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Commercial biostimulants based on humic substances and seaweed extracts improve growth, physiology, essential oil content, and biochemical quality of *Thymus vulgaris* L. grown under organic conditions

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Summary: The increasing demand for medicinal and aromatic plants (MAP) requires sustainable cultivation practices to improve both productivity and product quality. Their essential oils and secondary metabolites are used for flavoring and treating various ailments in humans and animals. However, information on the effects of humic substances and seaweed extracts on organically grown *Thymus vulgaris* cultivar 'Winter' under semi-arid conditions in the northeastern Morocco remains limited. Therefore, this study evaluated the effect of two commercial biostimulants based on seaweed extracts (SE) and humic substances (HS) on vegetative growth and biochemical traits, biomass yield, and essential oil content of *Thymus vulgaris* L. under open-field organic cultivation. The results showed that HS at 200 mg L^{-1} and SE at 2 g L^{-1} significantly ($p < 0.05$) enhanced most vegetative traits, yield, photosynthetic pigments, soluble sugars, and soluble protein content, compared with the control. The greatest improvements under HS200 and SE2 were observed in plant height (39% and 21%), root length (66% and 53%), biomass yield (30% and 33%), and total content of chlorophylls (153% and 46%), carotenoids (11% and 24%), glucose (280% and 230%), sucrose (360% and 250%), and soluble protein (34% and 45%). In addition, SE at 2 g L^{-1} enhanced essential oil content by 75%. These findings demonstrate that humic substances and seaweed extracts, particularly at 200 mg L^{-1} and 2 g L^{-1}, respectively, can improve biomass production and selected biochemical traits of *Thymus vulgaris* L. under the experimental conditions of this study.

Keywords: biostimulants, chlorophyll, essential oils, humic substances, organic cultivation, seaweed extract

Introduction

Medicinal and aromatic plants (MAPs) are high-value crops cultivated worldwide for their essential oils and bioactive secondary metabolites, which are extensively used in the pharmaceutical,

food, and cosmetic industries (Nurzyńska-Wierdak, 2023). These plants have played an essential role in human life for thousands of years. According to the World Health Organization (2019) almost 80% of the population in 170 WHO Member States relies on MAP as part of traditional medicine. Among these species, thyme (*Thymus vulgaris* L.) is a valuable aromatic and medicinal plant. This species belongs to the *Lamiaceae* family and is native to the scrublands and steppes of southern Europe and northern Africa.

Thyme is widely cultivated because of its high economic value, particularly in the pharmaceutical, food, cosmetic, and essential oil industries. Its essential oil is rich in bioactive compounds, mainly thymol and carvacrol, which are responsible for its antimicrobial, antioxidant, and anti-inflammatory properties (Al-Mijalli et al., 2025). In recent years, MAP plants have received increasing worldwide attention due to their utilization in several fields such as the food, pharmaceutical, and natural cosmetic products, as well as in the production of natural pesticides and repellents (Salamon, 2024). In addition, numerous studies have investigated their potential therapeutic properties, particularly their anticancer and hepatoprotective activities (Banerjee et al., 2023; Daoudi & Bnouham, 2020).

The essential oil (EO) from medicinal plants contains various phytochemicals, which have antiseptic, antifungal, and antioxidant properties and are used for medicinal purposes (Subudhi et al., 2025; Tohidi et al., 2019). Considerable efforts have been devoted to the use of chemical inputs to increase the yield and quality of MAP. However, their excessive application in MAP cultivation may adversely affect soil fertility and groundwater quality, while also leading to the accumulation of residues in medicinal plant products, thereby raising environmental and health concerns (Bijay-Singh & Craswell, 2021; Tripathy et al., 2015). It is therefore essential to implement sustainable measures to reduce the use of these chemical inputs to maintain crop productivity, protect the environment, and human health. Biostimulants have emerged as a promising approach to achieve this objective.

Ascophyllum nodosum seaweed extract is rich in bioactive compounds, including polysaccharides, phenolic compounds, phytohormones, essential minerals, and vitamins, while humic substances consist primarily of humic and fulvic acids. The use of these eco-friendly biostimulant can boost nutrient absorption, activate plant metabolism, increase resistance to abiotic stresses, and foster secondary metabolite production, leading to improved plant growth, productivity, and resilience to environmental stresses (Abdelkader et al., 2026; Canellas et al., 2024; Zulfiqar et al., 2024).

Unlike synthetic fertilizers, which primarily supply mineral elements, biostimulants can influence plants' physiological, metabolic, and hormonal processes while improving nutrient use efficiency and responses to environmental stress (Lau et al., 2022).

Several studies have reported beneficial effects of biostimulants based on algal extracts on *Thymus vulgaris*, particularly on growth, biomass production, and antioxidant defenses (Cardone et al., 2025; Muetasam Jafr et al., 2022). A recent study also showed that the application of biostimulants based on humic substances and seaweed extract improves agronomic performance, nutrient uptake, and biochemical quality in *Ocimum basilicum* (Kisvarga et al., 2024), highlighting their potential for improving other MAP plants such as *Thymus vulgaris*. However, the effects of these biostimulants on the growth, biochemical traits, and essential oil content of organically grown *Thymus vulgaris* (Winter), under open-field semi-arid conditions in northeastern Morocco, remain insufficiently documented.

Therefore, the present study aims to investigate the effects of two commercial biostimulants based on seaweed extracts (SE) and humic substances (HS) on growth, biochemical parameters, and essential oil (EO) content of organically grown thyme.

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Materials and methods

Plant material and growth conditions: Thyme plants (*Thymus vulgaris* L.), cultivar 'Winter', originating from the Mediterranean basin (Southern Europe, North Africa), were used in this work. The experiment was conducted under certified organic farming conditions at the Green Herbs ecological farm located in the Nador region, Northeastern Morocco (CCPB SRL, operator code MA-BIO-102; operator No. M0394), according to the official register of certified organic farming operators.

Thyme seeds purchased from an accredited nursery were sown on January 20, 2024, in 5 cm diameter cell trays at a depth of about 0.5 to 1 cm (the cell trays contain a mixture of peat and peat moss in a ratio of 1:3). These cell trays were placed in a simple shade-house under natural environmental conditions to provide the appropriate conditions, with the temperature maintained at 20 °C, until the seeds became seedlings, with a germination rate of approximately 70%. After 21 days from the sowing date, the emerged uniform seedlings were transplanted to the experimental plot of the open field.

During the experimental period (January–April 2024), the average air temperature was 15.2 °C and the total precipitation was 63.3 mm. The loamy sand soil was characterized by 1.95% organic matter, a pH of 8.12, an electrical conductivity (EC) of 0.31 dS m^{-1}, and available K_2O and P_2O_5 contents of 202 and 45 mg kg^{-1}, respectively.

At the time of planting, 10 L ha^{-1} of VIGORALMOL (Éléphant Vert, EV), an organic fertilizer of plant origin, containing 30% organic matter, 2% organic nitrogen, 18% humic substances, 5% K_2O and 5% Betaines, was applied to the field according to the EV recommendation. Subsequently, when the thyme plants reached the stem elongation period, sprays of this same fertilizer at 5 L ha-1 were carried out weekly.

Two commercial biostimulants were evaluated. The first was a seaweed extract (SE) (ALGALIV), containing 15% *Ascophyllum nodosum* extract, 7% P_2O_5, 11% K_2O, and 0.9% of micronutrients (B, Fe-EDTA, Mn-EDTA, Zn-EDTA, Cu-EDTA, and Mo). The second was a humic substance-based biostimulant (HS) (VIGORALMOL), containing 30% organic matter, 18% fulvic acids, 5% K_2O, 2% nitrogen, and 5% betaine. Both products were purchased from EV, Meknès Branch, Morocco.

In this manuscript, SE and HS refer to the commercial seaweed extract-based and humic substance-based biostimulants, respectively.

The plants were watered twice a week, using drip emitters with a flow rate of 1 L h^{-1} for 2–3 h per irrigation session, and treated by foliar application of humic substance 100 (T1) and 200 mg L-1 (T2) and *Ascophyllum nodosum* seaweed extract (SE) at 1 (T3) and 2 g L-1 (T4). The HS dose followed EV recommendation, whereas the SE concentrations were selected based on previous farm experience and published studies on MAP.

Subsequently, these concentrations were sprayed using a 20-L backpack sprayer at 9:00 am on the leaves until they were completely covered (approximately 1 L plot-1), and at an interval of 15 days between spraying and the next. The first spraying was carried out 21 days after planting, and the untreated plants were sprayed with tap water (T0).

Experimental design: The experiment was conducted using a randomized complete block design (RCBD) with five treatments (Control, HS100, HS200, SE1, and SE2) and three replicates per treatment. Each replicate consisted of one experimental plot (1 m^2), resulting in a total of 15 plots. Each plot contained 20 plants (300 plants in total). Adjacent plots within each block were separated by 1 m, whereas blocks were separated by 0.5 m. Within each plot, plants were spaced 23 cm between rows and 19 cm within rows.

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Plant analysis: Seventy days after transplanting, plants were harvested using hand pruners. For growth measurements, two plants were randomly selected from each of the three replicate plots (six plants per treatment in total). For biochemical analyses, one plant was randomly selected from each replicate plot (three plants per treatment). Border plants were excluded from sampling. The growth parameters such as branch number (n plant-1), plant height (cm), root length (cm), stem diameter (cm), shoot and root fresh weight (g), shoot and root dry weight (g) were determined using a graduated ruler, a digital scale, and a precision balance, respectively. Dry weights were obtained after oven-drying the samples at 70 °C for 48 h until constant weight.

Yield: At the time of harvest, the fresh aerial parts (stem, leaves, flowers) were measured using a digital scale and the average values were reported based on the number of g per m^2.

Chlorophyll determination: Samples of 0.1 g of *T. vulgaris* fresh materials used for the measurement of Amax were extracted in 5 mL of pure methanol (100%) for spectrophotometric determination of the concentration of total chlorophyll and carotenoids according to Lichtenthaler & Wellburn, (1983).

Absorbance was then measured at 470, 653, and 666 nm using a UV–Vis spectrophotometer and were calculated using the following equations. Results were expressed as mg g-1 fresh weight (FW).

$$\text{Chla} = 15.65\, A_{666} - 7.340\, A_{653}$$

$$\text{Chlb} = 27.05\, A_{653} - 11.21\, A_{666}$$

$$\text{Carotenoids} = \frac{(1000\, A_{470} - 2.860\, Chl(a) - 129.2 Chl(b))}{245}$$

Glucose and sucrose determination: Samples of 0.5 g of fresh plant tissues were crushed in 5 mL 95% (v/v) ethanol. The insoluble fraction of the extract was washed twice with 5 mL of 70% ethanol. All soluble fractions were centrifuged at 3500 × g for 10 min. The supernatants were collected and stored at 4 °C for glucose and sucrose determinations. Glucose and sucrose concentrations (µg g-1 FW) were determined spectrophotometrically at A650 using the colorimetric assay with the anthrone reagent (Irigoyen et al., 1992).

Soluble-protein determination: Fresh *T. vulgaris* samples (0.5 g) were crushed with cold phosphate buffer (50 mM KH2PO4, pH 7.0) and centrifuged at 12 000 × g for 15 min. The resulting supernatant was used for the determination of soluble proteins (µg g-1 FW) using Bradford G-250 reagent (Bradford, 1976).

The essential oil (EO) concentration was determined by hydro distillation of the air-dried leaves with a Clevenger apparatus after 3 hours. These measurements began when the first drop of liquid condensed in the cooling column and dripped into the graduated burette. Air-dried thyme leaves (75 g) from each sample were introduced into an extraction flask with 750 mL of distilled water. The extraction was carried out for 180 min (until no essential oil was present). After condensation, the essential oil from each sample was collected and placed in a bottle containing anhydrous sodium sulfate (Na_2SO_4) to remove any water and stored in a refrigerator at 4 °C until use (Gedikoğlu et al., 2019). The EO content was expressed as a percentage (%, v/w) based on the dry weight of the plant material and was calculated using the following equation (Ghatas et al., 2021).

$$Essential\ oil\ content\ (\%) \frac{oil\ volume\ in\ the\ graduated\ tube\ (ml)}{dry\ weight\ of\ sample\ (g)} \times 100$$

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Statistics: Statistical analysis was performed using IBM SPSS (version 26). For each parameter evaluated, replicates were taken for the treatments. Means and standard deviations were used to determine differences. Normality and homogeneity of variance were verified before performing ANOVA. Duncan's test was used, and the significance level was set at $p < 0.05$. Percentage increases were calculated relative to the control treatment using the following formula:

$$\frac{Treatment - Control}{Control} \times 100$$

A heatmap was generated using Python (version 3.12) with the Seaborn library to visualize the distribution and clustering patterns of the measured variables across treatments.

Results

The effects of HS and SE on plant growth parameters and yield are presented in Table 1. Plant height increased significantly ($p < 0.05$) under HS treatments, with a maximum value of 23.75 cm obtained at 200 mg L-1 (T2), compared to 17.10 cm for the control (T0). SE at 2 g L-1 (T4) also stimulated height to 20.75 cm ($p < 0.05$). Regarding shoot fresh weight, T2 treatment produced highest value of biomass (27.10 g), followed by T4 (26.27 g), compared to 17.55 g for the control plants ($p < 0.05$). Shoot dry weight followed a similar trend, however, differences among treatments were not statistically significant ($p > 0.05$). The stem diameter increased significantly ($p < 0.05$) with HS treatments, rising from 0.45 mm (control) to 0.78 mm (T2: 200 mg L^{-1}), while SE at 2 g/L resulted in an intermediate value of 0.63 mm ($p < 0.05$). All treatments increased significantly ($p < 0.05$) *Thymus vulgaris* branching, with a maximum value of 99.2 observed at 2g L^{-1} (SE), compared to 89 for the control.

Regarding root parameters, root length was significantly improved ($p < 0.05$) by 200 mg L^{-1} of HS (10.38 cm) and 2 g L^{-1} of SE (9.58 cm), compared to 6.25 cm for the control. For root fresh weight, the highest values ($p < 0.05$) were obtained with 2 g L-1 of seaweed extract (RFW = 3.27 g) and 200 mg L^{-1} of HS (RFW = 2.93 g), compared to 1.96 g for the control. A similar trend was observed for root dry weight, however, differences among treatments were not statistically significant ($p > 0.05$). The highest yield was observed with SE at 2 g L^{-1} (678.4 g m^{-2}), followed by HS at 200 mg L^{-1} (661.6 g m^{-2}), while the control yield was only 510.2 g m^{-2} (Table 1).

Table 1. Effect of biostimulants on the vegetative growth parameters of *Thymus vulgaris*

Treatments	PH (cm)	SD (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	RL (cm)	BN (n plant^{-1})	Yield (g m^{-2})
T0 (control)	17.10± 0.93e	0.45± 0.084c	17.55± 1.45d	1.96± 0.64b	5.24± 1.42a	1.32± 0.24a	6.25± 0.93c	89.0± 2.37d	510.2± 11.22d
T1 (HS100 mg L^{-1})	22.72± 0.92b	0.73± 0.100a	21.34± 7.97b	2.47± 0.72ab	6.60± 1.46a	1.52± 0.23a	8.00± 2.61b	95.6± 3.41bc	591.2± 97.28b
T2 (HS200 mg L^{-1})	23.75± 1.31a	0.78± 0.136a	27.10± 1.41a	2.93± 0.73a	7.00± 1.41a	1.75± 0.13a	10.38± 1.83a	94.6± 3.37c	661.6± 67.50a
T3 (SE1g L^{-1})	19.68± 1.37d	0.50± 0.110c	20.50± 1.45c	2.66± 0.86ab	5.55± 0.88a	1.56± 0.22a	6.75± 0.64 bc	97.2± 4.67ab	528.5± 140.80c
T4 (SE2g L^{-1})	20.75± 3.89c	0.63± 0.051b	26.27± 0.91ab	3.27± 0.38a	6.06± 1.25a	1.56± 0.12a	9.58± 0.65b	99.2± 1.86a	678.4± 23.71a

Means in the same column, followed by different letters, indicate significant differences ($p < 0.05$), while the same letters are not statistically different. Values are means ± SD.
Plant height: PH, Stem diameter: SD, Shoot fresh weight: SFW, Root fresh weight: RFW, Shoot dry weight: SDW, Root dry weight: RDW, Root length: RL, Branch number: BN

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Figure 1 shows the effect of HS and SE on total chlorophyll and carotenoids in thyme plants. The results showed that all treatments increase significantly total content of chlorophylls, with T2 treatment (200 mg L^{-1} of HS) led to the greatest increase ($p < 0.05$) in total chlorophyll, reaching 5.44 mg g^{-1} FW, representing a 153% increase compared to the control.

Regarding carotenoids, the response was less pronounced than that observed for total chlorophyll. Treatment T4 reached the highest concentration (1.94 mg g^{-1}), corresponding to a 24.4% increase compared to the control ($p < 0.05$). Conversely, treatments T1 and T3 slightly decreased carotenoid levels ($p > 0.05$).

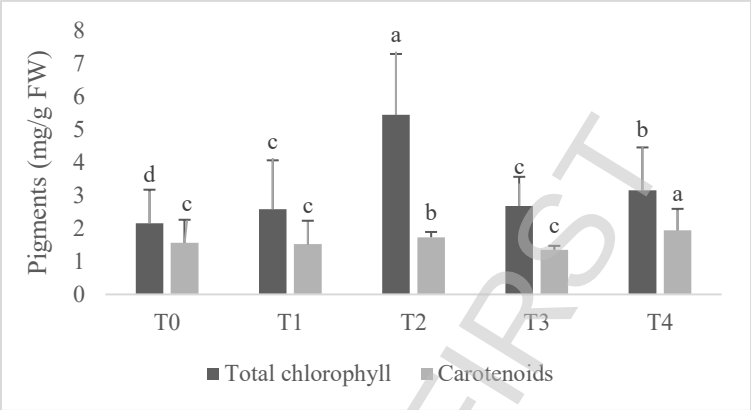


Figure 1. Effect of biostimulants on total chlorophyll and carotenoids in thyme plants. Error bars indicate standard deviation. Different letters above bars indicate significant differences ($p < 0.05$), while the same letters indicate no significant effect.

Figure 2 presents the concentration of essential oil in thyme plants treated with (HS) and (SE) biostimulants. Our results showed a dose-dependent increase in essential oil content for both biostimulants, with SE showing a significant increase ($p < 0.05$) (up to +75% at 2 g L^{-1}) compared to HS (+37.5% at 200 mg L^{-1}).

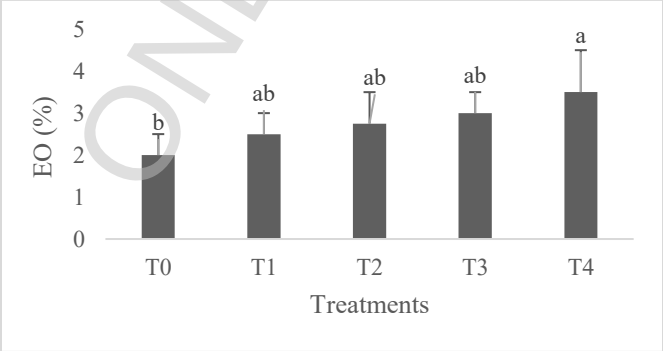


Figure 2. Effect of biostimulants on essential oil content in thyme plants. Error bars indicate standard deviation. Different letters above bars indicate significant differences ($p < 0.05$), while the same letters indicate no significant effect.

Soluble protein concentrations varied according to the treatment (Figure 3), with a minimum value in the control T0 (35.21 µg g-1 FW) and a maximum in T4 (51.09 µg g-1 FW). HS (T1, T2) increased ($p < 0.05$) protein levels to 44.79 µg g-1 FW and 47.04 µg g-1 FW, respectively, representing increases of +27% and +34% compared to the control. In relation to SE, statistical

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analysis showed that there was no significant difference ($p > 0.05$) between T0 and T3 (1 g SE L-1 H2O), while T4 (2 g L^{-1} SE L^{-1} H_2O) reached the highest concentration of soluble proteins (51.09 μg g^{-1} FW).

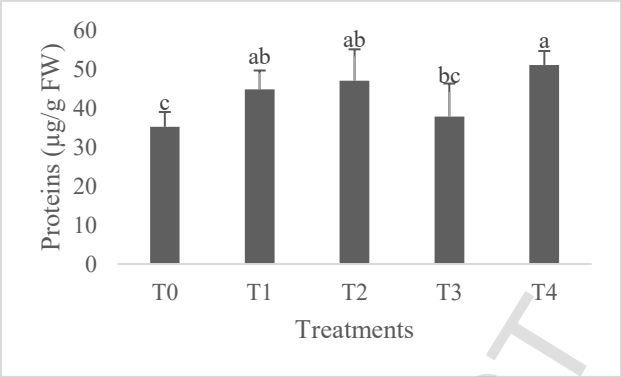


Figure 3. Effect of biostimulants on soluble proteins in thyme plants. Error bars indicate standard deviation. Different letters above bars indicate significant differences ($p < 0.05$), while the same letters indicate no significant effect.

Figure 4 shows that the control group had the lowest glucose (300 μg g^{-1} FW) and sucrose (94.67 μg g^{-1} FW) contents. All treatments significantly increased ($p < 0.05$) these levels, with the largest increase observed under T2, reaching 1140 and 438 μg g^{-1} FW for glucose and sucrose, respectively.

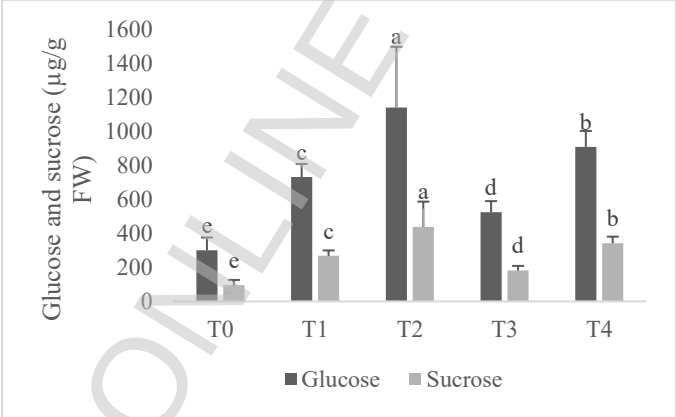
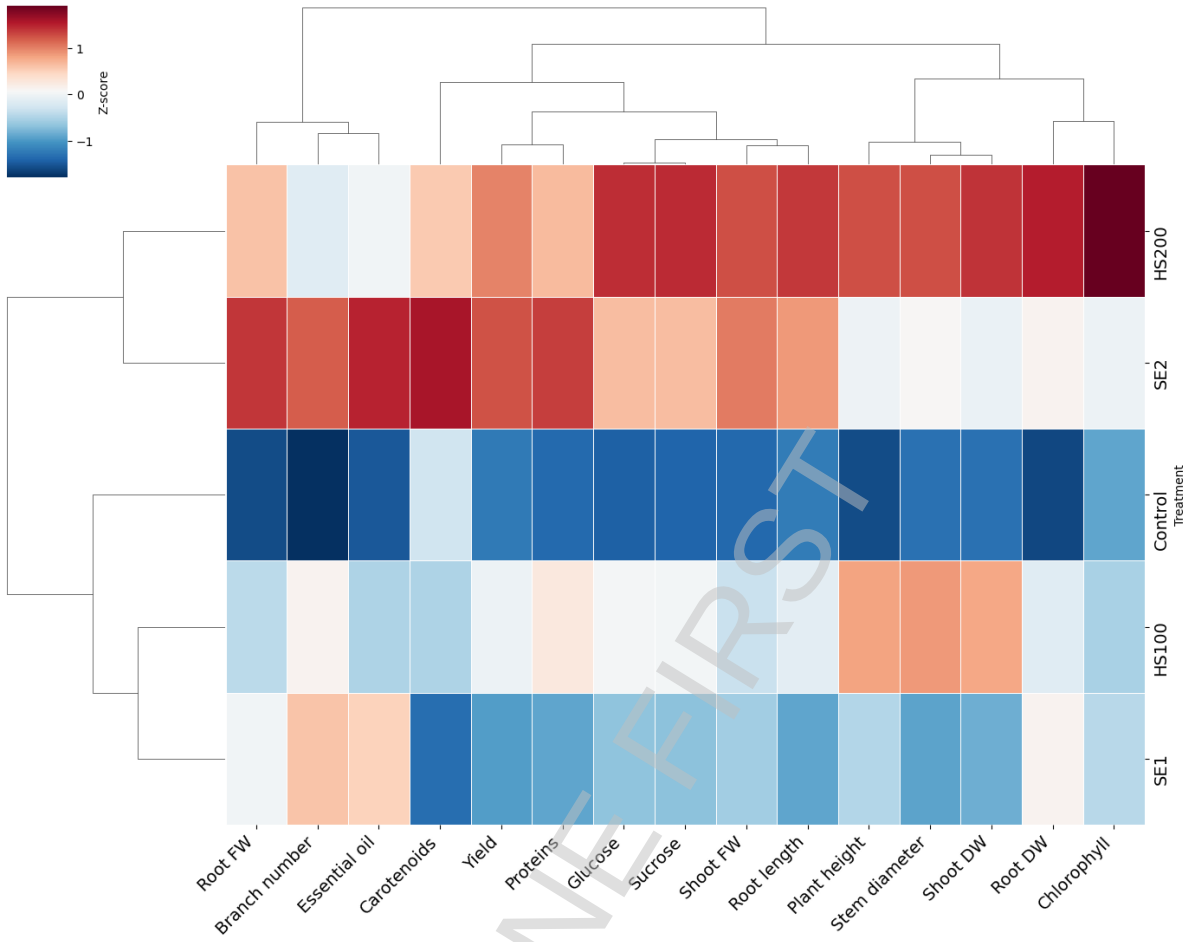


Figure 4. Effect of biostimulants on glucose and sucrose in thyme plants. Error bars indicate standard deviation. Different letters above bars indicate significant differences ($p < 0.05$), while the same letters indicate no significant effect.

Figure 5 shows that hierarchical clustering based on standardized (Z-score) mean values revealed clear discrimination among the treatments. HS200 was grouped separately and associated with the highest values for most measured traits. The control treatment, on the other hand, clustered independently and exhibited generally lower values. SE2 also exhibited favorable overall performance, particularly with regard to essential oil content and several plant growth parameters. In contrast, HS100 and SE1 displayed intermediate responses, reflecting moderate improvements relative to the control treatment.

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Figure 5. Hierarchical clustered heatmap shows the standardized (Z-score) responses of growth, yield, biochemical, and essential oil traits of *Thymus vulgaris* under different treatments. Red and blue colors indicate relatively high and low standardized values, respectively. Rows represent treatments and columns represent the measured variables.

Discussion

The beneficial effects of HS at 200 mg L^{-1} and SE at 2 g L^{-1} on thyme growth and yield can be attributed to the physiological and metabolic effects of these biostimulants, while the greater response observed at these highest concentrations is likely associated with the higher availability of bioactive compounds, resulting in stronger physiological and metabolic responses than those induced by the lower doses.

Humic substances have been reported to promote cell division, cell elongation, and root development, thereby enhancing water and nutrient uptake. They also have been reported to improve nutrient availability through their chelating properties while increasing soil fertility and water-holding capacity (Bera et al., 2024). Moreover, humic substances have been reported to exhibit hormone-like activity by modulating endogenous phytohormone levels and stimulating enzymatic activity, which enhances photosynthesis, respiration, and overall plant growth (Li et al., 2024).

Similar improvements in growth and yield following humic substances application were reported in organically *Thymus vulgaris* by Cardone et al. (2025), who observed increases in biomass

yield, branch number, and stem diameter of 20%, 18%, and 22%, respectively, compared with 30%, 6 %, and 73%, respectively, in the present study. Variations in environmental conditions, experimental duration, dose, and application methods may influence the magnitude of the response.

Similarly, seaweed extracts, rich in phytohormones (auxins and gibberellins), amino acids, and minerals, may enhance photosynthesis, nutrient uptake, and vegetative growth, thereby increasing biomass and yield (Alam et al., 2013). They may also regulate endogenous phytohormone levels by regulating genes involved in phytohormone biosynthesis, thereby promoting plant growth (De Saeger et al., 2020).

Similar trends have been reported in *Thymus vulgaris* by Rasuli et al. (2025), in which the application of *Laurencia caspica* seaweed extract significantly boosted vegetative and reproductive traits, including plant growth, total fresh and dry weight (shoots + roots) by 34%, 49%, and 41%, respectively, compared with 21%, 51%, and 16%, respectively, in the current study.

The similar trend between the two studies suggests that seaweed extracts may have a similar effect under different cultivation conditions. However, the magnitude of the response may vary depending on environmental conditions, the seaweed species, and application rates.

The increase in photosynthetic pigments, observed mainly under HS at 200 mg L^{-1} and EA at 2 g L^{-1}, may be attributed to enhanced uptake of nitrogen and magnesium, two essential elements for chlorophyll biosynthesis. Humic substances may stimulate nitrogen metabolism and chlorophyll synthesis (Bayat et al., 2021). They may also activate the plasma membrane H^+-ATPase, promoting root elongation, nitrate uptake, and protein synthesis, thereby indirectly enhancing photosynthetic pigment biosynthesis (Canellas et al., 2002). Whereas enhanced phosphorus and potassium uptake has been associated with improved photosynthetic performance (Jindo et al., 2020). Likewise, seaweed extracts provide cytokinins, auxins, and mineral nutrients that protect chloroplasts and enhance photosynthetic efficiency (Gupta et al., 2021). Similar increases in total content of chlorophylls (58–69%) were reported in *Thymus vulgaris* L. following the combined application of humic acid and mycorrhizal fungi (Taksera et al., 2023), although these increases were lower than the 153% increase observed in the present study.

Carotenoid content also increased significantly, although less than the total content of chlorophylls, suggesting a comparatively weaker response of carotenoid accumulation to biostimulant application under the study field conditions. The enhancement of photosynthetic activity subsequently promotes CO_2 assimilation and carbohydrate accumulation, which may explain the higher glucose and sucrose contents observed, particularly in plants treated with HS at 200 mg L^{-1}. This response may also be associated with improved phosphorus and potassium uptake induced by humic substances (Jindo et al., 2020). The increase in soluble protein content observed in this study could be attributed to the enhanced nitrogen uptake and metabolism induced by humic substances (Amerian et al., 2024). Likewise, seaweed extracts, rich in amino acids, betaines, phytohormones, and minerals, promote photoassimilation, soluble protein synthesis, and the accumulation of secondary metabolites (Rasuli et al., 2025). Similar findings under comparable conditions in another MAP (*Rosmarinus officinalis*) were reported by Nazih et al. (2026), in which the application of humic substances, alone or in combination with seaweed extracts, significantly increased soluble sugar contents by 51% and 97%, and soluble protein contents by 94% and 96%, respectively. In the present study, soluble sugars increased by 300% and 216%, while soluble proteins increased by 34% and 45%, respectively, under HS at 200 mg L^{-1} and EA at 2 g L^{-1}. Although the study was conducted on a different species, the similar responses suggest that these biostimulants may enhance primary metabolism in aromatic plants.

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The increase in essential oil content may also be attributed to enhanced photosynthesis and the availability of carbon precursors for terpene biosynthesis (Hashemifar et al., 2025). Similar improvements in essential oil content have also been reported in another aromatic species, *Trachyspermum ammi* L. (El-Nagar et al., 2025), with increases of 17.86% and 23.35% during the first and second seasons, respectively, following the application of seaweed extract. More specifically, Waly et al. (2020) reported that seaweed extract increased EO content in *Thymus vulgaris* L. by 74%, which was higher than 37.5% increase observed in the present study. This difference may be attributed to variations in growing conditions, dose, and experimental duration. However, only essential oil content was evaluated in the present study. Therefore, the influence of these biostimulants on the chemical composition and major constituents of thyme essential oil remains to be investigated.

Overall, these mechanisms enhanced vegetative growth, biomass yield, essential oil content, and the biochemical quality of *Thymus vulgaris*. However, these responses may be affected by climate, soil, irrigation, and seasonal factors. Nevertheless, the present findings were obtained during a single growing season under the specific soil and climatic conditions of northeastern Morocco, which may have influenced plant responses. Therefore, further multi-season and multi-location studies, including essential oil composition and economic feasibility analyses, are recommended to confirm the broader applicability of these biostimulants for sustainable thyme production.

The study results suggest the effectiveness of these algae extract and humic substance biostimulants, which could be used in commercial and organic thyme production to further improve growth, biomass, and essential oil content. This environmentally friendly and sustainable approach could contribute to crop productivity under a changing climate. These results are promising for thyme cultivation in semi-arid and Mediterranean regions, where more sustainable management practices are needed to cope with environmental constraints.

Conclusion

The application of HS and SE significantly improved the growth, physiological performance, biomass yield, essential oil content, and biochemical quality of *T. vulgaris*. Among the tested treatments, HS at 200 mg L^{-1} and seaweed extract at 2 g L^{-1} were the most effective, suggesting their potential as biostimulants for enhancing thyme productivity and quality under the experimental conditions of this study. These findings suggest that tested biostimulants may represent promising tools for sustainable thyme cultivation. However, further studies under different environmental conditions and over multiple growing seasons are needed to confirm their broader applicability, particularly in semi-arid regions.

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Author contributions: A.R.: Conceptualization, Writing – original draft, Writing – review & editing, Formal analysis; A.N.: Formal analysis, Software, Writing – original draft, Writing – review & editing; K.E.: Methodology, Data curation; H.E.H.: Conceptualization, Resources, Investigation; M.B.: Validation, Supervision, Project administration.

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References

Abdelkader, M., Bhuker, A., Malik, A., Punia, H., Koul, A., Ahmed, M., Elshamly, A. M. S., Iqbal, R., Aghayeva, S., & Ullah, S. (2026). Cultivating resilience from the ocean to the field: Leveraging seaweed biostimulants for sustainable and efficient farming systems. *Plant Stress*, 19, 101170. https://doi.org/10.1016/j.stress.2025.101170

Alam, M. Z., Braun, G., Norrie, J., & Hodges, D. M. (2013). Effect of Ascophyllum extract application on plant growth, fruit yield and soil microbial communities of strawberry. *Canadian Journal of Plant Science*, 93(1), 23-36. https://doi.org/10.4141/cjps2011-260

Al-Mijalli, S. H., Alshabrmi, F. M., Alnasser, S. M., El Hachlafi, N., Mrabti, H. N., Addi, M., Elbouzidi, A., Ashmawy, N. S., Batbat, A., Abdallah, E. M., Laaboudi, W., & Aladhadh, M. (2025). Thymus serpyllum L. Essential Oil : Phytochemistry and in Vitro and in Silico Screening of Its Antimicrobial, Antioxidant and Anti-Inflammatory Properties. *Phyton*, 94(1), 209-227. https://doi.org/10.32604/phyton.2025.060438

Amerian, M., Palangi, A., Gohari, G., & Ntatsi, G. (2024). Humic acid and grafting as sustainable agronomic practices for increased growth and secondary metabolism in cucumber subjected to salt stress. *Scientific Reports*, 14(1). https://doi.org/10.1038/s41598-024-66677-8

Banerjee, S., Nau, S., Hochwald, S. N., Xie, H., & Zhang, J. (2023). Anticancer properties and mechanisms of botanical derivatives. *Phytomedicine Plus*, 3(1), 100396. https://doi.org/10.1016/j.phyplu.2022.100396

Bayat, H., Shafie, F., Aminifard, M. H., & Daghighi, S. (2021). Comparative effects of humic and fulvic acids as biostimulants on growth, antioxidant activity and nutrient content of yarrow (Achillea millefolium L.). *Scientia Horticulturae*, 279, 109912. https://doi.org/10.1016/j.scienta.2021.109912

Bera, B., Bokado, K., Barkha, ., & Arambam, S. (2024). Effect of Humic Acid on Growth, Yield and Soil Properties in Rice : A Review. *International Journal of Plant & Soil Science*, 36(6), 26-35. https://doi.org/10.9734/ijpss/2024/v36i64603

Bijay-Singh, & Craswell, E. (2021). Fertilizers and nitrate pollution of surface and ground water : An increasingly pervasive global problem. *SN Applied Sciences*, 3(4), 518. https://doi.org/10.1007/s42452-021-04521-8

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Canellas, L. P., Da Silva, R. M., Busato, J. G., & Olivares, F. L. (2024). Humic substances and plant abiotic stress adaptation. *Chemical and Biological Technologies in Agriculture*, 11(1), 66. https://doi.org/10.1186/s40538-024-00575-z

Canellas, L. P., Olivares, F. L., Okorokova-Façanha, A. L., & Façanha, A. R. (2002). Humic Acids Isolated from Earthworm Compost Enhance Root Elongation, Lateral Root Emergence, and Plasma Membrane H+-ATPase Activity in Maize Roots. *Plant Physiology*, 130(4), 1951-1957. https://doi.org/10.1104/pp.007088

Cardone, L., Polito, F., Denora, M., Casiello, D., Castronuovo, D., Cicco, N., Perniola, M., De Feo, V., & Candido, V. (2025, février 11). Effects of Biostimulant Foliar Applications on Morphological Traits, Yield, Antioxidant Capacity, and Essential Oil Composition of Thymus vulgaris L. Under Field Conditions. *Agronomy*, 15(2), 442. https://doi.org/10.3390/agronomy15020442

Daoudi, N. E., & Bnouham, M. (2020). Hepatoprotective Essential Oils: A Review. *Journal of Pharmacopuncture*, 23(3), 124-141. https://doi.org/10.3831/KPI.2020.23.3.124

De Saeger, J., Van Praet, S., Vereecke, D., Park, J., Jacques, S., Han, T., & Depuydt, S. (2020). Toward the molecular understanding of the action mechanism of Ascophyllum nodosum extracts on plants. *Journal of Applied Phycology*, 32(1), 573-597. https://doi.org/10.1007/s10811-019-01903-9

El-Nagar, A. H., Ghanem, K. Z., Hassan, F. A. S., Fetouh, M. I., El-Serafy, R. S., & Moussa, M. M. (2025). The changes in growth, yield, and biologically active compounds of essential oil in Trachyspermum ammi L. upon rhizobacteria and seaweed applications. *Plant, Soil and Environment*, 71(8), 565-580. https://doi.org/10.17221/266/2025-PSE

Gedikoğlu, A., Sökmen, M., & Çivit, A. (2019). Evaluation of Thymus vulgaris and Thymbra spicata essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. *Food Science & Nutrition*, 7(5), 1704-1714. https://doi.org/10.1002/fsn3.1007

Ghatas, Y., Ali, M., Elsadek, M., & Mohamed, Y. (2021). Enhancing growth, productivity and artemisinin content of Artemisia annua L. Plant using seaweed extract and micronutrients. *Industrial Crops and Products*, 161, 113202. https://doi.org/10.1016/j.indcrop.2020.113202

Gupta, S., Stirk, W. A., Plačková, L., Kulkarni, M. G., Doležal, K., & Van Staden, J. (2021). Interactive effects

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of plant growth-promoting rhizobacteria and a seaweed extract on the growth and physiology of Allium cepa L. (onion). *Journal of Plant Physiology*, 262, 153437. https://doi.org/10.1016/j.jplph.2021.153437

Hashemifar, Z., Sanjarian, F., Naghdi Badi, H., & Mehrafarin, A. (2025). Impact of varying light intensities on morphology, phytochemistry, volatile compounds, and gene expression in Thymus vulgaris L. *PLOS ONE*, 20(2), e0317840. https://doi.org/10.1371/journal.pone.0317840

Irigoyen, J. J., Einerich, D. W., & Sánchez-Díaz, M. (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa) plants. *Physiologia Plantarum*, 84(1), 55-60. https://doi.org/10.1111/j.1399-3054.1992.tb08764.x

Jindo, K., Canellas, L., Albacete, A., Figueiredo Dos Santos, L., Frinhani Rocha, R., Carvalho Baia, D., Oliveira Aguiar Canellas, N., Goron, T., & Olivares, F. (2020). Interaction between Humic Substances and Plant Hormones for Phosphorous Acquisition. *Agronomy*, 10(5), 640. https://doi.org/10.3390/agronomy10050640

Kisvarga, S., Horotán, K., Hamar-Farkas, D., & Orlóci, L. (2024). Effects of Humic Acids, Seaweed Extract and Equisetum arvense L. Extracts on Morphological, Histological and Physiological Parameters of the Ornamental Plant Ocimum basilicum Rokokó. *Horticulturae*, 10(11), 1231. https://doi.org/10.3390/horticulturae10111231

Lau, S.-E., Teo, W. F. A., Teoh, E. Y., & Tan, B. C. (2022). Microbiome engineering and plant biostimulants for sustainable crop improvement and mitigation of biotic and abiotic stresses. *Discover Food*, 2(1), 9. https://doi.org/10.1007/s44187-022-00009-5

Li, X., Zhi, Y., Jia, M., Wang, X., Tao, M., Wang, Z., & Xing, B. (2024). Properties and photosynthetic promotion mechanisms of artificial humic acid are feedstock-dependent. *Carbon Research*, 3(1), 4. https://doi.org/10.1007/s44246-023-00085-x

Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 11(5), 591-592. https://doi.org/10.1042/bst0110591

Muetasam Jafr, S., Rahimi, A. R., Hashemi, M., & Rokhzadi, A. (2022). Influence of N, K, and Seaweed Extract Fertilization on Biomass, Photosynthetic Pigments, and Essential Oil of Thymus vulgaris: Optimization Study by Response Surface Methodology. *Agronomy*, 12(12), 3222. https://doi.org/10.3390/agronomy12123222

Nahed R. Taksera, Eman F. AbuEl-Leil, & Gamal M. Abdel-Fattah. (2023). Impact of bio-fertilizer and humic acid applications on productivity and some biochemical characteristics of thymus vulgais grow in sandy soil. *Minia Journal of Agricultural Research and Development*, 43(4), 853-872. https://doi.org/10.21608/mjard.2023.340131

Nazih, A., El Jafoufi, C., Akodad, M., Skalli, A., El Haddaji, H., & Baghour, M. (2026). Enhancing morphological traits, yield, and chemical quality of rosemary (Rosmarinus officinalis L.) using biostimulants. *Vegetos*. https://doi.org/10.1007/s42535-026-01744-1

Nurzyńska-Wierdak, R. (2023). Chemical Diversity, Yield, and Quality of Aromatic Plants. Agronomy, 13(6), 1614. https://doi.org/10.3390/agronomy13061614

Rasuli, N., Riahi, H., Shariatmadari, Z., Nohooji, M. G., MehrabanJoubani, P., & Dehestani, A. (2025). Enhancing thymol and carvacrol biosynthesis in Thymus vulgaris L. using Laurencia caspica seaweed extract : Biostimulant potential and gene expression insights. *Journal of Applied Phycology*, 37(1), 645-657. https://doi.org/10.1007/s10811-024-03386-9

Salamon, I. (2024). Medicinal, Aromatic, and Spice Plants : Biodiversity, Phytochemistry, Bioactivity, and Their Processing Innovation. *Horticulturae*, 10(3), 280. https://doi.org/10.3390/horticulturae10030280

Subudhi, L., Thatoi, H., & Banerjee, A. (2025). Anti-inflammatory activity of essential oil from medicinal plants : An insight into molecular mechanism, in-silico studies and signaling pathways. *Phytomedicine*, 138, 156364. https://doi.org/10.1016/j.phymed.2025.156364

Tohidi, B., Rahimmalek, M., & Trindade, H. (2019). Review on essential oil, extracts composition, molecular and phytochemical properties of Thymus species in Iran. *Industrial Crops and Products*, 134, 89-99. https://doi.org/10.1016/j.indcrop.2019.02.038

Tripathy, V., Basak, B. B., Varghese, T. S., & Saha, A. (2015). Residues and contaminants in medicinal herbs - A review. *Phytochemistry Letters*, 14, 67-78. https://doi.org/10.1016/j.phytol.2015.09.003

Waly, A., Abd El-Fattah, Y., Hassan, M., El-Ghadban, E.-M., & Abd Alla, A. (2020). Enhancing growth, productivity and essential oil percentage of thymus vulgaris l. Plant using seaweeds extract, chitosan and potassium silicate in sandy soil. *Scientific Journal of Flowers and Ornamental Plants*, 7(4), 549-562. https://doi.org/10.21608/sjfop.2020.148056

World Health Organization. (2019). *WHO global report on traditional and complementary medicine 2019*. World Health Organization. https://iris.who.int/items/0dc0d605-b1c3-4350-b62e-984b47688442

Zulfiqar, F., Moosa, A., Ali, H. M., Bermejo, N. F., & Munné-Bosch, S. (2024). Biostimulants : A sufficiently effective tool for sustainable agriculture in the era of climate change? *Plant Physiology and Biochemistry*, 211, 108699. https://doi.org/10.1016/j.plaphy.2024.108699

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Komercijalni biostimulatori na bazi huminskih supstanci i ekstrakata morskih algi poboljšavaju rast, fiziologiju, sadržaj etarskog ulja i biohemijski kvalitet vrste *Thymus vulgaris* L. gajene u uslovima organske proizvodnje

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Sažetak: Sve veća potražnja za lekovitim i aromatičnim biljem (LAB) zahteva primenu praksi održivog uzgoja kako bi se poboljšali produktivnost i kvalitet proizvoda. Njihova eterična ulja i sekundarni metaboliti koriste se za aromatizaciju i lečenje različitih oboljenja kod ljudi i životinja. Međutim, podaci o uticaju huminskih supstanci i ekstrakata morskih algi na organski uzgoj sorte *Thymus vulgaris* „Winter" u polusušnim uslovima severoistočnog Maroka i dalje su ograničeni. Stoga je u ovoj studiji procenjen uticaj dva komercijalna biostimulatora na bazi ekstrakata morskih algi (SE) i huminskih supstanci (HS) na vegetativni rast, biohemijske karakteristike, prinos biomase i sadržaj eteričnog ulja vrste *Thymus vulgaris* L. u uslovima organskog uzgoja na otvorenom polju. Rezultati su pokazali da su HS u koncentraciji od 200 mg L^{-1} i SE u koncentraciji od 2 g L^{-1} značajno ($p < 0,05$) poboljšali većinu vegetativnih karakteristika, prinos, sadržaj fotosintetičkih pigmenata, rastvorljivih šećera i rastvorljivih proteina u poređenju sa kontrolom. Najveća poboljšanja pri tretmanima HS200 i SE2 zabeležena su kod visine biljke (39% i 21%), dužine korena (66% i 53%), prinosa biomase (30% i 33%) i ukupnog sadržaja hlorofila (153% i 46%), karotenoida (11% i 24%), glukoze (280% i 230%), saharoze (360% i 250%) i rastvorljivih proteina (34% i 45%). Pored toga, SE u koncentraciji od 2 g L^{-1} povećao je sadržaj eteričnog ulja za 75%. Ovi rezultati pokazuju da huminske supstance i ekstrakti morskih algi, posebno u koncentracijama od 200 mg L^{-1} i 2 g L^{-1}, mogu poboljšati proizvodnju biomase i određene biohemijske karakteristike vrste *Thymus vulgaris* L. u eksperimentalnim uslovima ove studije.

Ključne reči: biostimulatori, hlorofil, eterična ulja, huminske supstance, organski uzgoj, ekstrakt morskih algi

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