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Amino acid based biostimulants mitigate salinity-induced yield loss and modulate phytochemical composition in hydroponic flat leaf parsley (*Petroselinum crispum* var. *neapolitanum*)

Tilen Zamljen^{1*}, Jerneja Jakopic¹ and Mariana Cecilia Grohar¹

Abstract

Background Soil salinization is a major constraint in horticulture, reducing yield and resource-use efficiency in high-value crops. While salinity generally inhibits plant growth, it can enhance the accumulation of bioactive compounds in medicinal and aromatic species. This study aimed to evaluate the effects of salinity and amino acid-based biostimulants on yield and phytochemical composition of hydroponically grown flat-leaf parsley (*Petroselinum crispum* var. *neapolitanum*). We hypothesized that salinity would reduce biomass while increasing phenolic and volatile compounds, and that biostimulants would mitigate yield loss under stress.

Results Salinity (40 mM NaCl) reduced yield nearly fivefold compared to control plants. Biostimulant application partially alleviated this effect, increasing yield by 73.3 and 54.6% relative to untreated salt-stressed plants. Salinity increased total phenolic content, particularly hydroxycinnamic acids and flavones (e.g., apigenin-6,8-di-C-glucoside), while decreasing chrysoeriol pentose-hexose. Volatile compounds, mainly monoterpenes, also increased under salinity, with higher levels of *p*-1,3,8-menthatriene and α -phellandrene. Biostimulants moderated these changes while supporting biomass recovery.

Conclusions Salinity reduced yield but enhanced phenolic and volatile compound accumulation in parsley. Amino acid-based biostimulants partially mitigated yield loss while maintaining phytochemical quality, highlighting their potential for hydroponic production under saline conditions.

Keywords Herbs, Hydroponics, LC-MS, GC-MS, Salinity, Metabolomics

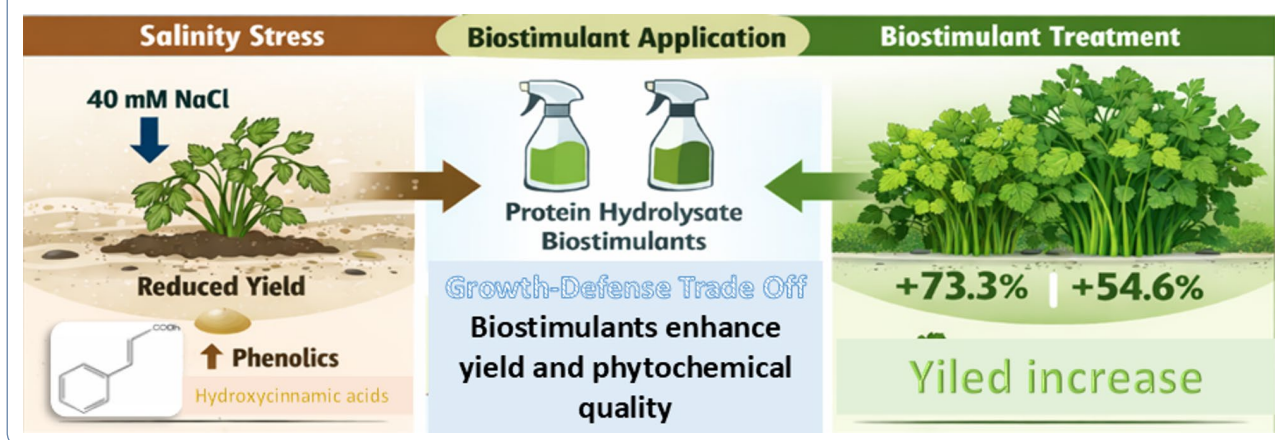
*Correspondence:

Tilen Zamljen
tilen.zamljen@bf.uni-lj.si

Full list of author information is available at the end of the article

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Graphical Abstract



Introduction

Parsley (*Petroselinum crispum* (Mill.) is a biennial herb native to Europe and Western Asia and is extensively cultivated worldwide due to its culinary, aromatic, and medicinal importance. Two major botanical types are commonly grown: curly leaf parsley (var. *crispum*) and flat leaf, or Italian, parsley (var. *neapolitanum*) [1]. The seeds, leaves, and essential oils of parsley have a wide range of applications and have traditionally been used as antispasmodic, carminative, diuretic, emmenagogue, and stomachic agents [2]. Parsley leaves are rich in volatile compounds that contribute to their characteristic aroma and medicinal value [3]. The accumulation of these compounds is influenced by environmental conditions, including abiotic stressors such as salinity. Salinity is one of the most pervasive abiotic stresses in agriculture and has the capacity to markedly reduce the growth and yield of numerous crop species, including medicinal and aromatic plants. Parsley is moderately susceptible to salinity, with severe negative effects occurring at 90 mM NaCl [4]. Under saline conditions, plant growth is negatively affected primarily due to increased osmotic stress, which limits water and nutrient uptake, as well as ion toxicity resulting from excessive accumulation of sodium (Na^+) and chloride (Cl^-). These effects disrupt plant metabolism and nutrient balance, ultimately impairing growth and productivity [5]. Despite the generally detrimental effects of salinity on plant growth, several studies have demonstrated that salinity stress can stimulate the accumulation of secondary metabolites [6]. These compounds are responsible for many of the medicinal and aromatic properties of plants, suggesting that controlled stress conditions may enhance phytochemical quality even when biomass production is reduced. This trade-off between yield and quality is particularly relevant for medicinal plants, where biochemical composition is often of greater importance than total biomass [7].

In parallel, increasing demand for natural and plant-based products, together with environmental challenges such as soil salinization, drought, and temperature fluctuations, has highlighted the limitations of conventional soil-based cultivation systems [8]. In this context, hydroponic cultivation has emerged as a promising alternative, as it allows precise control over nutrient composition, electrical conductivity, and growing conditions that directly influence plant growth and secondary metabolite production [9]. For medicinal and aromatic plants, hydroponic systems offer the opportunity to optimize biomass yield, essential oil content, and phytochemical quality, while enabling consistent, disease-free production throughout the year [10, 11]. Given the increasing prevalence of soil salinization in herb cultivation, recent research has focused on strategies to mitigate salinity-induced stress while maintaining or improving crop quality. These strategies include: (i) the use of plant growth regulators such as biostimulants, (ii) organic fertilization, (iii) beneficial microorganisms such as mycorrhizal fungi and plant growth-promoting rhizobacteria, and (iv) the selection of salt-tolerant species [12]. The choice of strategy should be guided by the intended purpose of cultivation, as controlled stress levels may reduce yield but enhance the accumulation of bioactive compounds, thereby increasing the medicinal value of the crop [13].

Although there is ample documentation of the enhancement of secondary metabolites in aromatic and medicinal plants in response to salinity, the majority of studies have focused on phenolic compounds, with comparatively little attention paid to changes in volatile profiles, particularly in parsley. In a similar manner, amino acid-based biostimulants have been the focus of extensive study in the context of soil-grown crops. However, the effects of these biostimulants under hydroponic conditions, particularly in conjunction with salinity stress, remain to be fully elucidated. Furthermore, the combined

impact of salinity and biostimulant application on both phenolic composition and volatile organic compounds has received limited attention.

It was hypothesised that (i) salinity would reduce biomass production while enhancing the accumulation of phenolic and volatile compounds, and (ii) amino acid-based biostimulants would mitigate yield loss under salinity while modulating phytochemical composition. Consequently, this study offers novel insights into the integrated response of hydroponically cultivated parsley to salinity and biostimulant application, with particular emphasis on yield, phenolic metabolites, and volatile profiles.

Materials and methods

Experimental design

A hydroponic experiment was conducted in a greenhouse at the laboratory field of Biotechnical faculty in Ljubljana (46° 3' 4" N; 14° 30' 18" E), Slovenia from 4th June to 1st September 2025. Italian or flat leaf type of parsley (*Petroselinum crispum* var. *neapolitanum* (Mill.)) 'Gigante D Italia' purchased from Austroaat, was sown in April into Styrofoam plugs. When the seedlings reached a sufficient height, they were transplanted (after 35 days) into 9 cm high hydroponic pots filled with mineral wool substrate. When the parsley roots overgrow the mineral wool, the plants were placed on a Nutrient Film Technique (NFT) hydroponic system. Each treatment was established in a single NFT system consisting of four independent channels (canals), each containing five plants. Although plants were spatially separated into channels, all channels within a treatment shared a common nutrient reservoir. Therefore, the channels represent subsamples within a single experimental unit rather than fully independent biological replicates.

The nutrient solution was based on Kacjan Maršič, Štolfa [14] and it contained the following ionic composition: 14.0 mM NO_3^- , 1.0 mM NH_4^+ , 1.0 mM H_2PO_4^- , 6.0 mM K^+ , 4.0 mM Ca^{2+} , 2.0 mM SO_4^{2-} , 2.0 mM Mg^{2+} , 2.0 mM Na^+ , 1.8 mM Cl^- , 10 μM Mn^{2+} , 5.0 μM Zn^{2+} , 30 μM B^{3+} , 15 μM Fe^{3+} , 0.75 μM Cu^{2+} , and 0.5 μM Mo^{6+} . pH and electrical conductivity (EC), were measured every five days.

The established treatments were: a) Control treatment with regular growing conditions (C); b) Control treatment with added 40 mM NaCl to the nutrient solution (C salt); c) 40 mM NaCl to nutrient solution and application of biostimulant Protifert® LMW (B1); d) 40 mM NaCl to nutrient solution and application of biostimulant Delfan Plus (B2).

The selected salinity level (40 mM NaCl) represents stress conditions, previously reported to induce physiological and metabolic responses in parsley without

causing complete growth inhibition [15]. This level was chosen to simulate realistic saline conditions while allowing the assessment of both stress-induced phytochemical changes and mitigation effects of biostimulants.

NaCl was added to the solution one the 12th June. The baseline electrical conductivity (EC) of the irrigation water was approximately 2.1 dS m^{-1} and pH of 6.1. Following the addition of 40 mM NaCl, EC increased to 5.9 dS m^{-1} (pH was 6.0), which represents relatively high salinity conditions.

Environmental conditions in the greenhouse

The greenhouse conditions were an average temperature of 23.3 °C and 71.2% humidity. The maximum daily photosynthetically active radiation inside the greenhouse, ranged from 820 to 1450 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Biostimulant application

The biostimulant Protifert® LMW contains 60 wt% dry matter and 8.5 wt% total nitrogen, of which 8.0 wt% is present 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.

The biostimulant 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.

Both biostimulants were applied foliar 4 times during the cultivation period, in 14-day intervals, starting on 20th June. The control treatments were sprayed with water.

Plant material harvesting and yield assessment

Parsley samples were collected several times for yield assessment (fully developed leaves were cut), while samples for metabolic analysis were collected at the last harvest on 1st September. Only leaves and stems were collected, and weight for yield quantity. They were immediately frozen using liquid nitrogen and transported to the Chair of Fruit Growing, Viticulture and Vegetable Growing laboratory. They were then ground into a fine powder using a pestle and mortar, before being stored in Eppendorf tubes at -80 °C. For the analysis of metabolites 5 biological replicants were used for each treatment.

Extraction, identification, and quantification of phenolic compounds in parsley

Phenolic compounds were extracted according to the method described by Zamljen, Veberic [16], with minor modifications. Fresh plant material (0.1 g) was extracted using 4 ml of 80% methanol containing 3% formic acid. The mixture was sonicated for 1 h at 0 °C, then centrifuged at 8000×g for 7 min and filtered through a 0.25 µm polyamide filter. The extracts were stored at −20 °C until analysis. The identification of the phenolic compounds was performed using UHPLC-MS/MS in negative ionisation mode. Quantification was carried out using UHPLC, as described by Zamljen, Veberic [16]. Quantification was based on authentic standards; when these were unavailable, the compounds were expressed as equivalents of structurally related phenolic compounds. All apigenin hexosides were calculated based on apigenin-7-*O*-glucoside, coumaric acid-*O*-hexosides were expressed as *p*-coumaric acid equivalents. Chrysoeriol pentose-hexose was expressed as kaempherol-3-*O*-rutinoside equivalent. Results were reported as mg kg^{−1} fresh weight (FW).

Extraction, identification, and quantification of volatile compounds in parsley

Volatile organic compounds (VOCs) were extracted and analysed by SPME–GC–MS following Zamljen, Zupanc [17] with minor modifications. Herb powder (1 g) was placed in 20 mL vials with 10 µL of 3-nonanone internal standard (8.1 mg/ml), sealed, and incubated at 50 °C for 5 min with agitation. VOCs were extracted from the headspace using a DVB/carbon WR/PDMS fibre for 40 min and thermally desorbed in the GC injector at 250 °C. Separation and detection were performed using a Shimadzu GC–MS QP2020 system equipped with a ZB-Wax PLUS column, using helium as the carrier gas. The oven temperature

program and MS parameters followed standard conditions as described by Zamljen, Zupanc [17]. Compound identification was based on mass spectral matching with NIST and FFNSC libraries, while quantification was achieved using analyte-to-internal-standard peak area ratios and corresponding external standards calibration curves (Table 2).

Chemical used in the experiment

The standards used in the experiment were: *p*-coumaric acid, kaempherol-3-*O*-rutinoside, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, 3-nonanone, α-pinene, 1-pentanol, eugenol, farnesol, limonene, methyl butyrate, myrcene, and NaCl (Sigma–Aldrich Chemie GmbH, Steinheim, Germany).

Statistical analysis

The statistical analyses were conducted utilising the R software (version 4.5.2). The data are presented as means ± standard error (SE). The statistical analysis employed to evaluate the observed differences among the treatments was a one-way analysis of variance (ANOVA), with treatment considered as a single factor with four levels (control, salinity control, Fitofert under salinity, and Delfan under salinity). When significant effects were detected, Tukey's honestly significant difference (HSD) test was applied for multiple comparisons among treatment means. Statistical significance was accepted at $p < 0.05$.

It is important to note that, given the experimental design was conducted under conditions of salinity, it does not represent a fully factorial arrangement. This is due to the fact that biostimulant treatments were only applied under these conditions (as their intended usage). Consequently, the analysis concentrated on overall treatment discrepancies, with particular attention to (i) the effect of salinity (control vs. salinity control) and (ii) the effect of biostimulants under salinity (salinity control vs. Fitofert and Delfan).

Results

Yield assessment in saline and biostimulant treated parsley

The yield of hydroponically grown parsley under saline conditions and treated with biostimulants is presented in Fig. 1. We observed drastic variation among treatments. In general, the C treatment had the highest yield contents with 531.0 g/plant. The B1 treatment had the second highest with 398.1 g/plant and the B2 treatment had 234.2 g/plant, respectively. The lowest yield was present at parsley grown only under saline conditions (C salt treatment), with a yield of 106.3 g/plant. The C treatment had almost five times higher yield than the C salt treatment. Biostimulants both increased yields compared to the C salt treatment

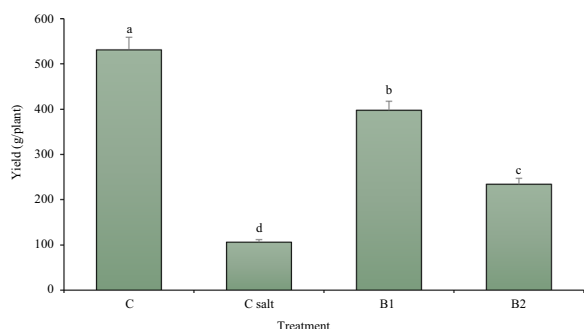


Fig. 1 Yield of hydroponically cultivated parsley under saline conditions and treated with two amino acid based biostimulants. **C**—Control plants; **C salt**—control plants and 40 mM salinity; **B1**—40 mM NaCl and Protifert®; **B2**—40 mM NaCl and Delfan Plus. Letters a–d denote statistically significant differences among treatments

Table 1 Tentative identification of phenolic compounds in parsley

Peak	Compound	Rt (min)	[M-H] ⁻ (m/z)	MS ² (m/z)	MS ³ (m/z)
1	Coumaric acid- <i>O</i> -hexoside 1	11.49	325	163 (100)	
2	Coumaric acid- <i>O</i> -hexoside 2	17.22	325	163 (100)	
3	Chrysoeriol pentose-hexose	21.60	593	299 (100)	
4	Apigenin- <i>O</i> -pentoside- <i>O</i> -hexoside	22.06	599	563 (100)	269 (100)
5	Apigenin-7- <i>O</i> -apiosyl-glucoside (Apiin)	22.20	563	269 (100)	
6	Kaempferol-3- <i>O</i> -rutinoside	22.63	593	285 (100)	153 (100)
7	Luteolin-7- <i>O</i> -glucoside	24.25	447	285 (100)	151 (100)
8	Apigenin-7- <i>O</i> -glucoside	24.71	431	269 (100)	
9	Apigenin-6,8-di- <i>C</i> -glucoside	25.17	593	473 (100)	353 (100), 269 (50)

Rt, retention time; [M-H]⁻, main mass of substance in negative ion mode; MS², first fragmentation; MS³, second fragmentation, (numbers) present relative abundance of ion

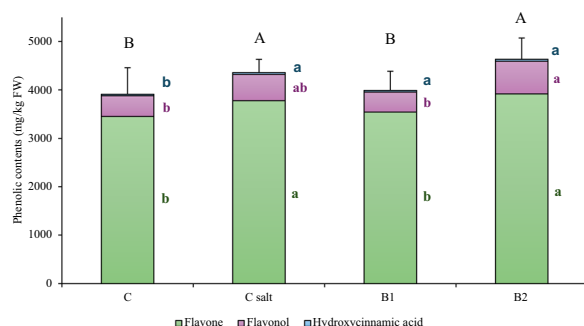


Fig. 2 Total analyzed flavones, flavonols and hydroxycinnamic acids contents in hydroponically grown parsley under saline and biostimulant treatments. **C**—Control plants; **C salt**—control plants and 40 mM salinity; **B1**—40 mM NaCl and biostimulant Protifert®; **B2**—40 mM NaCl and biostimulant Delfan Plus. Lowercase letters (a–b) denote statistical differences for each phenolic group among treatments. Upper case letters (A–B) denote statistical differences among total analyzed phenolics in all four treatments

with an 73.3 and 54.6% for B1 and B2 treatment respectively.

Tentative identification of phenolic compounds

Tentative identified individual phenolic compounds are presented in Table 1. Two hydroxycinnamic acids were identified based on the typical fragmentation pattern of MS² *m/z* 163 as reported by Kramberger, Barlič-Maganja [18]. The only flavanol identified was, with the pattern of MS *m/z* 593, MS² *m/z* 285 and MS³ *m/z* 153 [19]. Six flavones were identified. Luteolin-7-*O*-glucoside was identified based on its loss of glucose (– 162 Da), and external standard. Chrysoeriol pentose-hexose yielded a typical fragment of MS² *m/z* 299, as reported by Arsenov, Župunski [20]. Several apigenin derivatives were identified based on the characteristic MS² fragment at *m/z* 269 and comparison with literature data [21].

Phenolic contents in saline-grown parsley

As presented in Fig. 2 the most abundant phenolics were flavones with more than half of the total phenolic

contents, followed by flavonols. The total analyzed phenolics were highest in the C salt and B2 treatments, with 4357.4 mg/kg FW and 4632.9 mg/kg FW. Interestingly, the B1 treatment, had similar total analyzed phenolic contents as the C treatment. The flavone total contents was highest at the C salt and B2 treatments, with 3781.4 mg/kg FW and 3916.3 mg/kg FW, respectively. Both C and B1 treatment had lower flavone contents with 3455.6 mg/kg FW and 3546.1 mg/kg FW, respectively. In terms of flavonols the B2 treatment had the highest contents with 675.9 mg/kg FW, compared to the other three treatments. Hydroxycinnamic acids increased in all three treatments where NaCl was added (C salt, B1 and B2) and the increase ranged from 4 to 10 mg/kg FW, when compared to the C treatment.

In terms of individual phenolic presented in Table S1, apiin (apigenin-7-*O*-apiosyl-glucoside), was the most abundant in all four treatments. Chrysoeriol pentose-hexose decreased in all three treatments where salt was applied, when compared to the C treatment. On the other hand coumaric acid-*O*-hexoside 2 (4.0 to 6.0 mg/kg FW) and apigenin-6,8-di-*C*-glucoside (6.0 to 12.0 mg/kg FW) increased in all three saline treatments (C salt, B1 and B2), compared to the C treatment.

Volatile profile of parsley

The volatile profile of parsley is presented in Table 2. In general, 25 individual volatile compounds were identified. The most abundant were monoterpenes (13 compounds) and sesquiterpenes (7 compounds). Additionally, two aldehydes, one ester, one alcohol and one phenol ether were determined. The aroma is mostly characterised by fresh, green, citrusy, and resinous notes. Compounds such as *n*-hexanal, 2*E*-hexenal, 3*Z*-hexen-1-ol, and 3*Z*-hexenyl acetate, have been identified as key contributors to the fresh-cut grass and leafy odor that is characteristic of fresh plant material. The presence of monoterpenes, including limonene, γ -terpinene, α -terpinene, terpinolene,

Table 2 Volatile compound profile of parsley plants in the experiment

RT (min)	CAS no	Compound ID	Classification	Aroma	External standard
6.242	80-56-8	α -Pinene	Monoterpene	Resinous, woody	α -Pinene
8.14	66-25-1	<i>n</i> -hexanal	Aldehyde	Fresh green, grassy	Hexanal
8.809	127-91-3	β -Pinene	Monoterpene	Resinous, woody, slightly citrus	α -Pinene
9.288	3387-41-5	Sabinene	Monoterpene	Spicy, woody, spicy	α -Pinene
10.764	123-35-3	Myrcene	Monoterpene	Resinous, balsamic, herbal	Myrcene
11.31	99-86-5	α -Terpinene	Monoterpene	Herbal, citrusy	Limonene
11.982	138-86-3	Limonene	Monoterpene	Citrusy, fresh	Limonene
12.311	555-10-2	α -Phellandrene	Monoterpene	Minty, peppery, herbal	Limonene
12.815	6728-26-3	2 <i>E</i> -Hexenal	Aldehyde	Green, grassy	Hexanal
13.659	99-85-4	γ -Terpinene	Monoterpene	Citrusy	Limonene
14.531	99-87-6	<i>p</i> -Cymene	Monoterpene	Spicy, herbal, slightly citrus	eugenol
14.98	586-62-9	Terpinolene	Monoterpene	Citrus, pine	Limonene
16.236	3681-71-8	3 <i>Z</i> -Hexenyl-acetate	Ester	Green, fruity	Methyl butyrate
18.723	928-96-1	3 <i>Z</i> -Hexen-1-ol	Alcohol	Green, grassy	1-Pentanol
18.87	18,368-95-1	<i>p</i> -1,3,8-menthatriene	Monoterpene	Herbal, citrusy, minty, spicy	Limonene
19.941	21,195-59-5	<i>p</i> -1,5,8-Menthatriene	Monoterpene	Herbal, citrusy, minty, spicy	Limonene
20.291	1195-32-0	<i>p</i> -Cymenene	Monoterpene	Citrus, pine	Limonene
22.002	3856-25-5	Copaene	Sesquiterpene	Woody, spicy, resinous	Farnesol
23.482	13,744-15-5	β -Cubebene	Sesquiterpene	Spicy, woody	Farnesol
25.367	13,877-93-5	<i>E</i> -Caryophyllene	Sesquiterpene	Spicy, woody, clove-like	Farnesol
27.262	18,794-84-8	<i>E</i> - β -Farnesene	Sesquiterpene	Citrus, green	Farnesol
28.721	23,986-74-5	Germacrene D	Sesquiterpene	Woody, herbal, spicy-balsamic	Farnesol
30.014	16,729-01-4	δ -Cadinene	Sesquiterpene	Woody, smoky, spicy	Farnesol
30.267	20,307-83-9	β -Sesquiphellandrene	Sesquiterpene	Spicy, woody	Farnesol
37.826	607-91-0	Myristicin	Phenol ether	Spicy, sweet	Eugenol

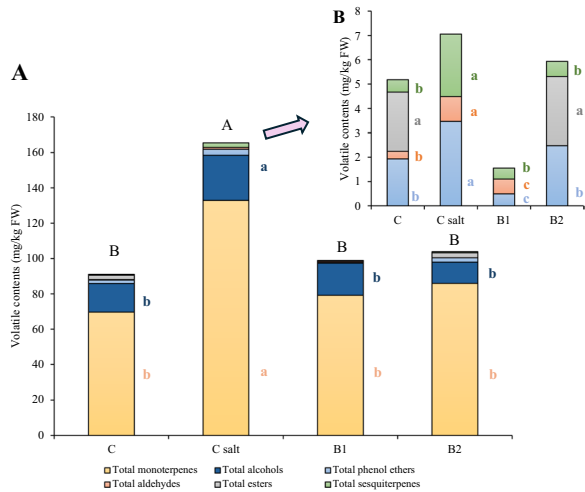


Fig. 3 Total analyzed monoterpenes, alcohols, phenol ethers, aldehydes, esters and sesquiterpenes content (**A** and **B**) in hydroponically grown parsley under saline and biostimulant treatments. **C**—Control plants; **C salt**—control plants and 40 mM salinity; **B1**—40 mM NaCl and biostimulant Protifert®; **B2**—40 mM NaCl and biostimulant Delfan Plus. Lowercase letters (**a–c**) denote statistical differences for each volatile group among treatments. Upper case letters (**A–B**) denote statistical differences among total analyzed volatiles in all four treatments

and *p*-cymene, has been identified as contributing to a range of sensory characteristics, including citrusy, herbal, and slightly spicy nuances, which have been shown to enhance the overall freshness of the aroma. Concurrently, α -pinene, β -pinene, sabinene, and myrcene contribute resinous, woody, and pine-like notes. The presence of *p*-1,3,8-menthatriene, *p*-1,5,8-menthatriene, and myristicin contributes to a distinctive herbal, minty and mildly sweet spicy profile, characteristics that are indicative of parsley. Sesquiterpenes such as *E*-caryophyllene, germacrene D, δ -cadinene, and copaene contribute to woody and balsamic base notes, adding depth and persistence to the overall aroma profile of parsley.

Volatile contents in saline grown parsley

Volatile contents are presented in Fig. 3A and B. Monoterpenes and alcohols were the most abundant volatiles, with a range from 69.66 to 132.09 mg/kg FW and from 11.96 to 25.50 mg/kg FW, respectively (Fig. 3A). Other volatile groups such as phenol esters, aldehydes, esters and sesquiterpenes presented only minor contents (up to 7.50 mg/kg FW), and were not present in all treatments (Fig. 3B). There were no aldehydes determined in the B2 treatment and no esters in the C salt and B1 treatment. In general, the C salt treatment had the highest total analyzed volatile contents with

165.47 mg/kg FW, followed by all other three treatments. Biostimulant treatments (B1 and B2) and the C treatment had similar total analyzed volatile contents.

In terms of individual volatiles, the most abundant volatile compound was *p*-1,3,8-menthatriene, α -phellandrene and 3Z-Hexen-1-ol in all four treatments. The C salt treatment significantly increased several volatile compounds compared to the other treatments. The most pronounced increase was observed for *p*-1,3,8-menthatriene, which was from 38.60 to 41.33 mg/kg FW higher than C, B1, and B2 (Table S2). Similarly, α -phellandrene showed a substantial increase ranging from 8.19 to 14.28 mg/kg FW under C salt compared to the other three treatments. The C salt treatment also had the highest contents of 3Z-hexen-1-ol (7.31 to 13.54 mg/kg FW higher), limonene (4.90 to 5.68 mg/kg FW higher), terpinolene (3.18 to 3.38 mg/kg FW higher), α -pinene (1.30 to 2.20 mg/kg FW higher), and myristicin (1.20 to 3.21 mg/kg FW higher) compared to the C, B1 and B2 treatments. The only volatile that decreased under saline conditions (C salt, B1 and B2) was α -terpinene, which decreased from 0.01 to 0.06 mg/kg FW compared to the C treatment. In general, both biostimulant treatments did not show any differences compared to the C treatment.

Discussion

As demonstrated in the present study, yield was found to be significantly influenced by salinity. This phenomenon can be attributed to the deleterious effects associated with the accumulation of sodium (Na^+) and chloride (Cl^-) in plant cells. Salinity has been demonstrated to disrupt key physiological and biochemical pathways [22], and excessive Na^+ accumulation has been shown to promote the efflux of cytosolic K^+ and Ca^{2+} in crop plants [23], thereby disturbing ionic homeostasis. This imbalance has been shown to result in a number of adverse outcomes, including, but not limited to, nutrient deficiencies, oxidative stress, impaired growth, and ultimately cell death [24]. Furthermore, elevated salinity has been demonstrated to directly impact photosynthetic processes, thereby reducing plant growth and development and consequently lowering yield [25]. The application of the two amino acids based biostimulants significantly mitigated the negative effects of salinity on yield, although the B1 biostimulant showed better efficacy than the B2 biostimulant (variability in effectiveness is common) [26]. Consistent findings were reported by Zuzunaga-Rosas, Calone [27], who observed enhanced lettuce growth under saline conditions following the application of a plant based hydrolyzed biostimulant. The role of biostimulants in modulating calcium (Ca^{2+}) accumulation, maintaining photosynthetic pigment levels,

and regulating sodium (Na^+) and chloride (Cl^-) concentrations within plant tissues has been demonstrated as reported by Pungin, Lartseva [28]. Furthermore, they have been demonstrated to reduce salt-induced stress biomarkers, including proline, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) [29]. The improvement of nutrient uptake and metabolic balance, even under conditions of severe salinity, is a key factor in the ultimate contribution of biostimulants to yield enhancement as reported by Zuzunaga-Rosas, Calone [27], which could explain our results regarding increased yield.

In contrast to the yield responses, the phenolic content exhibited greater variability. Salinity was found to increase the levels of hydroxycinnamic acids, particularly coumaric acid-*O*-hexoside 2, and apigenin-6,8-di-*C*-glucoside, while reducing chrysoeriol pentose-hexose concentrations, regardless of biostimulant treatment. It is noteworthy that the B2 treatment resulted in significantly higher concentrations of apigenin-7-*O*-glucoside and kaempferol-3-*O*-rutinoside compared to the other treatments. As demonstrated in the study by Pungin, Lartseva [28], hydroxycinnamic acids have been shown to exert protective effects under conditions of salinity stress. This may provide a rationale for the increased accumulation observed in this study.

The observed changes in phenolic compounds can be interpreted within the framework of the growth–defense trade-off hypothesis [6]. As reported by Rodrigues, Neng [30] In optimal conditions, carbon and energy derived from photosynthesis are primarily allocated to growth and maintenance, with surplus resources directed towards defense-related metabolites. However, salt and drought stress induce stomatal closure, thereby limiting CO_2 influx and suppressing Calvin cycle activity, which in turn reduces NADPH consumption [31]. Consequently, metabolic processes are reallocated towards the synthesis of highly reduced secondary metabolites, including phenolics, isoprenoids, and alkaloids [30]. These compounds have been demonstrated to play a crucial role in preventing excessive accumulation of reactive oxygen species (ROS) and maintaining cellular redox balance Świątek, Lu [32]. In the context of abiotic stress, the activation of the phenylpropanoid pathway plays a pivotal role in the detoxification of reactive oxygen species (ROS) and the protection of cellular components from oxidative damage [33]. Consequently, key genes encoding enzymes involved in phenolic biosynthesis, such as PAL (phenylalanine ammonia-lyase), C4H (cinnamate 4-hydroxylase), 4CL (4-coumarate: CoA ligase), CHI (chalcone isomerase), and F3H (flavanone

3-hydroxylase), are commonly upregulated in response to stress [34].

A more discernible pattern began to manifest in the volatile compounds. The C salt treatment was found to have the highest volatile content of all the treatments. Similar increases in volatiles such as *p*-cymene and γ -terpinene under saline conditions have been reported in oregano [13], while Tarchoune, Baâtour [35] observed a 22% increase in basil volatile content under NaCl treatment compared with control plants. These findings are consistent with our own results and provide further support for the concept of stress-induced metabolic reallocation. In the context of salinity stress, a greater proportion of photosynthetically fixed carbon is allocated to the synthesis of secondary metabolites rather than to growth [36]. In the present study, the C salt treatment resulted in a reduction in yield, accompanied by an increase in phenolic and volatile accumulation. In contrast, the application of biostimulants has been shown to alleviate stress, thereby enabling a greater proportion of carbon to be allocated to growth processes, while concurrently reducing the relative allocation to secondary metabolism, particularly volatile production and potentially changing the aroma of the parsley [26].

In summary, the application of hydrolyzed animal protein biostimulants resulted in a significant enhancement of plant performance under salt stress conditions. The application of these substances has been demonstrated to enhance photosynthetic activity, promote root and shoot growth, and strengthen antioxidant defense systems. Collectively, these effects have been shown to alleviate osmotic and oxidative stress [17, 37]. These products have been found to be rich in amino acids such as proline and glycine, which have been demonstrated to improve nutrient uptake and metabolic efficiency, thereby reducing salinity-induced yield losses [38]. These mechanisms provide substantial support for the findings of the present study and underscore the potential of amino acid based biostimulants as sustainable tools for enhancing crop performance under saline conditions.

Conclusion

Salinity (40 mM NaCl) significantly reduced parsley yield (up to fivefold), while concurrently enhancing the accumulation of phenolic and volatile compounds, thereby confirming a trade-off between growth and secondary metabolism. The application of amino acid-based biostimulants led to a partial alleviation of yield losses (up to 73%) while maintaining elevated levels of key phytochemicals, including flavones and monoterpenes.

The findings demonstrate that biostimulants can improve plant performance under saline conditions without compromising phytochemical quality, thus highlighting their potential for hydroponic cultivation of medicinal and aromatic plants. Significantly, this study offers original insights into the combined effects of salinity and biostimulants on both phenolic composition and volatile profiles in parsley, a subject that has been scarcely investigated.

However, it is imperative to exercise caution when interpreting the results, given the experimental limitations associated with system-level replication. Further studies incorporating replicated hydroponic systems and physiological measurements are required to confirm the underlying mechanisms and to optimize biostimulant application strategies under saline conditions.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-026-00966-4>.

Supplementary Material 1

Author contributions

Conceptualization, methodology, and investigation: T.Z., J.J., M.C.G.; writing—original draft preparation: T.Z., M.C.G.; writing—reviewing and editing: J.J., M.C.G.; funding acquisition and project administration: T.Z.

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Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

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