*, 2024; Panda *et al.*, 2024). Under drought stress, plants counteract these effects through ion exclusion, compartmentation, and antioxidant defences (Laxa *et al.*, 2019). Moreover, liquid seaweed extracts (LSEsp) have been reported to improve growth and yield traits of solanaceous crops, particularly bell pepper, under stress conditions (Salim *et al.*, 2025; Khan *et al.*, 2018).

Although the interaction between field capacity and seaweed extract was statistically significant, chlorophyll responses varied across treatment combinations, reflecting a complex interplay between water availability and biostimulant application (Table 2). Under drought, reduced water availability induces osmotic stress, impairing chloroplast function and chlorophyll synthesis. Seaweed extracts, containing cytokinins, betaines, and polyols, can partially mitigate these effects by enhancing chloroplast biogenesis, stabilising pigments, and delaying senescence (Jiménez-Aria *et al.*, 2021). They also boost antioxidant defences and osmolyte accumulation, such as proline, protecting cellular structures and sustaining photosynthesis under water-limited conditions. These physiological adjustments help maintain chlorophyll content and photosynthetic performance, supporting biomass and fruit yield. However, their effectiveness depends on the specific combination of extract concentration and soil moisture, emphasising the need for optimised application strategies (Jacomassi *et al.* 2022).

The study examined how drought stress influences growth, physiological traits, and fruit yield in hot pepper. Findings revealed that increasing field capacity levels markedly decreased plant height, stem diameter, and dichotomous height (Table 3). While foliar application of seaweed extract did not significantly enhance growth parameters or yield components—including plant height, stem diameter, dichotomous height, fruit number, fruit length, individual fruit weight, and total fruit yield—physiological traits such as total chlorophyll and proline content exhibited notable increases (Table 4). The present study indicated that LSEsp treatment did not enhance plant height, stem diameter, or dichotomous height, likely due to environmental conditions such as temperature and humidity, as well as factors affecting cell division and elongation that influence root development, nutrient and water uptake, and the net photosynthesis rate under drought stress (Seleiman *et al.*, 2021; Yang *et al.*, 2021; Aslam *et al.*, 2015). These findings contrast with previous reports where seaweed extracts have been shown to promote plant height, biomass accumulation, root hydraulic conductivity, and root activity, thereby improving

water and nutrient uptake and supporting carbon fixation for enhanced biomass production (Shukla *et al.*, 2019b; Khan *et al.*, 2009).

The foliar application of seaweed extract under varying field capacity levels (drought stress) significantly influenced physiological traits, notably total chlorophyll and proline content (Table 4). Plants treated with 5% LSEsp exhibited the highest chlorophyll concentration across field capacities. This enhancement is likely due to betaine-mediated stimulation of chloroplast biogenesis and delayed leaf senescence. Moreover, seaweed extracts promote the secretion of root exudates, including phospholipids and betaine, which improve soil aggregation, water infiltration, and aeration in the rhizosphere (Espinosa-Antón *et al.*, 2023; Ali *et al.*, 2021). Rich in growth regulators, seaweed extracts also boost endogenous phytohormone synthesis, particularly cytokinins, protecting chlorophyll from photodamage, increasing leaf greenness, and supporting a higher net photosynthetic rate, which ultimately contributes to improved fruit yield (Yao *et al.*, 2020).

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Conflict of interest

The authors declare no conflict of interest.

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