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ORIGINAL PAPER

Chitosan-enhanced hydroponic cultivation of *Ocimum basilicum* L.: a sustainable approach for improved growth, quality and antioxidant activity

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Abstract

Hydroponic cultivation presents a sustainable alternative to conventional farming, addressing water scarcity and environmental degradation. This study investigates the effects of chitosan from royal shrimp waste on the growth and biochemical characteristics of *Ocimum basilicum* L. (basil) in hydroponic systems. The study compared three cultivation methods: traditional soil-based (Control/B), hydroponic without chitosan (B.H), and hydroponic with chitosan treatment (B.H.CS). Chitosan treatment significantly enhanced growth parameters, including root length (+ 52%), shoot height (+ 52%) and leaf count (+ 65%) relative to the control and non-chitosan hydroponic conditions. The biochemical analyses revealed increased chlorophyll (*a* and *b*), total proteins, and soluble sugars in chitosan-treated plants. Furthermore, antioxidant activity, assessed using DPPH and FRAP assays, was markedly higher in the chitosan-treated group, indicating superior quality compared to both control and hydroponic basil. These results highlight the potential of shrimp-derived chitosan as a sustainable biostimulant to enhance growth and nutritional value in hydroponic basil cultivation, thereby contributing to sustainable agricultural practices and improved resource management.

Keywords Chitosan · Hydroponic · *Ocimum basilicum* L. · Sustainable agriculture · Water preservation

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Abbreviations

B	Traditional soil-based culture of basil/control
B.H	Hydroponic culture of basil without chitosan
B.H.CS	Hydroponic culture basil with chitosan treatment
SDG	Sustainable development goal
DPPH	2,2-Diphenyl-1 picrylhydrazyl
FRAP	Ferric-reducing antioxidant power

Introduction

Agriculture holds a predominant position in the Moroccan economy, serving not only as a major source of income but also as a cornerstone of food security and rural development (Ghanem 2015). Within this vital sector, aromatic and medicinal plants play a key role in the economy. Among these, *Ocimum basilicum* L. stands out for its aromatic (Du et al. 2023), medicinal (Zhakipbekov et al. 2024) and culinary properties (Shahrajabian et al. 2020), making it a crop of significant importance in Morocco. Aromatic plants, including basil, accounted for a significant share of the

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17,000 tons in terms of agricultural exports in 2018 (Fellahtrade 2019). This contributed to a total export value of nearly 40 billion dollars in 2019, highlighting the growing economic impact of Morocco's agricultural sector.

Moroccan agriculture faces significant challenges, including water scarcity, land degradation and the impacts of climate change. In this context, it is crucial to promote sustainable and innovative agricultural practices to enhance both productivity and resilience of agricultural systems. The adoption of innovative agricultural techniques such as hydroponics, offers promising solutions for improving crop yields and quality (Verdoliva et al. 2021). Hydroponics is a soilless gardening method that lets plants grow using nutrient solutions. It has many benefits, such as better use of water and nutrients (over 70% less water used for irrigation), fewer soil-borne diseases, and better use of cultivation space (Rajaseger 2023). This integrated approach aligns with several sustainable development goals (SDGs), addressing SDG 6 (clean water and sanitation), SDG 12 (responsible consumption and production), SDG 13 (climate action), and SDG 15 (life on land) by offering alternative agricultural solution to climate change challenges, such as drought.

This technique is particularly promising for aromatic and medicinal plants like *Ocimum basilicum* L., enabling consistent and high-quality production (Atherton and Li 2023). Soilless cultivation represents a significant technological advancement for modern agriculture, offering better adaptation to market demands through the optimization of several key factors (Gebreegziher 2023). However, hydroponic cultivation presents certain limitations, including lower agricultural yields compared to soil-based cultivation and increased susceptibility to pest attacks (Velazquez-Gonzalez et al. 2022; Agarwal et al. 2023). Therefore, the use of plant fortifiers and nutrient solutions is crucial for successful hydroponic farming. Chitosan, a biopolymer recognized for its stimulating and fortifying activity on plants, is widely used in soil-based cultivation and has demonstrated a natural protective and nutritive potentiality (El Amerany et al. 2020b). It finds applications in various fields, including biomedicine (Hamdan et al. 2023; Ait Hamdan et al. 2024b, a), wastewater treatment (Elouali et al. 2024a, b) and agriculture (El Amerany et al. 2020a, 2022). Furthermore, chitosan is biodegradable and biocompatible (Jiménez-Gómez and Cecilia 2020; Hamdan et al. 2024a), distinguishing it from traditional chemical fertilizers, which may cause environmental harm and increase chemical dependency (Sharif et al. 2018; Malerba and Cerana 2019). Table 1 provides a comparison between chitosan and traditional chemical fertilizers.

This study investigates the impact of incorporating a chitosan solution into hydroponic cultivation systems without altering the water solution. The choice of chitosan derived from shrimp waste serves a dual purpose: it offers a sustainable approach to valorizing waste biomass by converting it into a high-value natural biostimulant with the specific bioactive properties of shrimp-based chitosan, recognized for its effectiveness in promoting plant growth and enhancing stress resilience. For clarity, a comparison with both conventional hydroponic and field cultivation methods without using chitosan. Furthermore, the study examines the effect of hydroponic cultivation combined with chitosan on the morphological and biochemical growth parameters, as well as the antioxidant activity of *Ocimum basilicum L.* as a model of aromatic and medicinal plants that plays a fundamental role in the Moroccan economy.

Materials and methods

Chitosan extraction

Shrimp shells were collected from Safi coastline, washed to remove any adhering impurities and then dried at 50 °C to eliminate residual moisture. The dried shells were first subjected to demineralization using 0.55 M hydrochloric acid (HCl) at a solid-to-liquid ratio of 1:20 (w/v). This step was carried out at 25 °C for 1 h under continuous stirring. Following demineralization, the shells underwent deproteinization by immersion in a 1 M sodium hydroxide (NaOH) solution, also at a ratio of 1:20 (w/v). The mixture was heated to 90 °C under reflux and a repeated-bath technique was applied, with each bath lasting 1 h, to ensure effective removal of protein residues. Finally, chitosan was

Table 1 Comparison of chitosan and conventional chemical fertilizers

Feature	Chitosan	Traditional chemical fertilizers	References
Environmental impact	Biodegradable, eco-friendly	Often contributes to soil/water pollution	Sharif et al. (2018) and Malerba and Cerana (2019)
Nutrient uptake	Enhances nutrient absorption	Lead to nutrient runoff	Faqir et al. (2021)
Disease resistance	Induce plant defenses	Limited protective effects	Malerba and Cerana (2016)
Soil health	Promotes beneficial microbes	Harm soil microbiota	Sharif et al. (2018), Malerba and Cerana (2019)
Stress tolerance	Improves resilience to drought/salinity	No significance effect	Hidangmayum et al. (2019)

obtained by performing deacetylation, where the shells were treated with a mixture of potassium hydroxide (KOH) (50%), ethanol (25%) and ethylene glycol (25%) at 120 °C for 24 h under reflux (Fig. 1), following the procedure outlined by Hamdan et al. (2024b). The chitosan yield was calculated as follows:

$$\text{Chitosan}(\%) = \frac{m_{\text{chitosan}}}{m_{\text{rm}}} \times 100,$$

where m_{chitosan} represents the weight of chitosan obtained after deacetylation, m_{rm} represents the weight of the raw material.

Chitosan solution preparation

Chitosan was used in an aqueous solution as follows: 1 g of chitosan is dissolved in one liter of acetic acid solution (0.1%, v/v). Subsequently, the pH is adjusted to 5.6 by adding a NaOH solution (1N) (El Amerany et al. 2024). A total volume of 7.5 mL of the chitosan solution is used in 200 mL of water in the hydroponic culture.

Chitosan characterization

The chitosan samples were characterized for their degree of acetylation (DA) and molecular weight. For DA calculation, ^{1}H-NMR analysis was conducted using a 400-MHz Bruker instrument. Approximately 10 mg of chitosan was dissolved in 0.45 mL of 2% DCl in D_2O, heated at 70 °C for 1 h and the NMR chemical shifts were recorded in ppm. The DA was calculated using the formula provided below.

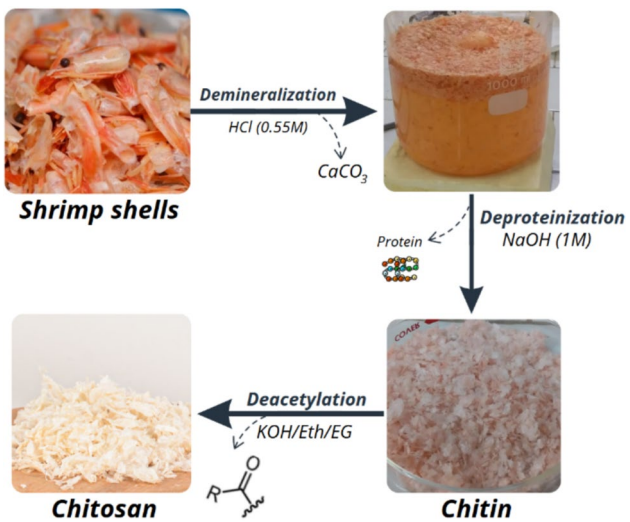

Fig. 1 Chitosan extraction process from royal shrimp shelling waste

$$\text{DA}(\%) = 1 - \frac{a}{(a + 1/3i)} \times 100.$$

The molecular weight was determined using an Ubbelohde capillary viscometer (0.5–3 mm^2/s, 20 mL). 0.3 M acetic acid and 0.2 M sodium acetate was used for this analysis. The intrinsic viscosity $[\eta]$ and viscometric molar mass (M) were calculated using the Mark–Houwink equation, as shown below, with K and a are the viscometric constants determined in the literature

$$[\eta] = K \cdot M^a.$$

Plant material and cultivation conditions

Basil (*Ocimum basilicum* L. cv. Genovese) seeds are soaked in a sodium hypochlorite solution (5%) for 3 min. Then, they are allowed to germinate in plastic trays containing a peat substrate at a temperature ranging from 25 to 28 °C. After 15 days of cultivation, uniform young seedlings are selected and transplanted into plastic pots (100 mm in width and 180 mm in height) containing a peat soil mixture (1:3; w/w), while others are placed in containers containing distilled water for hydroponic cultivation. Thus, the plants are divided into three categories: control (basil plants watered daily) (B), basil plants grown in a hydroponic system (B.H) and basil plants cultivated in a hydroponic system with an aqueous chitosan solution added every 7 days (B.H.CS) (Fig. 2).

Morphological parameters

All plant analyses were performed 7 weeks after transplanting. Following harvest, the treatment effects on plant growth were evaluated through a series of morphological parameters. Aerial height was measured from the plant base to the tip of the tallest leaf using measuring tape, while root elongation was assessed by carefully washing the roots to remove adhering soil and measuring the length from the root collar to the tip of the longest root using a ruler. Leaf area was quantified with a LI-3100C leaf area meter by individually analyzing detached leaves. The number of leaves per plant was counted manually and the fresh weight of leaves was determined immediately after harvest using a precision analytical balance.

Biochemical parameters

Quantification of chlorophyll pigments

To assess the impact of chitosan on chlorophyll and carotenoid production, the Arnon method was employed (Arnon 1949). Fresh leaves (50 mg) were mixed with 2.5 mL of

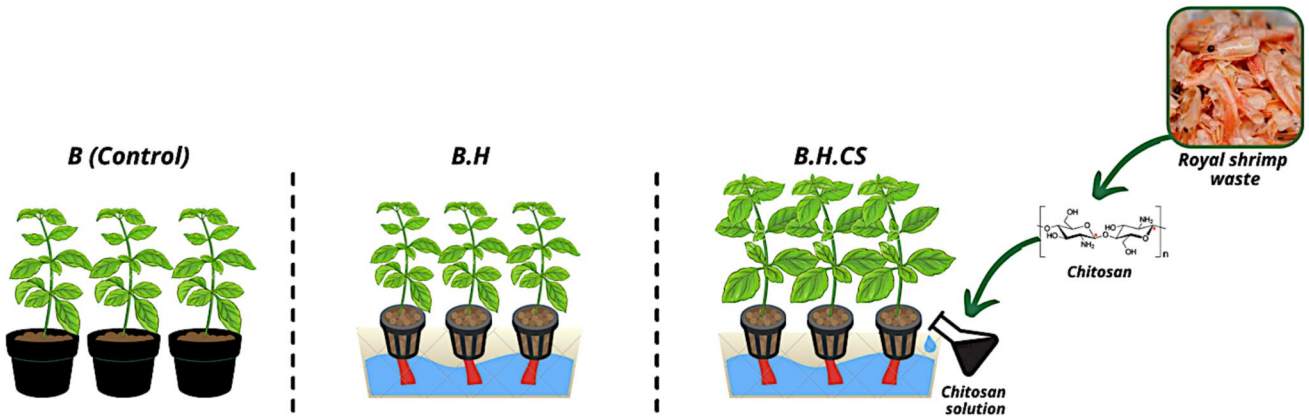

Fig. 2 Illustration of basil cultivation groups: control (B), hydroponic system (B.H) and hydroponic system with chitosan (B.H.CS)

acetone and incubated in darkness overnight. The extracts were then centrifuged at 4000 rpm for 6 min and the absorbance of the supernatant was measured between 663 and 470 nm using a UV-6300PC UV–visible spectrophotometer. The chlorophyll (*a* and *b*) and carotenoid contents were calculated using Arnon's formulas.

Quantification of sugars

Fresh material was placed in an 80% ethanol solution at a 1:25 (v/w) ratio. The mixture was kept in the dark for 30 min. Subsequently, 1 mL of the supernatant was mixed with 1 mL of 5% phenol and 5 mL of HCl. The mixture was heated in a water bath at 80 °C for 10 min, and the absorbance was measured using a UV-6300PC UV–visible spectrophotometer at 487 nm.

Proteins quantification

The proteins were quantified using the Bradford method (Bradford 1976) at 595 nm using a UV-6300PC UV–visible spectrophotometer. Fresh leaves (0.50 g) were homogenized in 1.50 mL of potassium phosphate buffer (0.02 M, pH 7.6) and then centrifuged at 4 °C for 5 min at 3800 rpm. The Bradford solution was added to the supernatant in a 1:1 (v/v) ratio. Protein content was determined using a BSA (bovine serum albumin) standard curve with a known concentration of 1 mg/mL.

Antioxidant activity

DPPH free radical scavenging activity

The antioxidant activity of the extracts was assessed using the DPPH assay, adapted from Şahin et al. (2004). A methanolic DPPH solution (60 µM) was combined with 50 µL of extract at concentrations of 2, 1, and 0.5 mg mL. The mixture was incubated in the dark at room temperature for 20 min and absorbance was measured at 517 nm. A control sample without extract was included for comparison. The percentage of DPPH inhibition was calculated using the formula:

$$\%\text{Inhibition of DPPH activity} = \frac{A_{\text{Blank}} - A_{\text{Test}}}{A_{\text{Blank}}}.$$

Ferric-reducing antioxidant power test (FRAP)

The reducing power of *Ocimum basilicum* L. was evaluated spectrophotometrically following the Oyaizu method (Oyaizu 1986). A 0.2 mL aliquot of each extract at varying concentrations, along with the reference compound, was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). After incubation at 50 °C for 20 min, 2.5 mL of 10% trichloroacetic acid was added. The resulting solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$). The absorbance of the blue-green complex was measured at 700 nm to determine reducing power.

Statistical analysis

Statistical analysis of the data was conducted using GraphPad Prism version 9.00 software (San Diego, California, USA). The results are expressed as the mean ± SEM (Standard Error of the Mean). All measurements were performed with three biological replicates ($n = 3$) and repeated twice under the same conditions. An analysis of variance (ANOVA) was applied, followed by Tukey post-hoc tests at a significance level of $p = 0.05$.

Results and discussion

Chitosan extraction and characterization

Annually, the coastal regions of Morocco discard approximately 21,150,054 tons of chitinous byproducts from shrimp processing (Arrouze et al. 2019, 2021). This large quantity of biowaste represents a valuable resource that can be sustainably managed through its conversion into chitosan. Chitosan is a multifunctional biopolymer**,** known for its biodegradability, biocompatibility, and antimicrobial properties, making it useful in sectors such as agriculture, pharmaceuticals and water treatment**.** Its global market value is projected to reach approximately USD 1.96 billion in 2024 (Elouali et al. 2025). In this study, royal shrimp shells sourced from the coastal city of Safi, Morocco, one of the regions with significant shrimp production, were used as the raw material. The chitosan obtained exhibited a yield of 20.12%, higher than previously reported yields of approximately 15.8%, 13.29% and 10% from *Parapenaeus longirostris* shells (El-araby et al. 2022), crab shells (Olaosebikan et al. 2021) and pupal exuviae of the Black soldier fly (Triunfo et al. 2022), respectively (Table 2).

Figure 3 presents the H-NMR spectrum of the chitosan sample. The amine proton peak (a) appears at 5.13 ppm with an integration value of 1.00. The protons at positions b to g in the molecule are observed between 4.16 and 3.98 ppm (Shin et al. 2019; Ait Hamdan et al. 2024b; Elouali et al.

Table 2 Physicochemical properties of chitosan sourced from royal shrimp shells and comparison with other studies

Study	Source	Chitosan yield (%)	DA (%)	Mv (g/mol)
This work	Royal shrimp	20.12	2.92	101 720
El-araby et al. (2022)	*Parapenaeus longirostris*	15.80	83.67	229 184
Olaosebikan et al. (2021)	*Callinectes amnicola*	13.29	84.20	–
Triunfo et al. (2022)	Black soldier fly	10	10%	35 000

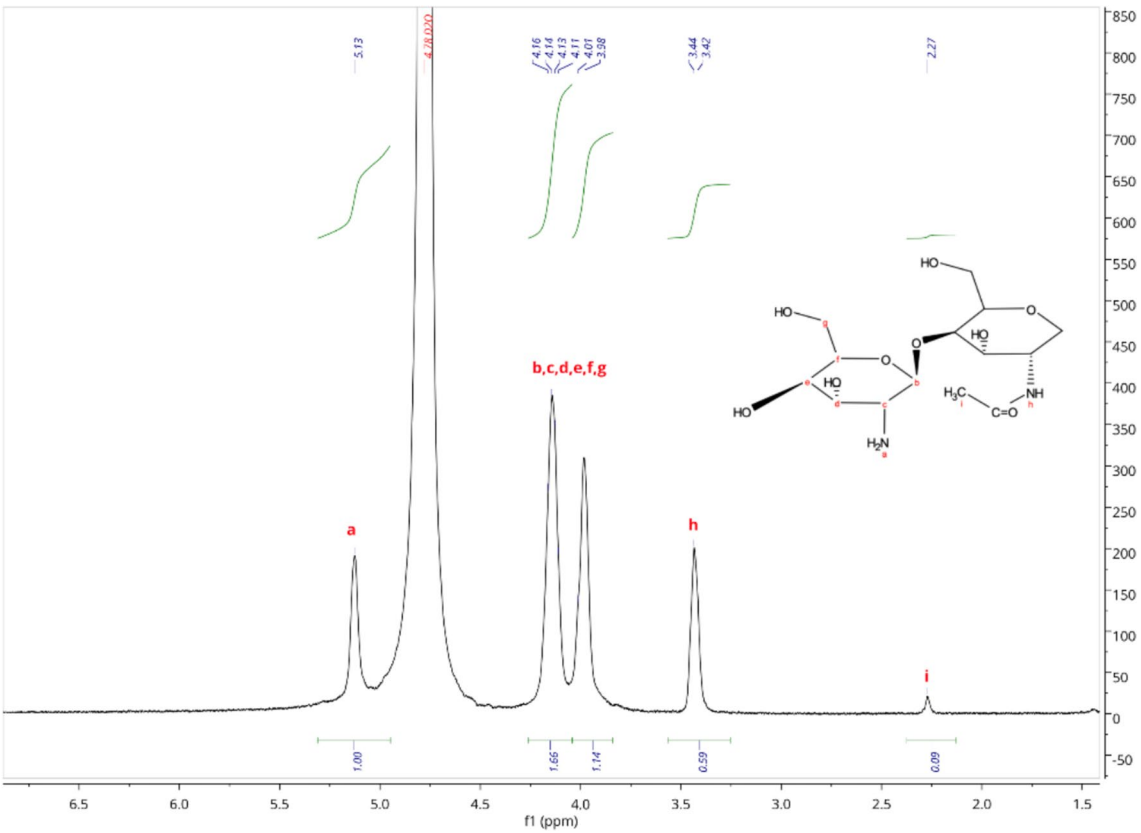

Fig. 3 ^{1}H-NMR spectrum of chitosan

2024b). The acetyl proton peak (i) is detected at 2.27 ppm and has an integration value of 0.09. A solvent peak is observed at 4.78 ppm. The degree of acetylation (DA) calculated from the spectrum is 2.9% (Table 2). Additionally, the chitosan obtained is characterized by a molecular weight of 101 720 g/mol (Fig. 4), which is considered a medium molecular weight compared to others reported in the literature (Olaosebikan et al. 2021; El-araby et al. 2022; Triunfo et al. 2022).

Morphological characterization

Root elongation

Figures 5 and 6 compare root elongation in basil across three basil cultivation methods: soil culture (B), hydroponic culture (B.H), and hydroponic culture under chitosan treatment (B.H.CS). Soil culture (B) demonstrates an average root elongation of approximately 8.23 cm. In the hydroponic basil culture (B.H), the average root elongation increases slightly to around 7.56 cm. Notably, the hydroponic basil culture treated with chitosan (B.H.CS) shows a marked increase in root elongation, with an average value of about 12.5 cm. This enhancement is statistically significant, with a p value < 0.001, indicating that chitosan treatment in hydroponic conditions significantly promotes root growth in basil compared to those grown in soil and a standard hydroponic system. The significant increase in root elongation in hydroponic basil treated with chitosan (B.H.CS) can be attributed to chitosan's multifaceted role in promoting auxin accumulation and translocation within roots (Lopez-Moya et al. 2019), a key plant hormone responsible for regulating root growth (Tanimoto 2005; Yun et al. 2023).

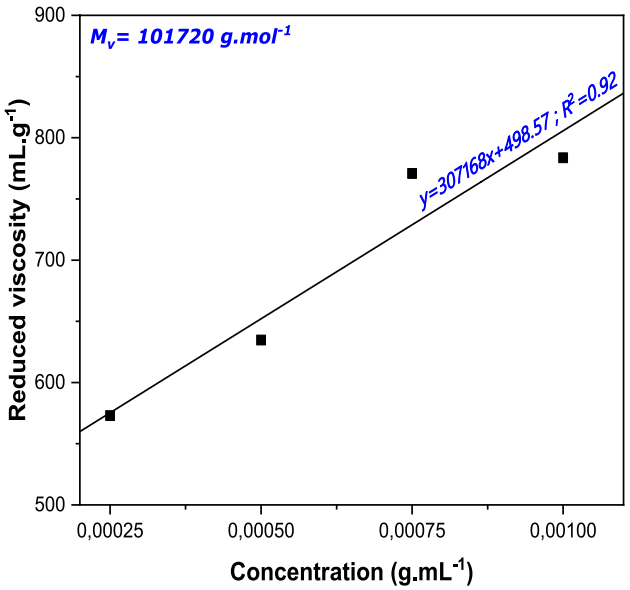

Fig. 4 Example of the viscosity curve of chitosan from royal shrimp shells

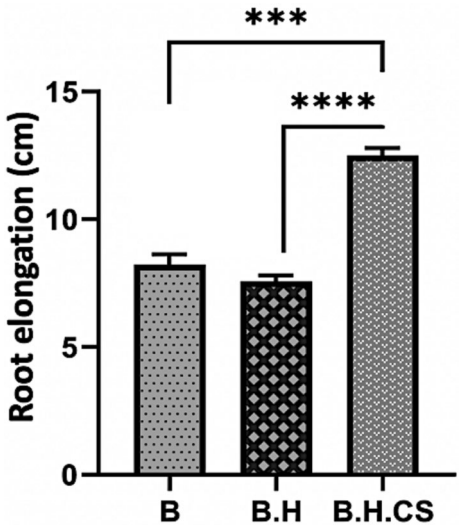

Fig. 5 The effect of chitosan on root elongation: B (control Basil), B.H. (Hydroponic Basil), and B.H.CS (Hydroponic Basil treated with Chitosan)

Shoot height

Figures 7 and 8 present the shoot height variations among the three basil cultivation methods. Basil grown in soil medium reaches an average shoot height of 7.4 cm, while the hydroponic method yields a slightly greater average height of 7.56 cm. The most notable increase is observed in hydroponic basil treated with chitosan, resulting into an average shoot height of 11.26 cm. This significant difference demonstrates that chitosan treatment greatly enhances shoot growth in hydroponic basil compared to both soil-grown and untreated hydroponic basil. This improvement may be

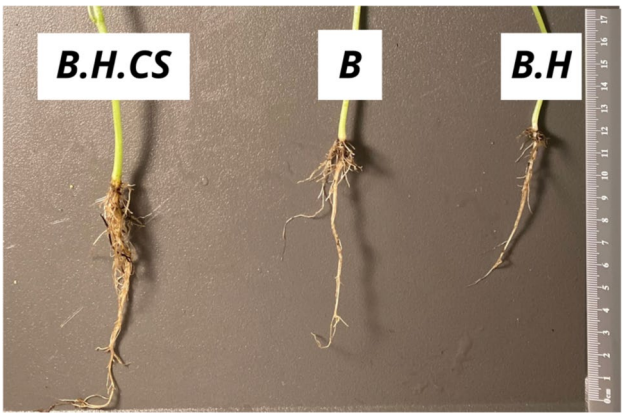

Fig. 6 Visual comparison of root elongation: control basil (B), hydroponic basil (B.H), and chitosan-treated basil (B.H.CS)

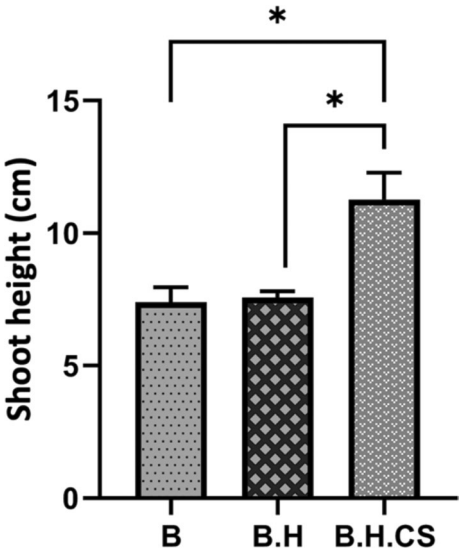

Fig. 7 Effect of Chitosan treatment on aerial height (cm): B (Control Basil), B.H (Hydroponic Basil), and B.H.CS (Hydroponic Basil Treated with Chitosan)

attributed to chitosan's role in elevating levels of indole-3-acetic acid (IAA), a plant hormone known to promote shoot growth (Mohite 2013; El Amerany et al. 2022). Chitosan was applied in a liquid form to the root system, promoting stem elongation. This improvement may be linked to the biosynthesis of phytohormones, particularly gibberellic acid (Sardoei et al. 2024). These results are consistent with those reported by (Suwanchaikasem et al. 2024). The fate of chitosan, with its medium molecular weight, after interacting with the plant cell wall is still unclear. However, it is likely that chitosan is absorbed by the plant and translocated via the xylem to areas of active cell division, thereby enhancing gibberellic acid synthesis.

Leaf count

The comparison of leaf counts among the three basil cultivation methods shows distinct differences (Fig. 9). Basil grown in soil medium has an average leaf count of 7.66, while hydroponic culture results in a slightly lower leaf count average of 6.66. However, basil grown in a hydroponic system with chitosan treatment exhibits a significantly higher leaf count, averaging 12.66, which is double that of the hydroponic culture without chitosan. This result also surpasses the leaf count observed in traditional soil-based cultivation (B), which is typically considered advantageous for agricultural yield when compared to hydroponic methods. The integration of chitosan into hydroponic systems presents a promising strategy for optimizing growth and yield, potentially offering significant advantages over both traditional field methods and standard hydroponic practices. The ability to produce a greater number of leaves is closely associated with the initiation of leaf primordium from the shoot apical meristem (SAM) (Lv et al. 2023). The previous studies have shown that auxin levels are elevated in the SAM, coinciding with the formation of leaf primordia (Bar and Ori 2014). This induction may be linked to chitosan application, as its interaction with plants has been demonstrated to stimulate auxin biosynthesis and accumulation (Lopez-Moya et al. 2019). Moreover, the positive effects of chitosan and hydroponic systems on leaf formation may not only attributed to phytohormone biosynthesis and accumulation but also to the availability of essential nutrients (such as nitrogen, phosphorus, potassium, and calcium) and the enhanced uptake of these nutrients by plants (Liaqat 2019; Walled Fouad 2023). These nutrients play a crucial role in promoting cell division by stimulating the synthesis of proteins and nucleic acids, providing energy for various cellular processes, activating enzymes, and maintaining cell turgor pressure and cell wall stability.

Specific leaf area (SLA)

The specific leaf area (SLA) of basil plants is illustrated in Fig. 10. In the hydroponic cultivation of basil treated with chitosan (B.H.CS), the SLA increases compared to the untreated control group grown in soil (B) and the hydroponic culture without chitosan treatment (B.H). Untreated basil grown in hydroponics shows a lower leaf area than those cultivated in soil, with a difference of ± 52%, while the addition of chitosan results in a highly significant increase, reaching up to 586 cm^2/g. The overall results confirm that chitosan treatment has positive effects on basil's SLA, favoring soilless cultivation over soil cultivation. The increase in SLA in B.H.CS may be attributed to the role of chitosan in activating cell division and enhancing plant physiology, including stomatal opening, gas exchange, photosynthesis, and energy transfer within photosystem II (El Amerany et al. 2022). Additionally, the release of its nitrogen may be utilized in chlorophyll biosynthesis (El Amerany et al. 2022). Furthermore, the hydroponic system supports all these processes by supplying water and essential mineral elements that are readily available in the solution (Rajendran et al. 2024).

Fresh leaf weight

Figure 11 illustrates the effect of treatments on the fresh leaf weight of basil (g). The average fresh leaf weight for the control group (B) is 1.14 g, while the hydroponic group (B.H) shows a similar value, with an average of 1.08 g. However, the chitosan-treated hydroponic basil (B.H.CS) exhibits a significantly higher fresh leaf weight of 2.32 g, marking it as the highest among the groups. The statistical analysis indicates a slight but significant difference

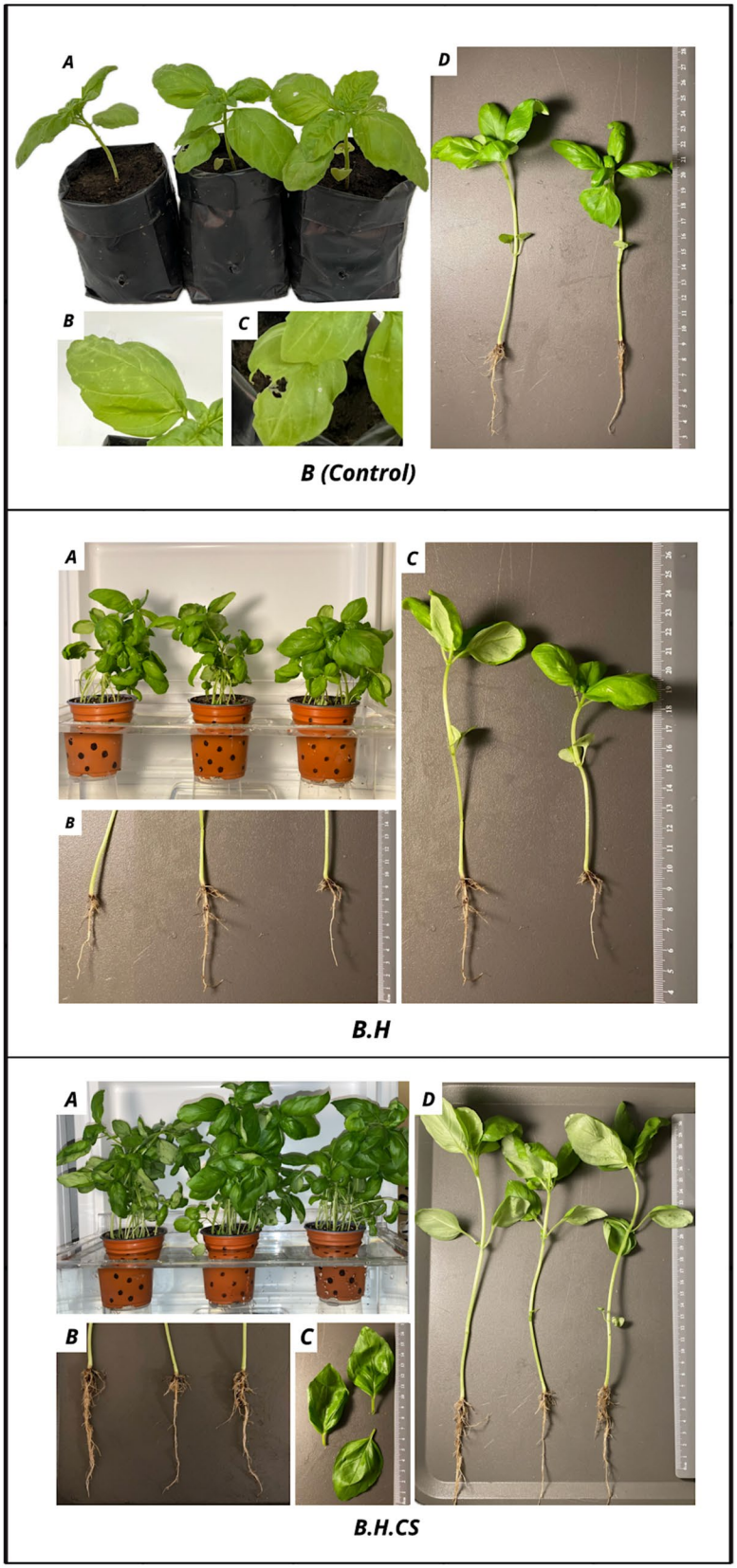

Fig. 8 Comparison of morphological parameters B (control Basil), B.H (Hydroponic Basil), and B.H.CS (Hydroponic Basil treated with Chitosan)

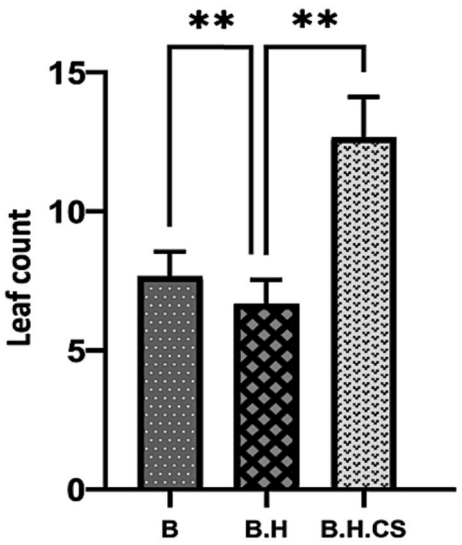

Fig. 9 Effect of chitosan treatment on number of leaves: B (control basil), B.H (hydroponic basil), and B.H.CS (hydroponic basil treated with chitosan)

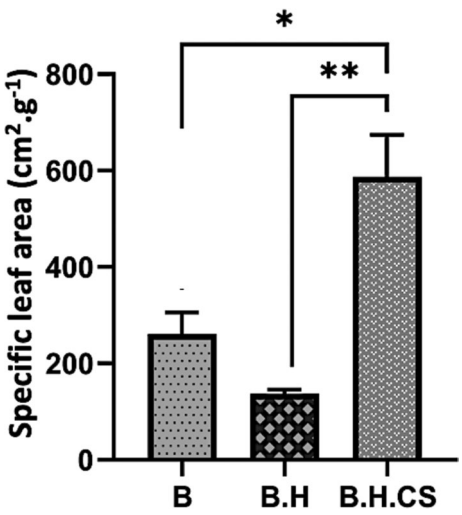

Fig. 10 Effect of chitosan treatment on specific leaf area (cm^2/g): B (control basil), B.H (hydroponic basil) and B.H.CS (hydroponic basil treated with chitosan)

($^*p < 0.05$) between the control group (B) and the hydroponic group (B.H), suggesting that hydroponic growth alone did not markedly increase leaf weight. However, a highly significant difference ($^{***}p < 0.001$) is observed between the hydroponic basil without (B.H) and with chitosan treatment (B.H.CS), highlighting the positive impact of chitosan on promoting leaf weight under hydroponic conditions. Interestingly, basil grown in different organic substrates like vermicompost (VER), compost (COM) and solarized manure (ES) did not achieve fresh leaf weights surpassing

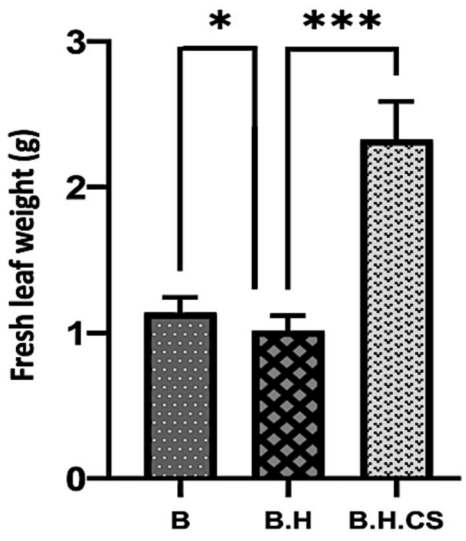

Fig. 11 Effect of chitosan treatment on fresh leaf weight (g): B (control basil), B.H (hydroponic basil) and B.H.CS (hydroponic basil treated with chitosan)

1.5 g (Vázquez-Vázquez et al. 2015), even under sulfur treatments or inoculation with Thiobacillus (Gandomkar 2022).

Biochemical characterization

Quantification of chlorophyll pigments

Table 3 presents a comparison of chlorophyll a, chlorophyll b, and carotenoid content in the three groups: control basil (B), hydroponic basil (B.H) and hydroponic basil treated with chitosan (B.H.CS). For chlorophyll a, the control basil (B) exhibits the lowest content, with an average of 0.16 mg/g. This value increases substantially in hydroponic basil (B.H) to 0.38 mg/g, suggesting that the hydroponic system enhances chlorophyll production. The highest value is observed in hydroponic basil treated with chitosan (B.H.CS), which reaches 0.63 mg/g. Regarding chlorophyll b, the control basil (B) again shows the lowest content at 0.18 mg/g, while hydroponic basil (B.H) displays a significant increase to 0.53 mg/g. The highest value is recorded in the chitosan-treated hydroponic basil (B.H.CS), with 0.99 mg/g. As shown above, B.H.CS positively influenced leaf count and SLA, and these results align with the observed increase in chlorophyll levels. This improvement can likely be attributed to the direct effect of B.H.CS on photosynthesis, chlorophyll synthesis, and chloroplast development. Similar findings have been reported in previous studies (El Amerany et al. 2023; Al-Gaadi et al. 2024).

In terms of carotenoids, control basil (B) has a content of 4.32 mg/g, which increases modestly in hydroponic basil (B.H) to 4.86 mg/g. However, the most significant

Table 3 The effect of chitosan on chlorophyll pigments *a* and *b* and carotenoids in mg/g. control basil (B), hydroponic basil (B.H), and hydroponic basil treated with chitosan (B.H.CS)

Treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Carotenoids (mg/g)
B	0.16 ± 0.08	0.18 ± 0.09	4.32 ± 0.38
B.H	0.38 ± 0.05 (**)	0.53 ± 0.07 (*)	4.86 ± 0.26 (*)
B.H.CS	0.63 ± 0.13 (*)	0.99 ± 0.08 (**)	6.65 ± 0.23 (*)

*Values are presented as means ± standard deviation. Statistically significant differences ($n = 3$; $p < 0.05$) compared with B (control) are indicated by *. Statistical evaluation was performed using ANOVA with $p < 0.05$ considered statistically significant between control and treated groups

increase occurs in the hydroponic basil treated with chitosan (B.H.CS), where carotenoid levels reach 6.65 mg/g. Carotenoids are group of molecules typically produced in plant tissues in response to blue and ultraviolet (UV) light, as well as stress conditions (Kim and Eom 2025). The data from this study suggest that chitosan may induce oxidative stress, thereby triggering the plant's defense mechanisms and elevating the levels of antioxidant molecules, such as carotenoids (Acemi et al. 2021). Additionally, the hydroponic system itself might contribute to this effect by increasing the plant's exposure to light and temperature. These findings contrast with those reported by Kimura and Rodriguez-Amaya (2003), which may be attributed to differences in the type of hydroponic system and greenhouse conditions used.

Proteins and sugars quantification

Figure 12 illustrates the effect of chitosan treatment on the concentrations of sugars (g/L) and proteins (µg/g) in basil under three different conditions: control basil (B), hydroponic basil (B.H), and hydroponic basil treated with chitosan (B.H.CS). For protein quantification, the control basil (B) displays a concentration of 66.55 µg/g, while hydroponic basil (B.H) shows a slightly higher concentration of 67.11 µg/g. Notably, hydroponic basil treated with chitosan (B.H.CS) exhibits the highest protein concentration at 71.11 µg/g, suggesting that chitosan treatment positively influences protein accumulation (Kumar Dutta et al. 2004), potentially enhancing the nutritional quality of the basil. In terms of sugar concentration, the control basil (B) has a sugar content of 0.08 g/L, which is higher than that of hydroponic basil (B.H), measured at 0.06 g/L. However, hydroponic basil treated with chitosan (B.H.CS) shows a significant increase in sugar concentration to 0.17 g/L, which is in line with the standard sugar concentration of basil, ranging between 0.1 and 0.3% (FAOSTAT 2017). This indicates that chitosan treatment not only enhances protein levels but also promotes higher sugar accumulation in basil. These variations demonstrate that chitosan treatment effectively boosts both protein and sugar concentrations in hydroponic basil, which may contribute to its improved nutritional and functional properties.

Antioxidant activity

Ocimum basilicum L. is renowned for its effective antioxidant activity (Soran et al. 2009), which allows it to be

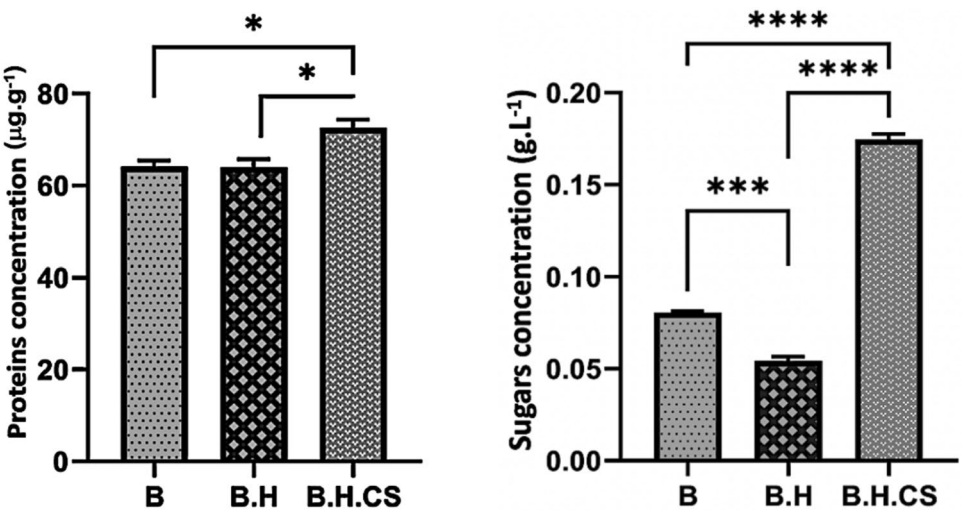

Fig. 12 Effect of chitosan treatment on sugars (g/L) and protein concentration (µg/g) in B (control basil), B.H (hydroponic basil), and B.H.CS (hydroponic basil treated with chitosan)

used in various biomedical and pharmaceutical treatments (Telci et al. 2009). Chitosan, employed as a biostimulant and biofertilizer in this study, also contributes to enhancing antioxidant properties (Hajji et al. 2015). The antioxidant activity assays conducted in this study and presented in Table 4 reveal insightful differences in antioxidant capabilities among the three groups: control basil (B), hydroponic basil (B.H), and hydroponic basil treated with chitosan (B.H.CS), as well as the reference standards, quercetin, and ascorbic acid.

In the DPPH assay, the lower values indicate higher antioxidant activity. Hydroponic basil treated with chitosan (B.H.CS) exhibited the lowest DPPH value of 1.25 ± 0.02 mg/mL, indicating the highest antioxidant activity among the samples tested. Following this, the control basil (B) showed a DPPH value of 3.49 ± 0.01 mg/mL, indicating a moderate level of antioxidant activity. Hydroponic basil (B.H) had a DPPH value of 3.09 ± 0.03 mg/mL, suggesting that while it retains some antioxidant properties, it is not as effective as the chitosan-treated variant. In the FRAP assay, the trend mirrors that of the DPPH results, with hydroponic basil treated with chitosan (B.H.CS) again showing the lowest value of 1.51 ± 0.02 mg/mL, reflecting the highest antioxidant activity. The control basil (B) exhibited a FRAP value of 4.04 ± 0.01 mg/mL, while hydroponic basil (B.H) recorded a value of 3.66 ± 0.03 mg/mL. Previous studies on the antioxidant activity of two basil varieties (*Ocimum basilicum* L.), "Italiano Classico" and "Genovese," report DPPH levels ranging from 1.14 ± 0.08 to 1.86 ± 0.17, as well as FRAP values (Romano et al. 2022). In comparison with our results, hydroponic cultivation in the presence of chitosan significantly enhances antioxidant activity, surpassing that observed in soil-cultivated extracts of the same plant.

Table 4 Antioxidant activity of *Ocimum basilicum* L. measured by DPPH and FRAP assays in control basil (B), hydroponic basil (B.H), and hydroponic basil treated with chitosan (B.H.CS)

		DPPH (mg/mL)	FRAP (mg/mL)
Treatments	B	3.49 ± 0.01	4.04 ± 0.01
	B.H	3.09 ± 0.03 (***)	3.66 ± 0.03 (**)
	B.H.CS	1.25 ± 0.02 (*)	1.51 ± 0.02 (*)
Standards	Quercetin	1.01 ± 0.03	2.48 ± 0.01
	Ascorbic acid	1.28 ± 0.01	3.75 ± 0.02

*Values are presented as means ± standard deviation. Statistically significant differences ($n = 3$; $p < 0.05$) compared with B (control) are indicated by *. Statistical evaluation was performed using ANOVA with $p < 0.05$ considered statistically significant between control and treated groups

Sustainability impact of chitosan-enhanced hydroponic/bioponic cultivation

The valorization of shrimp shell waste into chitosan presents a promising solution to address both environmental and agricultural challenges. In Morocco, vast amounts of crustacean waste are discarded annually, particularly from shrimp decortication units in the northern region. These units alone generate substantial waste streams that could be effectively repurposed. It is estimated that approximately 950 tons of pure chitin can be extracted from these residues, yielding up to 700 tons of highly to totally deacetylated chitosan (Arrouze et al. 2019). This transformation mitigates waste disposal issues and provides a high-value biopolymer with extensive applications. Chitosan's role in agriculture, particularly in hydroponic cultivation, represents an innovative and sustainable approach (Rojas-Pirela et al. 2024). Hydroponic systems are recognized for their efficient water use, reducing irrigation needs while maintaining optimal plant growth conditions. Integrating chitosan into such systems enhances nutrient uptake, promotes plant resilience and improves overall yield quality. This method aligns with the concept of bioponics, where biological materials replace synthetic additives to support plant development. Given Morocco's increasing water scarcity, adopting chitosan-based hydroponics offers a viable alternative to conventional farming by preserving water resources without compromising productivity.

Additionally, this technology is also cost-effective, it required less equipment compared to the soil cultivation (i.e., irrigation systems, fertilize spreaders, tractors, and plows). The integration of recycled plastic as a material for hydroponic pots could also further strengthens the sustainability aspect, reducing plastic waste while lowering production costs. This dual strategy utilizing chitosan as a bioenhancer and repurposing plastic for hydroponic infrastructure fosters a circular economy approach, making the method more accessible for large scale agricultural implementation.

Conclusion

This study investigated the effects of chitosan derived from royal shrimp shells with an acetylation degree of 2.92% and a molecular weight of 101 kDa on the growth and biochemical properties of *Ocimum basilicum* L. cultivated in hydroponics. The results indicate that chitosan-treated hydroponic basil (B.H.CS) outperformed both soil-grown and untreated hydroponic plants. Root elongation increased by 65%, reaching 12.5 cm, while shoot height rose to 11.26 cm, representing a 49% increase over untreated basil. Additionally, the leaf count doubled, averaging 12.66 leaves, and the fresh leaf weight reached 2.32 g compared to 1.08 g without treatment.

Biochemically, chlorophyll a and b content increased to 0.63 mg/g and 0.99 mg/g, respectively, indicating enhanced photosynthetic efficiency. The protein levels also rose, reaching 71.11 µg/g versus 66.55 µg/g in the control group. Sugar content increased from 0.06 to 0.17 g/L in the B.H.CS group. In terms of antioxidant activity, the B.H.CS group had the lowest DPPH and FRAP values (1.25 mg/mL and 1.51 mg/mL, respectively), which means it had better antioxidant properties. These findings suggest that chitosan holds strong potential for promoting bioponic basil growth and improving its biochemical quality, thereby contributing to more sustainable agricultural practices and improved food security in Morocco. However, further investigations are needed to evaluate the long-term effects of chitosan application under varying concentrations and to assess its commercial viability across different crop systems. Among our future perspectives, applying this approach to economically important crops in Morocco such as tomatoes will be of particular interest.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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