

Research Paper

Biostimulants enhance phenolic and volatile accumulation in common thyme under mild salinity stress

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ARTICLE INFO

Keywords:

Thymus vulgaris
Biostimulants
Salinity stress
Phenolic compounds
Volatile compounds
HPLC-MS
GC-MS
Secondary metabolism

ABSTRACT

Common thyme (*Thymus vulgaris* L.) is a widely cultivated aromatic herb that is valued for its high content of phenolic and volatile compounds, which contribute to its nutritional and sensory quality. A range of agronomic strategies have been investigated for the purpose of enhancing the quality of common thyme, including the implementation of mild abiotic stress. In this study, mild salinity stress was combined with four biostimulants to evaluate their effects on the phenolic and volatile profiles of common thyme. The identification of 14 phenolic compounds and 32 volatile compounds was achieved through the utilisation of HPLC-MS and GC-MS analysis, respectively. Salinity exhibited a significant effect on the total phenolic content, with a marked increase in comparison with the control. The incorporation of biostimulants served to augment phenolic accumulation, with Protifert® LMW exhibiting the most pronounced effect, resulting in an escalation of the total phenolic content by 44–98% relative to the control. It was established that rosmarinic acid and luteolin-3-O-glucuronide were the most responsive compounds. Concurrently, the volatile profile exhibited a substantial response to biostimulant application. The Delfan Plus and Protifert® LMW treatments resulted in the highest total volatile contents, with Delfan Plus reaching 167.31 mg kg⁻¹ FW, corresponding to increases of 129% and 52% compared with saline and non-saline conditions, respectively. Overall, mild salinity stress modulated phenolic and volatile metabolism in common thyme, while selected biostimulants enhanced metabolite accumulation. These findings underscore the efficacy of integrating controlled salinity stress with biostimulant application as a strategy to enhance the phytochemical quality of common thyme.

1. Introduction

Thymus vulgaris L., commonly known as common thyme, is an aromatic and medicinal plant belonging to the family Lamiaceae (Mamadalieva et al., 2017). It is indigenous to the Mediterranean region and has gained increasing economic importance in North America, Europe, and North Africa, where it is cultivated on a commercial scale in numerous countries. Common thyme can be cultivated in soil-based or hydroponic systems (Al-Ramamneh, 2009). Common thyme is distinguished by its high levels of volatile and phenolic compounds, which substantially contribute to its economic value (Nieto, 2020). Common thyme essential oil is considered among the ten most important essential oils and is widely used in the food and cosmetic industries (Mamadalieva et al., 2017). Common thyme represents a rich source of phenolic acids and flavonoids. The most prevalent phenolic acids include rosmarinic, caffeic, chlorogenic and protocatechuic acids, while flavonoids such as

luteolin and quercetin are also present. The principal volatile compounds responsible for the characteristic aroma of common thyme include thymol, carvacrol, *p*-cymene, β -myrcene and α -pinene, which have been shown to possess antioxidant, antimicrobial, anti-inflammatory and antiseptic properties (Waheed et al., 2024). Thymol, in particular, is the main component responsible for the antioxidant, antifungal, and antiseptic activity of common thyme essential oil. Several approaches have been employed to enhance the accumulation of volatile and phenolic compounds in common thyme (Vaičiulytė et al., 2022), including modulation of environmental conditions, cultivation practices, genetic variation, and harvest time. Elevated temperatures and increased potassium and calcium availability in the soil have been shown to stimulate thymol production (Yavari et al., 2010; Cianfaglione et al., 2022). In addition, the induction of mild controlled stress, such as salinity, has proven effective in improving common thyme quality (Karim et al., 2025). Common thyme exhibits moderate

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<https://doi.org/10.1016/j.scienta.2026.114889>

Received 23 March 2026; Received in revised form 21 April 2026; Accepted 12 May 2026

Available online 16 May 2026

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tolerance to salinity, however, salinity levels exceeding 60 mM NaCl negatively affect biomass production and yield (Etri et al., 2025, Zamljen et al., 2025). Mild stress is defined as exposure to an environmental factor that remains below the threshold of damage, eliciting a reversible plant response that is primarily governed by physiological plasticity and adaptive mechanisms rather than irreversible injury (Biswas et al., 2023). From a physiological perspective, the key distinction between mild and severe stress lies in the reversibility and flexibility of the plant response (Bistgani et al., 2019). Mild abiotic stress includes non-biological factors, including water deficit, salinity, temperature extremes, and light and UV radiation, which act within a sub-threshold range where the stress signal is perceived but does not exceed the plant's homeostatic capacity. In such conditions, core physiological processes are maintained, and stress-responsive pathways are transiently activated, enabling acclimation and enhanced metabolite production without detrimental effects on plant performance (Niinemets, 2010). In recent years, the application of plant biostimulants has emerged as a predominant strategy for mitigating plant stress. The effects of biostimulants under mild stress conditions have been extensively documented in horticultural crops (Win et al., 2025). However, despite the increasing use of biostimulants in horticultural systems, their effects on the secondary metabolism of aromatic plants, including common thyme (*Thymus vulgaris* L.), remain insufficiently explored. The majority of studies on common thyme have focused on the plant's growth performance, the yield of essential oil, and responses to abiotic stress (Kamyab and Samsampour, 2025). Only limited attention has been paid to the modulation of phenolic and volatile compounds under biostimulant application, particularly in combination with salinity stress conditions. In this context, the present study aimed to evaluate the efficacy of four distinct biostimulants applied to common thyme plants subjected to mild salt stress. A concentration of 40 mM NaCl was selected to induce controlled stress conditions, as it has been demonstrated to elicit measurable physiological and metabolic responses without causing severe growth inhibition or plant mortality (Ma et al., 2026). Such conditions allow for the assessment of early stress responses and treatment effects, and have been extensively utilised to simulate mild salinity in herbaceous species. The novelty of this study lies in its integrated evaluation of biostimulant-induced changes in both phenolic and volatile compounds in common thyme under mild salinity conditions. This approach provides novel insights into the potential of biostimulants to enhance the functional quality of aromatic plants, contributing to their nutritional and health-promoting value.

2. Materials and methods

2.1. Experimental design

A hydroponic experiment was conducted at the Biotechnical Faculty in Ljubljana (46°03'04" N, 14°30'18" E) from 1 June to 14 August 2025. Common thyme was chosen as the model plant, due to its specific cultivation and fast growth. Common thyme was cultivated in perlite and allocated to twelve 60-L systems (each treatment was represented by two systems to avoid pseudo repetitions). Each system comprised four plastic pots (20×20×30 cm) standing on a plastic plate. Two plants of common thyme were planted in each pot, so each treatment was represented by 16 plants ($n = 16$). The pots were connected to a drip system, the purpose of which was to supply water and minerals. The water and fertilisers were replaced at two- to three-week intervals. The mineral solution was derived from the formulation described by Kacjan Maršič et al. (2021). The pH and electrical conductivity (EC) of the nutrient solution were on average 6.0 and 2.1 dS m⁻¹ for the control treatments and 6.2 and 5.8 dS m⁻¹ for the saline condition, respectively. Sodium-chloride (NaCl; Sigma-Aldrich, Germany) was added after the establishment of the hydroponic system (40 mM, or 150g per system). The pH and electrical conductivity (EC) of the nutrient solutions were measured on a weekly basis using an electronic meter (CyberScan

PCD650; Eutech Instruments, Singapore). In instances where the pH was found to be outside the optimal range, a solution of 74% H₂SO₄ was administered. The greenhouse conditions were characterised by an average temperature of 23.1 °C and 72.1% relative humidity. Six treatments were established:

- control, with normal growing conditions (C)
- control + 40mM NaCl (C salt)
- 40mM NaCl + Amalgerol-essence (B1)
- 40mM NaCl + Delfan Plus (B2)
- 40mM NaCl + Biimore (B3)
- 40mM NaCl + Protifert® LMW (B4)

2.2. Biostimulant characteristics and applications

2.2.1. Amalgerol-essence

Amalgerol-essence characteristics were as follows: seaweed extract, molasses, herbal extract and hydrolyzed proteins. The substance contains 3% nitrogen (N) and 3% potassium oxide (K₂O), which are water-soluble. The total organic matter content was 39%, which corresponds to 80% of the dry matter. The pH value ranged from 5 and 6. The application concentration was 0.02% per treatment, as suggested by the producer.

2.2.2. Delfan Plus

Delfan Plus is derived from hydrolyzed animal protein and contains 24% amino acids, 44.4% organic matter, and 10.8% total nitrogen, of which 6% is in organic form. The product also contains 27.6% organic carbon and has a pH of 7.2. The application concentration was 0.01% per treatment, as suggested by the producer.

2.2.3. Biimore

Biimore includes 5.5% (m/m) total nitrogen, of which 3.0% (m/m) is in organic form and 2.5% (m/m) in nitrate form. In addition, it contains 20% (m/m) of the amino acid glutamine. The application concentration was 0.003% per treatment, as suggested by the producer.

2.2.4. Protifert® LMW

Protifert® LMW contains 60 wt.% dry matter and 8.5 wt.% total nitrogen, of which 8.0 wt.% is pre-sent in organic form (derived from 19 amino acids) and 0.5 wt.% in ammonium form. The product also contains 26 wt.% organic carbon (C). The application concentration was 0.02% per treatment, as suggested by the producer.

All biostimulants were applied foliar applications 3 times during the cultivation period. The control treatments were sprayed with distilled water.

2.3. Sampling of plant material

The common thyme samples were collected when the plants were fully grown, immediately before the generative phase. They were frozen using liquid nitrogen, transferred to the laboratory at the Chair for Fruit Growing, Viticulture and Vegetable Growing, Biotechnical Faculty, University of Ljubljana, Slovenia. The samples were ground to a fine powder using a mortar and pestle and stored in Eppendorf tubes at -80 °C.

2.4. Extraction, identification, and quantification of phenolic compounds in common thyme

The extraction of phenolic compounds was conducted in accordance with the protocol established by Zamljen et al. (2025) with minor modifications. Fresh common thyme powder (0.1 g) was extracted with 4 mL of 80% methanol containing 3% formic acid in bi-distilled water. The extracts were then subjected to sonication for a duration of one hour in an ultrasonic bath maintained at a temperature of 0 °C. This was followed by centrifugation at a speed of 8000 × g for a duration of seven

minutes. The resultant extracts were then filtered through a 0.25 µm polyamide filter (Chromafil AO-20/25, Macherey-Nagel, Düren, Germany) and stored in 1.5 mL vials at −20 °C until analysis.

The individual phenolic compounds were identified through the use of tandem mass spectrometry (MS/MS; LTQ XL, Thermo Scientific, Waltham, MA, USA) in negative ionization mode. Quantification was performed using an ultra-high-performance liquid chromatography system (UHPLC; Vanquish, Thermo Scientific, Waltham, MA, USA). The detailed chromatographic and mass spectrometric parameters were as described by Zamljen et al. (2025).

The quantification of individual phenolics was based on their respective analytical standards. In instances where standards were not available, the compounds were quantified as equivalents of structurally related phenolics. Derivatives of caffeoylhexaric acid were expressed as caffeic acid equivalents; eriodictyol-O-hexosides and eriodictyol-O-glucuronide as epicatechin equivalents; luteolin-3-O-glucuronide as luteolin-3-O-glucoside equivalent; rosmarinic acid isomer and salvianolic acid A as rosmarinic acid equivalents. The contents of individual phenolic compounds were expressed as milligrams per kilogram of fresh weight (FW).

2.5. Extraction, identification, and quantification of volatile compounds in common thyme

The volatile organic compounds (VOCs) were extracted and analysed using solid-phase microextraction coupled with gas chromatography–mass spectrometry (SPME-GC-MS), according to the method described by Zamljen et al. (2024) with minor modifications. For each replicate, 1 g of herb sample powder was placed into a 20 mL glass vial, and 10 µL of internal standard (3-nonanone, 2.7 mg/mL in acetonitrile) was added. The vials were hermetically sealed with screw caps fitted with PTFE–silicone septa and placed in a Shimadzu AOC-20s autosampler. The samples were then subjected to incubation at 50 °C for a duration of five minutes, with agitation at a speed of 250 revolutions per minute (rpm).

The DVB/carbon WR/PDMS SPME fibre was conditioned at 60 °C for 5 min and then exposed to the vial headspace for 40 min under continuous agitation at 250 rpm to allow volatile extraction. Thermal desorption was performed in the GC injector at 250 °C for 3 min using a split ratio of 1:50.

The separation of VOCs was achieved using a Shimadzu GC-MS QP2020 system equipped with a single quadrupole mass detector and a ZB-Wax PLUS capillary column (30 m × 0.25 mm i.d., 0.5 µm film thickness). Helium was used as the carrier gas, with a constant flow rate of 1 mL/min. The oven temperature program commenced at 40 °C (maintained for 3 min), elevated to 150 °C at a rate of 4 °C/min, then to 220 °C at a rate of 10 °C/min, and maintained at this temperature for a duration of 10 min. The ion source and interface temperatures were maintained at 240 °C. The mass spectrometric detection process was conducted at an ionisation energy of 70 eV, encompassing a mass scan range from 50 to 500 mass-to-charge ratios, with a scan rate of 2.0 scans per second.

The identification of volatile compounds was achieved through comparison of their mass spectra with those from commercial libraries (NIST 11 and FFNSC 4). Quantification was carried out using peak area ratios of analytes to the internal standard, in combination with external calibration standards and sample weight. If no standard existed the data were expressed as equivalents to similar substances.

2.6. Statistical analysis

The statistical analysis was carried out using R Commander (version 4.5.2). Data are expressed as means ± standard error (SE). Prior to analysis, data were tested for homogeneity of variances using Levene's test to ensure that assumptions of parametric analysis were met. Treatment effects were evaluated using one-way analysis of variance

(ANOVA). When significant differences were detected ($p < 0.05$), Tukey's test was applied for pairwise comparisons among treatments. Each treatment was replicated in two independent hydroponic systems, which were considered as the experimental units, while individual plants within each system were treated as subsamples. Hierarchical clustering was performed using Ward's method based on Euclidean distance. Results were visualized using clustered heatmaps.

3. Results

3.1. Tentative identification of phenolic compounds

In common thyme, 14 individual phenolic compounds were identified (Table 1): 5 phenolic acids and 9 flavonoids. Caffeoylhexaric acid derivative 1 and 2 were identified based on their typical fragmentation pattern of MS m/z 371 and MS2 m/z 353 as reported by (Gevrenova et al., 2023). Rosmarinic acid and its isomer were identified by the typical fragmentation pattern of MS m/z 359 and MS2 161, 179, and 197 as reported by Ielciu et al. (2024). Salvianolic acid A was identified by its typical fragment of MS2 m/z 295. Eriodictyol-O-hexoside 1, 2, 3, and 4 were identified, with a fragmentation pattern of MS m/z 449, MS2 287, as reported by Babotă et al. (2023). Kaempferol-3-O-hexoside, isorhamnetin-3-O-glucoside and luteolin-3-O-glucuronide were determined based on the fragmentation pattern of MS m/z 447, MS2 285, MS2 m/z 477, MS2 315, and MS m/z 461, MS2 285, respectively as reported by Sonmezdag et al. (2016) and Čulina et al. (2021). Kaempferol was identified by its typical fragmentation of MS m/z 285, MS2 153 as reported by Bera and Patel (2023).

3.2. Phenolic contents in common thyme

The data in Table 2 indicate that biostimulant application markedly affected phenolic accumulation in common thyme grown under saline conditions. Total analysed phenolics increased under B4 (9650.26 mg/kg FW) application compared with the C (4862.56 mg/kg FW) and C salt treatments (6713.69 mg/kg FW), with 98% and 44% increase

Table 1
Tentative identification of phenolic compounds in common thyme.

Compound	Rt (min)	[M-H] [−] (m/z)	MS ² (m/z)	MS ³ (m/z)
Kaempferol	12.28	285	153 (100)	
Caffeoylhexaric acid derivative 1	14.40	389	371 (100), 353 (73), 209 (60)	353 (20), 209 (100)
Eriodictyol-O-hexoside 1	15.53	449	287 (100)	
Eriodictyol-O-hexoside 2	16.40	449	287 (100)	
Caffeoylhexaric acid derivative 2	17.17	371	353 (100)	209 (100)
Kaempferol-3-O-hexoside	18.69	447	285 (100)	153 (100)
Isorhamnetin-3-O-glucoside	20.10	477	315 (100)	
Eriodictyol-O-glucuronide	20.98	463	287 (100)	
Luteolin-3-O-glucuronide	21.64	461	285 (100)	151 (100)
Eriodictyol-O-hexoside 3	21.97	449	287 (100)	
Eriodictyol-O-hexoside 4	22.30	449	287 (100)	
Rosmarinic acid isomer	23.50	359	161 (100), 179 (27), 197 (22)	
Rosmarinic acid	23.72	359	161 (100), 179 (27), 197 (22)	
Salvianolic acid A	24.32	493	295 (100)	

Rt = retention time; [M-H][−] = main mass of substance in negative ion mode; MS² = first fragmentation; MS³ = second fragmentation

Table 2

Individual and total analyzed phenolic contents in common thyme treated with biostimulants under saline conditions.

	C	C salt	B1	B2	B3	B4
Kaempferol	136.71 ± 16.51 ab*	129.09 ± 14.46 ab	94.14 ± 17.98 b	132.47 ± 24.80 ab	121.19 ± 16.93 ab	186.65 ± 3.99 a
Caffeoylhexaric acid derivative 1	41.56 ± 3.03 a	47.31 ± 2.25 a	43.04 ± 8.97 a	49.70 ± 3.39 a	26.06 ± 13.98 a	55.06 ± 5.44 a
Eriodictyol-O-hexoside 1	380.91 ± 40.10 a	488.31 ± 16.71 a	428.88 ± 115.77 a	465.43 ± 10.15 a	280.70 ± 77.34 a	479.62 ± 62.82 a
Eriodictyol-O-hexoside 2	1185.73 ± 244.72 ab	1399.74 ± 136.72 ab	1732.21 ± 565.94 ab	1237.62 ± 362.54 ab	199.31 ± 8.39 b	2081.52 ± 598.16 a
Caffeoylhexaric acid derivative 2	49.28 ± 2.35	89.80 ± 11.86	156.40 ± 49.60	89.34 ± 33.16	166.80 ± 26.71	150.05 ± 11.56
Kaempferol-3-O-hexoside	384.72 ± 22.33 b	375.64 ± 17.11 b	318.68 ± 51.99 b	349.98 ± 16.19 b	300.34 ± 95.24 b	523.94 ± 33.14 a
Isorhamnetin-3-O-glucoside	78.02 ± 11.64 a	149.84 ± 34.06 a	87.25 ± 18.35 a	117.79 ± 1.00 a	80.96 ± 22.51 a	135.22 ± 10.92 a
Eriodictyol-O-glucuronide	476.23 ± 32.00 b	684.87 ± 60.38 ab	632.68 ± 121.07 ab	644.18 ± 9.49 ab	428.32 ± 32.76 b	794.08 ± 48.64 a
Luteolin-3-O-glucuronide	708.42 ± 24.69 c	980.42 ± 81.45 b	1171.86 ± 140.29 ab	1332.42 ± 235.24 a	809.16 ± 44.58 b	1101.93 ± 148.18 ab
Eriodictyol-O-hexoside 3	651.15 ± 16.95 b	1048.68 ± 113.23 ab	1163.00 ± 154.66 ab	1309.42 ± 113.46 a	658.79 ± 103.86 b	1088.76 ± 116.81 ab
Eriodictyol-O-hexoside 4	174.40 ± 14.47 a	326.05 ± 28.08 a	208.80 ± 69.98 a	259.01 ± 6.21 a	149.32 ± 78.81 a	325.56 ± 24.55 a
Rosmarinic acid isomer	29.01 ± 3.30 a	32.65 ± 5.29 a	40.74 ± 14.22 a	27.54 ± 6.65 a	32.80 ± 7.00 a	50.72 ± 2.10 a
Rosmarinic acid	499.17 ± 86.47 d	847.49 ± 68.20 c	1265.50 ± 186.67 b	1466.19 ± 384.23 b	1901.21 ± 426.46 a	2162.36 ± 203.20 a
Salvianolic acid A	67.24 ± 2.80 c	113.79 ± 10.10 c	206.98 ± 70.75 b	206.06 ± 56.46 b	368.35 ± 102.36 b	514.79 ± 34.74 a
Total analyzed phenolics	4862.56 ± 302.66 c	6713.69 ± 206.07 b	7586.62 ± 1399.79 ab	7687.15 ± 738.59 ab	5523.31 ± 369.68 c	9650.26 ± 773.69 a

control, with normal growing conditions (C); control + 40mM NaCl (C salt); 40mM NaCl + first biostimulant Amalgerol-essence (B1); 40mM NaCl + second biostimulant Delfan Plus (B2); 40mM NaCl + third biostimulant Biimore (B3); 40mM NaCl + fourth biostimulant Protifert® LMW (B4).

* lowercase letters a-d denote statistical significant differences among treatments.

respectively.

Rosmarinic acid was strongly enhanced by salinity and even further

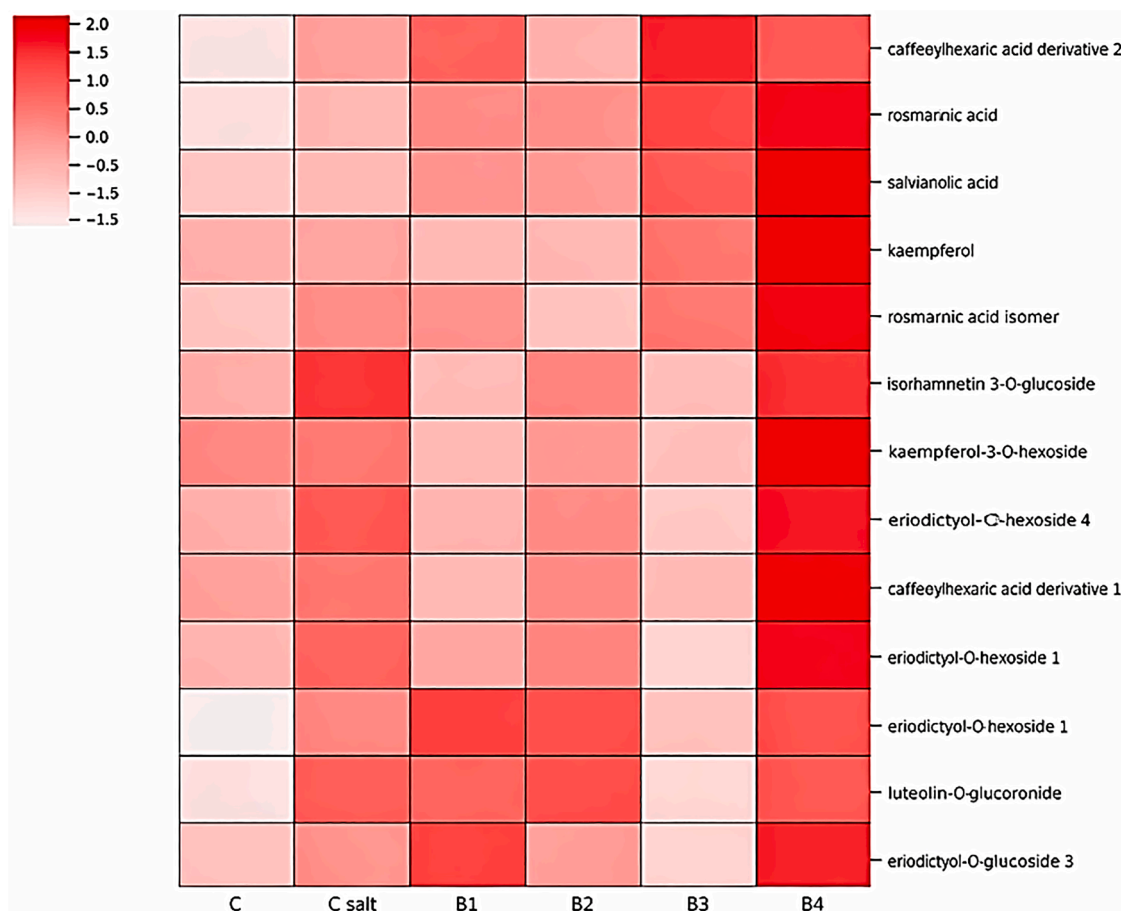


Fig. 1. Heatmap of common thyme individual phenolic contents. Rows represent treatments, and columns represent individual phenolics. Dark red colours indicate relatively high and white colours low metabolite concentrations. Dendrograms show hierarchical clustering of the samples using Ward's method based on Euclidean distance. The treatments are: control, with normal growing conditions (C); control + 40mM NaCl (C salt); 40mM NaCl + Amalgerol-essence (B1); 40mM NaCl + Delfan Plus (B2); 40mM NaCl + Biimore (B3); 40mM NaCl + Protifert® LMW (B4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by biostimulants, reaching 2162.36 mg/kg FW in B4, representing an approximate 333% increase compared to the C treatment and 155% increase compared to the C salt treatments. Kaempferol-3-*O*-hexoside increased under B4 by 36% and 26.5% compared to the C and C salt treatments (Supplementary Table 2). Luteolin-3-*O*-glucuronide at treatment B2 rose by 55% compared to the C treatment and 27% compared to the C salt treatment.

Fig. 1 presents the results of the individual phenolic clustering analysis, showing that the B4 biostimulant had the highest individual phenolic contents, while B3 and C treatments had the lowest. Overall, phenolic compounds were observed to cluster in three groups with distinctive content patterns. Group 1 comprises caffeoylhexaric acid derivative 1, rosmarinic acid isomer, salvianolic acid, and kaempferol. These compounds are the most abundant in B3 and B4 treatments and the least abundant in the C treatment. Group 2 comprises isorhamnetin-3-*O*-glucoside, kaempferol-3-*O*-hexoside, eriodictyol-*O*-hexoside isomer 4, eriodictyol-*O*-hexoside isomer 1, and caffeoylhexaric acid derivative 2. These compounds are the most abundant in C salt and B4, and the least abundant in B3. Group 3 includes eriodictyol-*O*-glucuronide, eriodictyol-*O*-hexoside isomer 3, eriodictyol-*O*-hexoside isomer 2, luteolin-3-*O*-glucuronide, and rosmarinic acid, which are the most abundant in B4, followed by C salt, B1 and B2, and the least abundant in C and B3.

3.3. Identification of volatiles in common thyme

GC-MS analysis of common thyme revealed a complex profile dominated by terpenoid compounds, particularly monoterpenes such as α -pinene, limonene, γ -terpinene, eucalyptol, linalool, thymol, and carvacrol, which are known to contribute resinous, herbal, citrusy, and spicy notes to the aroma of common thyme (Table 3). Sesquiterpenes (e. g., β -caryophyllene, germacrene D, and β -bisabolene) were also detected, imparting woody and balsamic notes. In addition, several aldehydes, alcohols, ketones, and esters (including hexanal, 2E-hexenal, 1-

octen-3-ol, and linalyl acetate) were identified, contributing green, mushroom-like, and fruity nuances. Minor amounts of furanoids and phenolic ethers were also determined. Overall, the volatile composition reflects the characteristic herbaceous, spicy, and aromatic profile of thyme.

3.2. Volatile contents in common thyme

The volatile individual and total contents are presented in Table 4. Salinity by itself (C salt) markedly reduced volatile organic compound (VOC) accumulation in common thyme compared to the C treatment, with total VOCs decreasing from 109.74 mg/kg FW to 72.79 mg/kg FW, corresponding to a 34% decrease. Among biostimulant treatments, B2 strongly enhanced VOC production under saline conditions, reaching 167.31 mg/kg FW, which represents a 129% and 52% increase compared to the C salt and C treatments, respectively. This stimulation was mainly associated with higher total monoterpenes (107% and 33% increase), sesquiterpenes (115% and 27.6% increase), and phenolic ethers (140% and 72.2% increase) compared to the C salt and C treatments, respectively. B3 treatment showed the lowest VOC accumulation (62.35 mg/kg FW), with a 43% decrease compared to the C treatment, largely due to strong reductions in monoterpenes (64% reduction compared to the C treatment) and sesquiterpenes (55% reduction compared to the C treatment). B1 and B4 partially alleviated salt stress effects, restoring total VOCs to levels comparable to the control but remaining 46–48% lower than B2.

In terms of individual volatiles, salinity generally reduced major monoterpenes such as γ -terpinene (58% decrease), *p*-cymene (14% decrease), and myrcene (34% decrease) compared with the C treatment (Table 4). B2 increased several key aroma compounds, including *p*-cymene (72% increase), γ -terpinene (21% increase), α -thujene (91% increase), myrcene (54% increase), and *E*- β -ocimene (59% increase) compared to the C and C salt treatments. In contrast, B3 caused a complete depletion of α -pinene, α -thujene, sabinene, limonene, and

Table 3
Identification of volatiles in common thyme.

RT	CAS No.	Compound ID	Classification	Aroma (based on PubChem data)	External standard
4.725	3208-16-0	2-methyl-furan	furanoid	Roasted, caramel-like, sweet	Methyl butyrate
6.328	80-56-8	α -pinene	monoterpene	Resinous, woody	α -Pinene
6.459	2867-05-2	α -thujene	monoterpene	Herbal, spicy, pine-like	α -Pinene
7.626	79-92-5	Camphene	hydrocarbon	Camphoraceous, resinous	α -Pinene
8.235	66-25-1	<i>n</i> -hexanal	aldehyde	Fresh green, grassy	Hexanal
8.911	127-91-3	β -pinene	monoterpene	Resinous, woody, slightly citrus	α -Pinene
9.376	3387-41-5	Sabinene	monoterpene	Spicy, woody, spicy	α -Pinene
10.346	13466-78-9	3-carene	monoterpene	Sweet pine, resin, citrusy	α -Pinene
10.855	123-35-3	Myrcene	monoterpene	Resinous, balsamic, herbal	Myrcene
10.993	616-25-1	1-Pentanol	alcohol	Slightly fruity, green	1-Pentanol
11.407	99-86-5	α -terpinene	monoterpene	Herbal, citrusy	Limonene
12.088	138-86-3	Limonene	monoterpene	Citrusy, fresh	Limonene
12.412	99-83-2	α -phellandrene	monoterpene	Minty, peppery, herbal	Limonene
12.561	470-82-6	Eucalyptol	monoterpene	Minty, camphoraceous	α -Pinene
12.916	6728-26-3	2E-hexenal	aldehyde	Green, grassy	Hexanal
13.362	3779-61-1	<i>E</i> - β -ocimene	monoterpene	Sweet, floral	Myrcene
13.766	99-85-4	γ -terpinene	monoterpene	Citrusy	Limonene
13.949	502-99-8	δ -3-carene	monoterpene	Fresh, green	Myrcene
14.158	106-68-3	3-octanone	ketone	Mushroom-like-like, earthy, fatty	2-Octanone
14.645	99-87-6	<i>p</i> -cymene	monoterpene	Spicy, herbal, slightly citrus	eugenol
18.374	2442-10-6	1-octen-3-yl-acetate	ester	Mushroom, fruity, green, earthy	Hexyl acetate
20.93	3391-86-4	1-octen-3-ol	alcohol	Mushroom, earthy, green	1-Pentanol
21.5	17699-16-0	Sabinene hydrate	monoterpene	Woody, herbal, slightly minty	α -Pinene
24.021	78-70-6	Linalool	monoterpene	Floral, citrus, -floral	Linalool
24.197	115-95-7	Linalyl acetate	ester	Sweet floral, bergamot-like-like, fruity	Hexyl acetate
25.381	1076-56-8	Thymol methyl ether	phenolic ether	Sweet, -herbal	Eugenol
25.5	13877-93-5	<i>E</i> -caryophyllene	sesquiterpene	Spicy, woody, clove-like	Farnesol
25.707	31574-44-4	Isothymol methyl ether	phenolic ether	Sweet, -herbal	Eugenol
28.858	105453-16-5	Germacrene D	sesquiterpene	Woody, herbal, spicy, -balsamic	Farnesol
29.308	4891-79-6	β -bisabolene	sesquiterpene	Sweet, balsamic, woody	Farnesol
36.693	89-83-8	Thymol	monoterpene	Herbal	Eugenol
37.087	499-75-2	Carvacrol	monoterpene	Spicy, sharp	Eugenol

Table 4
Individual and total analyzed volatile contents in common thyme treated with biostimulants under saline conditions.

	C	C salt	B1	B2	B3	B4
2-methyl-furan	0.31 ± 0.03 b*	0.31 ± 0.01 b	0.45 ± 0.08 a	0.25 ± 0.01 b	0.38 ± 0.10 b	0.26 ± 0.04 b
α-pinene	0.62 ± 0.13 b	0.53 ± 0.08 b	0.65 ± 0.10 b	1.21 ± 0.41 a	0.00 ± 0.00	0.44 ± 0.16 b
α-thujene	1.73 ± 0.50 b	1.42 ± 0.20 b	1.76 ± 0.24 b	3.30 ± 1.19 a	0.00 ± 0.00	1.33 ± 0.45 b
Camphene	0.19 ± 0.06 ab	0.11 ± 0.04 ab	0.08 ± 0.01 ab	0.24 ± 0.10 a	0.00 ± 0.00 b	0.07 ± 0.01 ab
n-hexanal	3.77 ± 0.78 a	4.37 ± 0.65 a	4.39 ± 0.74 a	3.34 ± 0.88 a	2.53 ± 0.42 a	3.13 ± 0.65 a
β-pinene	0.16 ± 0.03 b	0.16 ± 0.05 b	0.25 ± 0.05 b	0.36 ± 0.13 a	0.00 ± 0.00	0.16 ± 0.05 b
Sabinene	0.25 ± 0.05 ab	0.21 ± 0.05 ab	0.32 ± 0.06 ab	0.45 ± 0.17 a	0.00 ± 0.00	0.22 ± 0.07 b
3-carene	0.09 ± 0.01 a	0.08 ± 0.01 a	0.07 ± 0.01 a	0.10 ± 0.03 a	0.00 ± 0.00	0.07 ± 0.02 a
Myrcene	2.09 ± 0.55 b	1.37 ± 0.20 b	1.79 ± 0.18 b	3.22 ± 1.09 a	0.21 ± 0.05 c	1.48 ± 0.42 b
1-Pentanol	7.23 ± 1.12 a	6.80 ± 1.21 a	8.14 ± 2.18 a	7.15 ± 1.57 a	4.66 ± 1.10 a	5.97 ± 0.61 a
α-terpinene	1.44 ± 0.45 ab	0.65 ± 0.15 b	0.93 ± 0.22 b	1.98 ± 0.87 a	0.00 ± 0.00	0.68 ± 0.20 b
Limonene	0.28 ± 0.07 b	0.20 ± 0.04 b	0.29 ± 0.03 b	0.50 ± 0.17 a	0.02 ± 0.02 c	0.26 ± 0.08 b
α-phellandrene	0.14 ± 0.04 b	0.09 ± 0.01 b	0.12 ± 0.01 b	0.22 ± 0.08 a	0.00 ± 0.00	0.13 ± 0.05 b
Eucalyptol	0.59 ± 0.03 b	0.60 ± 0.33 b	1.13 ± 0.26 a	0.63 ± 0.32 b	0.00 ± 0.00	0.63 ± 0.27 b
2E-hexenal	5.43 ± 0.41 a	4.27 ± 0.49 a	4.60 ± 0.98 a	4.72 ± 1.18 a	4.45 ± 0.97 a	5.46 ± 0.93 a
E-β-ocimene	0.32 ± 0.07 b	0.14 ± 0.03 b	0.23 ± 0.04 b	0.51 ± 0.17 a	0.02 ± 0.02 c	0.29 ± 0.09 b
γ-terpinene	12.02 ± 3.21 a	5.04 ± 1.22 b	7.16 ± 1.93 b	14.55 ± 6.35 a	0.02 ± 0.02 c	5.74 ± 1.78 b
δ-3-carene	0.19 ± 0.02 b	0.08 ± 0.01 b	0.17 ± 0.02 b	0.30 ± 0.08 a	0.09 ± 0.02 b	0.18 ± 0.02 b
3-octanone	2.21 ± 1.93 b	2.51 ± 1.16 b	3.06 ± 1.45 a	0.34 ± 0.12 c	0.67 ± 0.46 c	1.08 ± 0.88 b
p-cymene	17.93 ± 4.66 b	20.37 ± 3.96 b	24.23 ± 4.55 b	35.03 ± 12.73 a	0.11 ± 0.01 c	20.04 ± 7.26 b
1-octen-3-yl-acetate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.26 ± 0.07 b	0.88 ± 0.10 a
1-octen-3-ol	32.53 ± 5.74 a	17.21 ± 4.87 a	16.85 ± 1.24 a	41.61 ± 13.96 a	14.06 ± 2.93 a	18.49 ± 4.48 a
Sabinene hydrate	0.11 ± 0.04 b	0.10 ± 0.02 b	0.05 ± 0.01 b	0.15 ± 0.04 a	0.00 ± 0.00	0.04 ± 0.02 b
Linalool	0.20 ± 0.06 b	0.00 ± 0.00	0.77 ± 0.38 b	0.59 ± 0.35 b	15.42 ± 4.72 a	0.65 ± 0.23 b
Linalyl acetate	7.38 ± 5.17 a	0.00 ± 0.00	10.75 ± 6.10 a	31.79 ± 26.90 a	17.17 ± 6.80 a	16.12 ± 6.90 a
Thymol methyl ether	0.00 ± 0.00 a	0.00 ± 0.00 a	1.09 ± 0.63 a	2.68 ± 1.41 a	0.00 ± 0.00 a	1.64 ± 0.58 a
E-caryophyllene	3.26 ± 0.40 ab	2.31 ± 0.19 b	2.57 ± 0.09 b	4.97 ± 0.87 a	1.70 ± 0.39 b	2.36 ± 0.52 b
Isothymol methyl ether	1.34 ± 0.30 a	2.01 ± 0.49 a	1.44 ± 0.64 a	2.14 ± 0.79 a	0.00 ± 0.00 a	0.60 ± 0.25 a
Germacrene D	1.23 ± 0.25 a	0.47 ± 0.13 b	0.57 ± 0.14 b	1.24 ± 0.21 a	0.59 ± 0.11 b	0.69 ± 0.10 b
β-bisabolene	0.65 ± 0.16 a	0.52 ± 0.07 a	0.68 ± 0.14 a	0.89 ± 0.14 a	0.00 ± 0.00	0.00 ± 0.00
Thymol	2.90 ± 2.77 a	0.00 ± 0.00 a	0.30 ± 0.17 a	2.20 ± 0.91 a	0.00 ± 0.00 a	0.78 ± 0.33 a
Carvacrol	3.13 ± 0.93 a	0.87 ± 0.20 b	0.62 ± 0.38 b	0.80 ± 0.41 b	0.00 ± 0.00 b	0.47 ± 0.47 b
TOTAL ALCOHOLS	39.76 ± 5.07 ab	24.01 ± 4.83 b	24.99 ± 3.39 b	48.76 ± 15.12 a	18.72 ± 2.36 b	24.46 ± 4.89 b
TOTAL ALDEHYDES	9.20 ± 1.18 a	8.64 ± 1.02 a	8.99 ± 1.64 a	8.06 ± 1.88 a	6.97 ± 1.34 a	8.59 ± 1.58 a
TOTAL ESTERS	7.38 ± 5.17 b	0.00 ± 0.00	10.75 ± 6.10 b	31.79 ± 26.90 a	17.44 ± 6.87 b	16.99 ± 6.91 b
TOTAL MONOTERPENES	44.19 ± 11.84 b	31.91 ± 6.18 b	40.85 ± 7.45 b	66.08 ± 23.91 a	15.89 ± 4.60 c	33.61 ± 10.75 b
TOTAL SESQUITERPENES	5.14 ± 0.67 b	3.30 ± 0.30 b	3.83 ± 0.29 b	7.10 ± 0.79 a	2.29 ± 0.50 c	3.05 ± 0.55 b
TOTAL PHENOLIC ETHERS	1.34 ± 0.30 b	2.01 ± 0.49 b	2.52 ± 0.03 b	4.82 ± 1.73 a	0.00 ± 0.00	2.24 ± 0.75 b
OTHERS	2.72 ± 1.88 a	2.93 ± 1.14 a	3.60 ± 1.47 a	0.70 ± 0.25 a	1.04 ± 0.56 a	1.40 ± 0.92 a
TOTAL VOC	109.74 ± 14.80 b	72.79 ± 10.61 c	95.53 ± 8.58 b	167.31 ± 24.05 a	62.35 ± 3.60 c	90.34 ± 13.32 b

control, with normal growing conditions (C); control + 40mM NaCl (C salt); 40mM NaCl + first biostimulant Amalgerol-essence (B1); 40mM NaCl + second biostimulant Delfan Plus (B2); 40mM NaCl + third biostimulant Biimore (B3); 40mM NaCl + fourth biostimulant Protifert® LMW (B4).

* Lowercase letters a-d denote statistical significant differences among treatments.

γ-terpinene, while uniquely inducing an increase in linalool (15.42 mg/kg FW), which was negligible or absent in other treatments. Esters such as linalyl acetate and 1-octen-3-yl acetate were particularly promoted by B2 and B4, contributing to the higher ester fraction under these treatments. Phenolic monoterpenes thymol and carvacrol showed high variability but were generally reduced under salinity and biostimulant treatments, especially under B3, where both compounds were absent, which can be observed also from the clustering analysis in Fig. 2.

4. Discussion

Our study investigated the effects of mild salinity stress combined with four different biostimulants on the quality of common thyme, with a particular focus on phenolic and volatile compounds. Compared with the control treatment, salinity significantly increased total phenolic content, which is consistent with previous findings reporting enhanced phenolic acid and flavonoid accumulation in *Thymus vulgaris* and *Thymus daenensis* under saline conditions (Bistgani et al., 2019). Biostimulants are primarily applied under stress conditions to mitigate adverse environmental effects; therefore, in this study, their application was focused on salinity stress treatments. However, the absence of non-saline biostimulant treatments limits the ability to distinguish between general biostimulant effects and stress-specific responses. The application of biostimulants further increased phenolic accumulation, with treatment B4 showing the strongest effect. Notably, all

biostimulants increased the levels of rosmarinic acid and luteolin-3-O-glucuronide relative to both control treatments. These compounds are widely reported as important antioxidants in plants and are commonly associated with stress responses and protective functions (Sharma et al., 2012). Salinity stress is known to induce the production of reactive oxygen species (ROS), which can negatively affect plant cells and tissues (Salehi-Arjmand et al., 2025). Phenolic compounds are widely reported to contribute to oxidative stress mitigation through their ability to scavenge ROS, including superoxide anion radicals (O₂⁻), singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), and hydroxyl radicals (·OH) (Shomali et al., 2022). In the present study, the observed increase in phenolic compounds following biostimulant application may indicate an enhanced metabolic response under salinity conditions. This response is consistent with previous reports describing coordinated changes in antioxidant-related metabolites in thyme grown under saline hydroponic conditions (Azeem et al., 2023). However, these interpretations are based on metabolite profiles and do not provide direct evidence of antioxidant activity or ROS regulation. Different biostimulants vary in composition and may influence nutrient availability, which in turn can affect plant metabolism (Ivancic et al., 2022). For example, B3 and B4 are characterized by higher nitrogen availability, which may promote amino acid synthesis, including phenylalanine, a key precursor of the phenylpropanoid pathway (Fritz et al., 2006). This may partially explain the increased accumulation of phenolic acids such as rosmarinic and salvianolic acids observed under these treatments. Similarly, the

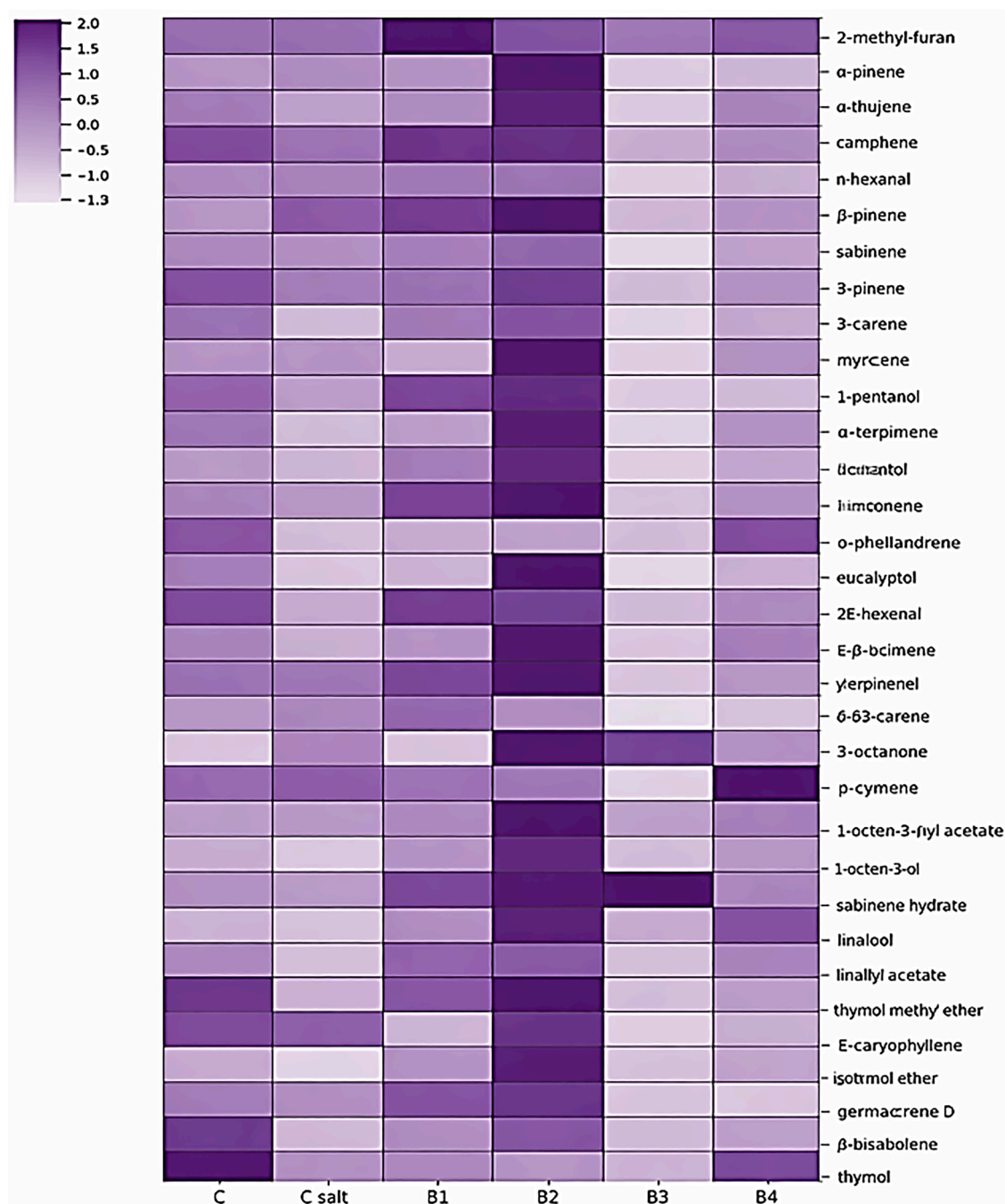


Fig. 2. Heatmap of common thyme individual volatile contents. Rows represent treatments, and columns represent individual volatiles. Purple colours indicate relatively high and white colours low metabolite concentrations. The treatments are: control, with normal growing conditions (C); control + 40mM NaCl (C salt); 40mM NaCl + first biostimulant Amalgerol-essence (B1); 40mM NaCl + second biostimulant Delfan Plus (B2); 40mM NaCl + third biostimulant Biimore (B3); 40mM NaCl + fourth biostimulant Protifert® LMW (B4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

increased levels of glycosylated flavonoids (e.g., isorhamnetin-3-O-glucoside and kaempferol-3-O-hexoside) under stress conditions may reflect enhanced stability of these compounds (Pungin et al., 2023). The higher accumulation of flavanones under B4 may be associated with the combined effect of nutrient availability and stress conditions; however, these interpretations remain speculative and would require further biochemical or molecular validation. Biostimulant treatments also affected the volatile profile of common thyme, supporting previous reports that biostimulants can modulate secondary metabolite composition (Omoarelojie et al., 2021). The responses were

treatment-specific, with B2 showing the strongest increase in total volatile content, while B3 resulted in the lowest accumulation. Under salinity stress, reductions in certain monoterpenes (e.g., thymol) and increases in methylated derivatives were observed. While thymol has been associated with antioxidant functions in plants (Xu et al., 2022; Sun et al., 2024), the present study does not provide direct evidence linking these changes to antioxidant activity or enzyme regulation. Therefore, such interpretations should be considered with caution. Distinct metabolic patterns were observed among the biostimulants. For instance, B2 and B4 treatments were associated with higher levels of certain volatiles,

including esters such as linalyl acetate, whereas B3 showed a general reduction in volatile compounds. These differences may reflect variations in nutrient composition and plant metabolic responses; however, the underlying biochemical mechanisms cannot be conclusively determined based on metabolite profiling alone. Additionally, changes in the ratios of key volatile compounds (e.g., linalool/linalyl acetate, thymol/carvacrol) may influence the aroma profile of thyme. While these shifts suggest that biostimulant application under salinity conditions can affect sensory-related traits, their direct relationship with plant stress tolerance or resilience remains to be further investigated.

5. Conclusions

In conclusion, mild salinity stress significantly influenced the accumulation of phenolic and volatile compounds in common thyme (*Thymus vulgaris* L.). Among the treatments that were examined, B4 demonstrated the most significant enhancement of the total phenolic content, while B2 exhibited the greatest efficacy in increasing the total volatile compounds. Conversely, B3 typically led to a decrease in the accumulation of both phenolic and volatile metabolites, suggesting significant variation in biostimulant efficacy based on their composition. The observed changes in phenolic and volatile profiles suggest that the combined application of mild salinity stress and biostimulants can modulate secondary metabolism in thyme. However, it should be noted that these results are based on metabolite profiling under controlled conditions and do not provide direct evidence of underlying physiological, biochemical, or molecular mechanisms. The present study demonstrates the potential of integrating mild salinity stress with selected biostimulants as an agronomic strategy to influence the phytochemical composition of common thyme. Further research is required, incorporating physiological and molecular analyses, to more effectively elucidate the mechanisms underlying these responses and to validate their practical applications (Krause et al., 2021; Piga et al., 2025; Reinecke et al., 2024; Zheng et al., 2025).

CRedit authorship contribution statement

Tilen Zamljen: Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Sergeja Adamić Zamljen:** Validation, Visualization, Data curation, Writing – review & editing. **Mariana Cecilia Grohar:** Writing – review & editing, Validation, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing 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.

Acknowledgment

This work is part of the program Horticulture P4–0013–0481 supported by the Slovenian Research Agency and the infrastructural center IC RRC-AG (IO-0022–0481–001).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2026.114889](https://doi.org/10.1016/j.scienta.2026.114889).

Data availability

Data will be made available on request.

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