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EFFECTS OF FOLIAR SPRAYS OF IODINE AND SELENIUM ON VEGETATIVE, REPRODUCTIVE, AND FRUIT RESPONSES OF KORDIA SWEET CHERRY

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ABSTRACT

Iodine (I) and selenium (Se) deficiency in the human body constitutes a significant health, social, and economic challenge. This study aimed to examine the effects of sprays of I and Se, applied alone or combined, on vegetative growth, yield, fruit quality, and fruit biofortification of Kordia sweet cherry (*Prunus avium* L.). The trees were sprayed with: (i) I and/or Se, four times in the early season at rates of 25 g ha^{-1} and 15 g ha^{-1} per spray, respectively; (ii) I and Se, once, 3 days before harvest, at rates of 50–100 g ha^{-1} and 30–60 g ha^{-1}, respectively; and (iii) I and Se in the early season as in combination (i), plus before harvest as in combination (ii), using the lowest rates of these nutrients. It was found that none of the spray combinations affected tree vigour, fruit yield, mean fruit weight, and fruit firmness and acidity. Early-season Se sprays delayed leaf fall and increased both chlorophyll level in leaves and soluble solid concentration in fruit. Pre-harvest spray treatments decreased leaf chlorophyll concentration, and caused leaf damage and defoliation. The highest amounts of I and Se in fruit were found in trees sprayed with these nutrients in the early season plus before harvest. Lower efficiency in biofortifying fruit with I and Se was observed for early-season sprays and for pre-harvest spray applied at the highest rates of I and Se. It is concluded that early-season sprays, alone or combined with a pre-harvest spray of I and Se, can be recommended for late-ripening sweet cherry cultivars.

Keywords: chlorophyll level, fruit biofortification, nutrients, *Prunus avium* L., soluble solid concentration

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a deciduous stone fruit tree widely cultivated in temperate zones [Habib et al. 2017]. Its fruit is valued not only for exceptional organoleptic properties, such as flavour, aroma, and texture, but also for its nutritional and dietary value, as it is a rich source of vitamins (e.g., vitamin C and provitamin A), minerals, dietary fibre, and bioactive compounds, including flavonols and anthocyanins [Díaz-Mula et al. 2010, Serra et al. 2011]. For these reasons, sweet cherry is recognised as a functional food with health-promoting properties [Blando and Oomah 2019].

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Selenium (Se) and iodine (I) are essential trace elements for humans. Iodine deficiency results in endemic goitre and impairment of growth, reproductive capacity, and cognitive functions [Hatch-McChesney and Lieberman 2022]. Selenium plays a crucial role in preventing cardiovascular diseases and certain types of cancer, as well as in supporting brain development, reproduction, and immune function [Rayman 2000]. It is estimated that nearly 2 billion individuals are at risk of I deficiency disorders [Ittermann et al. 2020], while 0.5–1 billion people worldwide exhibit clinical signs of Se deficiency [Oumer et al. 2024].

It is believed that the primary cause of human malnutrition in I and Se is the limited phytoavailability of these microelements in soil, which is multifactorial and depends on numerous factors such as pH, salinity, redox potential, microbiological activity, and the contents of clay particles, iron-manganese oxides, organic matter, and antagonistic ions in the soil solution [Eich-Greatorex et al. 2007, Stroud et al. 2010, Medrano-Macias et al. 2016, Gonzali et al. 2017, Shahid et al. 2018, Song et al. 2018].

One of the strategies to mitigate human malnutrition in I and Se is the enrichment of edible crops with these nutrients through the application of I- and Se-containing fertilisers (agronomic biofortification). The use of such fertilisers is recommended in many I- and Se-deficient regions, as they are easy to apply and highly effective in biofortifying plants [Hartikainen 2005, White and Broadley 2009, Cakmak et al. 2017, Smoleń et al. 2019a, Oztekin and Buyuktuncer 2025]. Since foliar absorption of exogenously applied nutrients is generally more efficient than soil application [Fageria et al. 2009, Fernández and Eichert 2009, Kannan 2010], foliar fertilisation of I and Se should be preferred, at least for field crops with edible parts above the soil surface [White and Broadley 2009, Medrano-Macias et al. 2016, Cakmak et al. 2017, Gonzali et al. 2017, Smoleń et al. 2019b].

To date, no research has been conducted on the responses of sweet cherry to pre-harvest I sprays. Despite numerous studies on foliar sprays of Se in agricultural and horticultural crops, only two reports have addressed the effectiveness of sprays of this nutrient in sweet cherry cultivation [Wójcik 2024, Zhang et al. 2025]. These studies demonstrated that pre-harvest Se sprays were highly effective in biofortifying the fruit. However, the effects of combined Se and I sprays have not been investigated, despite the fact that, from an economic point of view, the simultaneous use of multiple nutrients in spray solutions should be preferable [Prom-u-thai et al. 2020]. Therefore, the goals of this study were to examine the effects of I sprays alone and combined I and Se sprays, applied either early in the season or shortly before harvest, on vegetative growth, yield, fruit quality, and fruit biofortification in sweet cherry.

MATERIAL AND METHODS

Study site and soil conditions

The study was conducted over two consecutive growing seasons (2024 and 2025) at the Dąbrowice Experimental Station (latitude 51°55'N, longitude 20°6'E, elevation 136 m above sea level), affiliated with the National Institute of Horticultural Research in Skierniewice, Poland. In the first year of the study, the mean annual air temperature and total precipitation were 10.5 °C and 367 mm, whereas in the following year they were 9.7 °C and 496 mm, respectively. During the vegetation period (April–September), the average air temperature and rainfall were 17 °C and 202 mm in 2024, and 15.5 °C and 318 mm in 2025, respectively (Table 1).

The experimental sweet cherry field was planted in 2015 in Albic Luvisols with the following properties in the 0–30 cm layer: 65% sand (0.05–2 mm), 19% silt (0.002–0.05 mm), 16% clay (<0.002 mm), bulk density 1.3 g cm^{-3}, organic carbon 14 g kg^{-1}, pH 6.7, redox potential 473 mV, 3.1 g kg^{-1} active iron (Fe) oxides, 1.8 g kg^{-1} active aluminium (Al) oxides, 13 mg kg^{-1} active manganese (Mn) oxides, 67 mg kg^{-1} available phosphorus (P), 103 mg kg^{-1} available potassium (K), 51 mg kg^{-1} available magnesium (Mg), 2.6 mg kg^{-1} total I, 0.17 mg kg^{-1} water-soluble I, 137 µg kg^{-1} total Se, and 4.1 µg kg^{-1} KCl-soluble Se. The soil parameters were determined on a composite sample consisting of 10 subsamples taken in autumn of 2023 from the surface of the herbicide strips along tree rows. Except for the determination of total and soluble Se in the soil, the analytical procedures and apparatus used to assess soil parameters were described previously by Wójcik and Wójcik [2021]. Protocols for determining total and soluble Se in the soil were provided by Wójcik [2024].

Plant material and orchard management

Kordia sweet cherry trees grafted onto the Mazzard F12/1 rootstock (*Prunus avium*) were used as the experimental plant material. Kordia is commonly grown in many sweet cherry-producing regions because the fruits are large, firm, sweet, and resistant to splitting and brown rot caused by *Monilinia* spp. [Quero-García et al. 2017].

Table 1. Monthly air temperatures and precipitation in Kordia sweet cherry orchard

Parameter	I*	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
	2024											
Average air temperature (°C)	–1.0	5.2	5.8	10.5	16.3	18.7	20.6	19.4	16.2	9.3	3.0	2.2
Precipitation (mm)	32.6	32.8	31.8	23.8	39.8	48.6	23.2	30.6	36.2	20.8	27.6	19.4
	2025											
Average air temperature (°C)	2.5	–0.5	6.2	11.0	11.3	18.0	19.2	18.3	15.3	8.5	4.1	2.2
Precipitation (mm)	19.6	6.8	40.8	34.4	71.8	53.4	90.2	6.8	61.6	61.4	35.8	13.6

* Month: I – January, II – February, III – March, IV – April, V – May, VI – June, VII – July, VIII – August, IX – September, X – October, XI – November, XII – December

The trees were planted in rows oriented north-south (each 150 m long) at a spacing of 4.5 m between rows and 2.5 m between trees within rows (888 trees ha^{-1}). The tree canopies were trained as a spindle to a height of 3.5 m.

In the experimental field, Regina sweet cherry trees, planted in every second row, served as pollinators for the Kordia trees. To ensure successful pollination, two beehives were placed in the field before the onset of flowering.

Protection treatments against pathogens and pests were carried out according to the recommendations for integrated fruit production provided by Czarnocka et al. [2023].

Soil management involved maintaining herbicide-treated strips along the tree rows and mowing the sod in the alleyways. The 1.5-m-wide herbicide strips were maintained from early spring until current-season shoot growth was completed. The sod was mowed until autumn, when it reached a height of 15–20 cm.

Soil moisture was maintained at nearly field capacity through surface drip irrigation using a tensiometer (Soil-moisture Equipment Corp., Santa Barbara, CA, USA) inserted at a depth of 30 cm, 15 cm from an emitter.

Annually, ammonium nitrate and K-sulphate were broadcast together at the swollen-breaking bud stage over the soil surface of the herbicide strips at rates of 60 kg nitrogen (N) ha^{-1} and 80 kg K ha^{-1}. The amounts of N and K fertilisers used, as well as the mode and timing of application, were in accordance with the recommendations of Wójcik [2009] for integrated fruit production carried out under conditions of low organic matter content and optimal K availability in the soil.

The combinations and experimental layout

Trees were sprayed with I as K-iodate (KIO_3, 59.3% I, Sigma-Aldrich, Poznań, Poland) and/or Se as sodium selenate (Na_2SeO_4, 41.8% Se; Sigma-Aldrich, Poznań, Poland) in the following combinations: (i) four early-season sprays of I or Se, applied from 14 days after petal fall (BBCH 72) to 6 days before harvest (BBCH 81), at 12–15 day intervals, at a rate of 25 g I ha^{-1} or 15 g Se ha^{-1} per spray (100 g ha^{-1} and 60 g ha^{-1} per season, respectively), (ii) early-season combined I and Se sprays applied according to the schedule and rates described above, (iii) pre-harvest I and Se spray, applied 3 days before picking, at the rates per ha: 50 g I + 30 g Se, 75 g I + 45 g Se, or 100 g I + 60 g Se, referred to as low, medium, and high rates, respectively, and (iv) early-season I and Se sprays as in combination (ii) plus pre-harvest I and Se sprays at the rates of 50 g $ha^{-1}$and 30 g ha^{-1}, respectively. Trees that were not sprayed with either I or Se served as controls. The seasonal rates of I and Se fertilisers and the solution concentrations of the given spray treatments are presented in Table 2.

The spray treatments were applied by means of a motorised backpack sprayer (Solo 433H, Kleinmotoren GmbH, Sindelfingen, Germany), equipped with a flat-fan nozzle, at a pressure of 10 bar, using approximately 750 L of water per ha. To improve the wetting of the fruit surface, the non-ionic surfactant Tween® 20 (polyoxyethylene sorbitan monolaurate; Sigma-Aldrich, St. Louis, Missouri, USA) was added to the spray solutions at a rate of 0.3 L ha^{-1}.

Table 2. Iodine and selenium rates and spray solution concentrations used in foliar sprays in Kordia sweet cherry orchard

Combination	I rate (g ha^{-1} per season)	Se rate (g ha^{-1} per season)	Spray solution concentration (%)
Early-season sprays of I	100	–	0.0055
Early-season sprays of Se	–	60	0.0047
Early-season sprays of I and Se	100	60	0.0102
Pre-harvest spray of I and Se	50	30	0.0204
	75	45	0.0306
	100	60	0.0408
Early-season sprays of I and Se plus pre-harvest spray of I and Se at low rates	150	90	0.0102* 0.0204**
Control	–	–	–

* for early-season sprays, ** for preharvest spray

In both years of the study, the same trees were used for the tested combinations. The experiment was conducted using a completely randomised block design with four replications, with one replicate per combination in each row. Each plot consisted of three trees. To minimise cross-contamination of the applied sprays, plots within a row were separated by one tree, and a buffer row was placed between every two adjacent treated rows.

Measurements and observations

Leaf blade injury, leaf chlorophyll concentration, leaf drop, and tree vigour were used as vegetative indices. Leaf spray damage was evaluated 20 days after harvest on a sample of 60 leaves from each plot, collected from the middle portion of current-season shoots. Damage was rated on a scale of 1 to 5, where 1 indicated no visible injury and 5 represented chlorosis/necrosis covering more than 75% of the leaf blade area.

Leaf chlorophyll level was assessed on the samples used for the determination of spray damage, using a handheld SPAD-502 Plus Chlorophyll Meter (Konica Minolta Optics Inc., Osaka, Japan). Two readings per leaf were taken on opposite sides of the blade, at the midpoint between the tip and the base of the lamina.

Leaf drop was assessed on 40 labelled current-season shoots per plot, located outside the tree canopy, at two time points per season: when 5–10% of the leaves had fallen from the control trees, and 30 days later.

Vigour was evaluated 4 weeks after harvest by measuring the total length of current-season shoots from the central tree within each plot.

Fruit yield was measured for each plot by harvesting fruits with stems attached at the stage of commercial maturity, evaluated based on fruit size and skin colour using the Royal Horticultural Society Colour Charts.

The fruit quality parameters included the mean fruit weight (with pedicel), firmness, soluble solid concentration (SSC), and titratable acidity (TA). These parameters were determined on 30 fruits for firmness measurement and 100 fruits for the remaining characteristics, randomly selected from 10-kg bulk samples per plot.

The average fruit weight was calculated using an electronic balance with an accuracy of 0.01 g (WPS2100/C/2; Radwag, Radom, Poland).

The firmness of the fruit, defined as the maximum force required to puncture the skin, was measured using a texture analyser (Zwick Roell Z010; Ulm, Germany) equipped with a 50 N load cell and a flat-ended cylindrical probe with a diameter of 3.18 mm. Firmness was measured once per fruit, placed horizontally on a stationary steel plate at mid-height, near the suture, at a crosshead speed of 50 mm min^{-1}. Results were expressed in newtons (N).

The soluble solid concentration was measured in fruit juice using a temperature-compensated digital refractometer (Atago Co., Ltd., Tokyo, Japan), and TA was determined by titrating the fruit homogenate with 0.1 *N* sodium hydroxide to pH 8.1 using a Mettler Toledo DL 50 Graphix automatic titrator (Mettler-Toledo AG, Schwerzenbach, Switzerland). Results were expressed as malic acid content.

Iodine and Se in the fruit were determined in the edible parts (skin and flesh) based on 100 fruits, uniform in size and colour, free of defects, randomly taken from a 10-kg bulk sample from each plot. Fruit samples were rinsed with

0.01 M HCl, followed by double-deionised water. After drying with a paper towel, seeds and pedicels were removed, and the samples were then homogenised using a mill (Retsch GM 200, RETSCH GmbH, Haan, Germany).

Iodine was determined according to the procedure proposed by the Polish Committee for Standardisation [2008]. One gram of homogenised fruit, 15 mL of distilled water, and 1 mL of 25% TMAH (tetra-methylammonium hydroxide) were added to 30-mL Falcon tubes. After thorough mixing, the samples were heated at 90 °C for 3 h. The tubes were then filled with deionised water, mixed, centrifuged at 4500 rpm for 15 min and filtered through a 0.24 µm membrane.

Selenium was determined according to the protocol recommended by the Polish Committee for Standardisation [2012]. Briefly, 1 g of homogenised fruit was placed in a Teflon vessel with 5 mL of 65% HNO_3 and 2.5 mL of 30% H_2O_2, and microwave digested in a closed system using a three-step temperature programme: 130 °C for 5 min, 160 °C for 10 min, and 200 °C for 20 min. After cooling, the digest was transferred to 25-mL volumetric flasks, diluted with double-deionised water, and filtered through a 0.24 µm membrane filter.

The measurements of I and Se in the obtained filtrates were performed by inductively coupled plasma mass spectrometry (XSeries 2 ICP-MS; Thermo Fisher Scientific, Waltham, MA, USA), and their concentrations in the fruit were expressed on a fresh weight (FW) basis.

The accuracy of I and Se measurements was verified using tomato (*Solanum lycopersicum*) leaves (SRM 1573a) with a concentration of 0.085 mg I kg^{-1} on a dry weight (DW) basis, and apple (*Malus domestica*) leaves (Standard Reference Material – SRM 1515) containing 0.05 mg Se kg^{-1} DW.

All data were subjected to one-way analysis of variance (ANOVA). Leaf drop data were transformed using the formula y = arcsin(x). Analyses were performed separately for each growing season, and mean comparisons were analysed using Tukey's multiple range test at $P < 0.05$ with Statistica 10 software (StatSoft Polska, Krakow, Poland).

RESULTS

The early-season sprays of I and/or Se caused no leaf injury. However, combinations of pre-harvest sprays of I and Se, regardless of the doses used, resulted in leaf damage, with injury severity increasing as the rates of these nutrients increased. Leaf injury from trees sprayed with I and Se early in the season plus before harvest was comparable to that observed after pre-harvest sprays of I and Se at low rates (Table 3).

At the first assessment time point of leaf fall, only the pre-harvest sprays of Se and I at medium and high rates led to defoliation (Table 3). At the second time point, all pre-harvest spray combinations caused defoliation, with the greatest leaf loss observed in trees treated with I and Se at high rates. Compared to the control, early-season Se sprays, with or without I, inhibited the leaf fall process, whereas the combination of early-season I and Se sprays together with pre-harvest sprays of these nutrients did not affect leaf drop (Table 3).

Early-season I sprays did not influence leaf SPAD readings, while early-season Se sprays, with or without I, led to increased values of this parameter (Table 3). The pre-harvest spray combinations resulted in a decrease in leaf SPAD readings, with the greatest decline found in trees treated with the highest rates of Se and I (Table 3).

The total length of current-season shoots and fruit yield per tree did not differ among the tested combinations, averaging 44.9 m and 9.4 kg in 2024, and 50 m and 7.8 kg in 2025, respectively (Tables 3 and 4).

The mean fruit weight was not affected by the tested treatments (Table 4). The spray combinations also had no effect on fruit firmness and acidity (Table 4). Only early-season Se sprays, with or without I, resulted in increased SSC in fruit (Table 4).

All I spray treatments enhanced fruit I concentration (Table 5). Early-season I sprays, with or without Se, were more effective in improving fruit I levels than pre-harvest I and Se sprays at low and medium rates, and were as effective as a single pre-harvest spray of these nutrients at high rates. The highest fruit I concentrations were found when early-season Se and I sprays together with a pre-harvest spray of these nutrients were applied (Table 5).

All Se spray combinations increased fruit Se concentrations (Table 5). The highest fruit Se concentrations were found in the combination of early-season I and Se sprays combined with a pre-harvest spray of these nutrients. Compared to the above combination, pre-harvest spray treatments had a weaker impact on fruit Se concentration, although their effectiveness positively corresponded to the Se rate used. Early-season Se sprays, with or without I, had a greater influence on fruit Se concentration than pre-harvest I and Se sprays at low and medium rates, and simultaneously had a comparable impact to pre-harvest I and Se sprays at high rates (Table 5).

Table 3. Effects of iodine and selenium sprays on phytotoxicity, leaf drop, leaf greenness and vigour of Kordia sweet cherry trees

Spray treatment	Leaf injury (1–5)		Defoliation (%)				SPAD value**		Total length of one-year old shoots per tree (m)	
	2024	2025	2024		2025		2024	2025	2024	2025
			I term*	II term*	I term	II term				
Early-season sprays of I	1.0 ±0d	1.0 ±0d	7 ±1b	36 ±2c	7 ±2b	32 ±4b	41 ±1b	42 ±1b	44 ±4a	51 ±4a
Early-season sprays of Se	1.0 ±0d	1.0 ±0d	6 ±1b	27 ±3d	6 ±1b	20 ±3c	45 ±2a	45 ±1a	46 ±3a	54 ±6a
Early-season sprays of I and Se	1.0 ±0d	1.0 ±0d	7 ±1b	25 ±2d	7 ±2b	24 ±4c	45 ±2a	46 ±2a	47 ±3a	49 ±2a
Pre-harvest spray of I and Se at low rates	1.5 ±0.1c	1.6 ±0.3c	8 ±1b	44 ±4b	7 ±1b	33 ±3b	38 ±1c	39 ±1c	44 ±5a	49 ±2a
Pre-harvest spray of I and Se at medium rates	2.1 ±0.2b	2.2 ±0.2b	17 ±2a	45 ±2b	15 ±3a	41 ±4a	38 ±1c	37 ±1c	43 ±3a	51 ±6a
Pre-harvest spray of I and Se at high rates	2.9 ±0.1a	2.9 ±0.1a	17 ±2a	56 ±4a	20 ±3a	42 ±2a	35 ±1d	33 ±1d	44 ±4a	49 ±2a
Early-season sprays of I and Se plus pre-harvest spray of I and Se at low rates	1.6 ±0.1c	1.4 ±0.1c	7 ±2b	35 ±2c	9 ±1b	32 ±5b	38 ±1c	37 ±2c	46 ±2a	48 ±4a
Control	1.0 ±0d	1.0 ±0d	7 ±2b	37 ±2c	7 ±2b	32 ±4b	41 ±1b	42 ±1b	45 ±5a	49 ±3a

* I and II terms of defoliation were assessed when 5–10% of leaves from the control trees fallen and 30 d later, respectively.
** The higher value the more intense the green colour of the leaves.
Means with the same letter within each column are not significantly different by Tukeys multiple range test at $\alpha = 0.05$.

Table 4. Effects of iodine and selenium sprays on yielding and fruit quality of the Kordia sweet cherry trees

Spray treatment	Fruit yield (kg tree^{-1})		Mean fruit weight (g)		Firmness (N)		Soluble solid concentration (%)		Titratable acidity (%)	
	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025
Early-season sprays of I	9.4 ±0.6a	8.1 ±0.3a	9.7 ±0.4a	10.1 ±0.3a	4.7 ±0.2a	4.8 ±0.2a	17.0 ±0.2b	17.3 ±0.2b	0.88 ±0.02a	0.83 ±0.02a
Early-season sprays of Se	9.2 ±0.6a	7.9 ±0.7a	9.9 ±0.3a	10.3 ±0.4a	4.8 ±0.2a	4.7 ±0.2a	19.5 ±0.3a	19.1 ±0.2a	0.85 ±0.02a	0.81 ±0.02a
Early-season sprays of I and Se	9.2 ±0.6a	7.5 ±0.5a	9.8 ±0.4a	10.5 ±0.4a	4.7 ±0.3a	4.9 ±0.1a	19.1 ±0.2a	19.2 ±0.2a	0.87 ±0.02a	0.84 ±0.02a
Pre-harvest spray of I and Se at low rates	9.4 ±0.5a	7.7 ±0.4a	9.7 ±0.3a	10.3 ±0.4a	4.6 ±0.3a	4.8 ±0.1a	17.4 ±0.3b	17.1 ±0.2b	0.86 ±0.02a	0.84 ±0.02a
Pre-harvest spray of I and Se at medium rates	9.3 ±0.4a	7.6 ±0.4a	9.7 ±0.3a	10.3 ±0.2a	4.7 ±0.3a	4.8 ±0.1a	17.3 ±0.4b	17.5 ±0.2b	0.86 ±0.04a	0.83 ±0.02a
Pre-harvest spray of I and Se at high rates	9.3 ±0.6a	7.8 ±0.5a	9.4 ±0.3a	10.4 ±0.2a	4.6 ±0.2a	4.9 ±0.1a	17.3 ±0.4b	17.3 ±0.2b	0.87 ±0.04a	0.84 ±0.04a
Early-season sprays of I and Se plus pre-harvest spray of I and Se at low rates	9.6 ±0.5a	7.8 ±0.4a	9.7 ±0.3a	10.3 ±0.1a	4.6 ±0.3a	4.8 ±0.1a	17.3 ±0.2b	17.2 ±0.2b	0.86 ±0.04a	0.82 ±0.02a
Control	9.7 ±0.4a	7.7 ±0.5a	9.7 ±0.3a	10.3 ±0.2a	4.7 ±0.2a	4.8 ±0.2a	17.4 ±0.4b	17.3 ±0.2b	0.86 ±0.03a	0.84 ±0.02a

Means with the same letter within each column are not significantly different by Tukeys multiple range test at $\alpha = 0.05$.

Table 5. Effects of iodine and selenium sprays in Kordia sweet cherry orchard on fruit concentration of these nutrients

Spray treatment	I concentration (µg 100 g FW)		Se concentration (µg 100 g FW)	
	2024	2025	2024	2025
Early-season sprays of I	17.3 ±1.2b	17.6 ±1.6b	0.1 ±0e	0.1 ±0e
Early-season sprays of Se	1.1 ±0.1e	1.0 ±0.1e	3.6 ±0.2b	3.6 ±0.1b
Early-season sprays of I and Se	17.8 ±1.3b	17.2 ±1.3b	3.4 ±0.3b	3.5 ±0.2b
Pre-harvest spray of I and Se at low rates	9.1 ±0.8d	9.4 ±0.6d	2.4 ±0.1d	2.4 ±0.2d
Pre-harvest spray of I and Se at medium rates	12.1 ±1.5c	13.5 ±1.6c	2.9 ±0.1c	2.9 ±0.1c
Pre-harvest spray of I and Se at high rates	15.9 ±1.3b	16.6 ±1.1b	3.5 ±0.2b	3.5 ±0.2b
Early-season sprays of I and Se plus pre-harvest spray of I and Se at low rates	23.7 ±1.2a	23.5 ±1.2a	5.6 ±0.2a	5.5 ±0.3a
Control	0.7 ±0.4e	0.9 ±0.2e	0.1 ± 0 e	0.09 ±0e

Means with the same letter within each column are not significantly different by Tukeys multiple range test at α = 0.05.

DISCUSSION

Soil I and Se

The amounts of I and Se in edible plant tissues primarily depend on their bioavailability in the soil [Weng et al. 2009, Lopes et al. 2017]. Typical total I concentrations in soils range from 0.5 mg to 20 mg kg^{-1}, with an average of approximately 5 mg kg^{-1} [Whitehead 1984]. In Poland, agricultural soils contain 0.8–10 mg kg^{-1} of I [Kabata-Pendias and Pendias 1999]. In our study, the total soil I concentration (2.6 mg kg^{-1}) was slightly above the lower limit of this range, and may be considered a low I status.

In most non-seleniferous soils, total Se concentrations range from 10 µg to 2000 µg kg^{-1} [White and Broadley 2009], with an average of 400 µg kg^{-1} [Shahid et al. 2018]. Soil Se concentrations below 125 µg kg^{-1} are considered deficient [Broadley et al. 2006], although Lyons et al. [2003] suggest that under certain soil conditions, plant Se deficiency may occur even at total soil Se concentrations as high as 600 µg kg^{-1}. In the present study, the total soil Se concentration was 137 µg kg^{-1}, indicating that this level can be regarded as deficient or low. Notably, the total soil Se concentration in our study was lower than the average Se concentration in coarse-textured soils (172 µg kg^{-1}) reported in Finland, where Se deficiency in edible plant parts was widespread until the late 1980s [Yläranta 1983].

In the present study, the proportion of water-soluble I relative to its total concentration was 6.5%, which is consistent with the statement by Duborská et al. [2021] that, in most mineral soils, the soluble I pool accounts for up to 10% of total soil I content.

In the case of Se, the KCl-soluble fraction in the soil, considered plant-available, was low (4.1 µg kg^{-1}), representing approximately 3% of the total soil Se content. Similar proportions of the KCl-soluble Se fraction in mineral soils have been reported by Yläranta [1983], Martens and Suarez [1997], Yanai et al. [2015], and Ali et al. [2017].

Tree responses

Although I and Se are not considered essential micronutrients for plants, numerous studies have demonstrated that under certain soil and climatic conditions, they can exert beneficial effects on some physiological, biochemical, and/or metabolic processes in plant tissues [Gupta and Gupta 2017, Zhang et al. 2023].

Given the limited phloem mobility of both I and Se [Gupta and Gupta 2017, Shahid et al. 2018], which restricts their retranslocation from leaves and fruits to woody tissues, the tested sprays of these micronutrients in 2024 likely had a negligible effect on sweet cherry tree responses in the following year.

In our study, none of the early-season spray combinations caused leaf burn and defoliation (Table 3), indicating that the applied rates of I and Se (25 g and 15 g ha^{-1} spray^{-1}, respectively) were safe for cherry trees. However, Zhang et al. [2025] reported that in sweet cherries grown under semi-arid conditions, Se spray treatments at a dose of 8 g ha^{-1} spray^{-1} and a solution concentration of 0.005% resulted in leaf and fruit damage. In our study, the Se rate applied in early-season sprays was nearly twice that used by Zhang et al. [2025)] while the spray solution concentrations were comparable. Thus, we suggest that the absence of spray burn in our study may be attributed to the use of the surfactant Tween 20 in the spray solutions, as this adjuvant is commonly known to reduce the surface tension between above-ground plant parts and aqueous solutions, thereby improving, among other things, the uniformity of spray deposition on plant surfaces. As a result, the amount of spray solution deposited per unit area of leaf and fruit is reduced, thereby lowering the risk of phytotoxicity. This suggestion is particularly justified for sweet cherry, which produces relatively large leaves. Another factor that might differentiate the susceptibility of sweet cherry leaves and fruits to spray-induced damage is the sprayer used in our study (a motorised backpack sprayer operating at a high water volume). On one hand, this sprayer ensures thorough coverage of all leaves and fruits within the tree canopy, especially in the upper zone, but on the other hand it results in considerable solution loss due to spray drift beyond the canopy as demonstrated by Godyń et al. [2025].

In our study, only early-season Se sprays, with or without I, increased leaf SPAD readings (Table 3), indicating an enhanced chlorophyll level. Because early-season I sprays caused no change in leaf SPAD readings, the increased leaf chlorophyll levels were related to the application of Se in early-season sprays. Similarly, Zhang et al. [2025] demonstrated an increase in chlorophyll content in the leaves of sweet cherry grown under Se-deficient conditions following sprays of this nutrient at a rate of 4.8 g ha^{-1} per spray. An increase in leaf chlorophyll induced by exogenously applied Se has also been reported for chicory (*Cichorium intybus* L.) by Malorgio et al. [2009], Chinese boxthorn (*Lycium chinense*) by Dong et al. [2013], spinach (*Spinacia oleracea* L.) by Saffaryazdi et al. [2012], and sweet basil (*Ocimum basilicum* L.) and lettuce (*Lactuca sativa* L.) by Hawrylak-Nowak [2008, 2013]. The positive relationship between Se content in leaves and chlorophyll level can be attributed to the maintenance of the structural integrity and functional stability of chlorophyll molecules by Se, which results from the enhanced capacity to scavenge reactive oxygen species, as reported by Khan et al. [2023] and Zhang et al. [2025].

Despite the elevated chlorophyll levels in the leaves of trees sprayed with Se early in the season, neither tree vigour nor cropping was affected by this treatment (Tables 3 and 4). In contrast, Zhang et al. [2025] reported that under Se-deficient conditions, pre-harvest sprays of this nutrient at a rate of 4.8 g ha^{-1} per spray increased the fruit yield of sweet cherries, which was related to an enhanced photosynthesis rate. In the above-mentioned studies, the growth of sweet cherry trees was not evaluated.

In our study, only early-season Se sprays, regardless of the presence or absence of I, increased SSC in fruit (Table 4), suggesting that greater production of assimilates, resulting from enhanced leaf chlorophyll levels, was primarily transported to the fruit. Under similar soil conditions, Wójcik [2024] also demonstrated elevated SSC in Burlat sweet cherry fruit from trees sprayed with Se early in the season, despite the lack of vegetative response. Elevated SSC in Se-sprayed trees has also been reported for sweet cherry by Zhang et al. [2025], peach (*Prunus persica* Batsch.) by Pezzarossa et al. [2012], pear (*Pyrus communis* L.) by Deng et al. [2019], and citrus (*Citrus reticulata*) by Wen et al. [2021].

In contrast to early-season Se sprays, with or without I, all pre-harvest spray combinations of I and Se decreased leaf SPAD readings, likely due to spray burns that consequently reduced leaf chlorophyll content (Table 3) and photosynthetic potential. Simultaneously, pre-harvest sprays of I and Se had no effect on fruit size and internal quality characteristics (Table 4), indicating that leaf burns, induced during 3 days before harvest (even up to about 25% of the leaf blade) do not pose a risk of changes in the basic commercial attributes of sweet cherry fruit, at least for the Kordia variety. Under similar soil and climatic conditions, Wójcik et al. [2014] and Wójcik and Wójcik [2021] also found no negative effects of foliar sprays of I or Ca applied 7–10 days before harvest on the appearance and quality of apples and pears, despite the observed leaf injury.

It is worth noting that, despite leaf damage and partial defoliation of trees sprayed with I and Se before harvest, their growth and fruit yields over the subsequent two seasons were not affected (Tables 3 and 4). This suggests that the reduction in leaf assimilatory area immediately after the termination of shoot elongation in mature sweet cherry trees did not affect flower bud formation and, presumably, the reserve of assimilates in the woody organs of the tree. Sitarek [2004] also reported that, under Polish conditions, partial leaf damage immediately before or after the harvest of late-ripening sweet cherry varieties did not affect the yield potential of trees and fruit quality, including size, SSC, and acidity.

Fruit biofortification

Sweet cherry fruit, as a fleshy fruit, is regarded as a poor dietary source of both I and Se [Kunachowicz et al. 2017], which was confirmed in our experiment, as the concentrations of these nutrients in fruit from the control trees averaged only 0.8 μg I 100 g^{-1} FW and 0.1 μg Se 100 g^{-1} FW (Table 4). Under similar soil conditions, Wójcik [2023] reported that the native concentrations of I and Se in apples were 2 μg 100 g^{-1} FW and 0.2 μg 100 g^{-1} FW, respectively. In Germany, Budke et al. [2020, 2021] reported that I concentrations in apples varied from 0.14 µg to 0.63 µg 100 g^{-1} FW. Pappa et al. [2006] found that Se concentrations in fleshy fruits of different species were within the range of 0.14–0.63 µg 100 g^{-1} FW.

According to the European Commission [2011], the recommended dietary intakes of I and Se for adults are 150 µg and 55 µg per day, respectively. Instytut Żywności i Żywienia (National Food and Nutrition Institute) [2017], along with many national and international organizations promoting healthy eating, recommend that, for adults, fruits and vegetables should constitute half of the daily diet, with fruit accounting for one-fourth of this portion. Accordingly, the daily intakes of I and Se from fruit are 18.7 µg and 6.9 µg, respectively. Thus, based on these calculations, the amounts of I and Se in one portion of sweet cherry fruit (100 g) from the control trees accounted for 3.7% and 1.4% of the target daily intakes of fruit-derived I and Se, respectively.

In our study, early-season I sprays increased fruit I level up to 17.5 µg 100 g^{-1} FW on average (Table 5), covering 94% of the daily requirement for fruit-derived I. In the case of Se, fruit sprayed with this nutrient in the early season contained, on average, 3.5 µg Se 100 g^{-1} FW (Table 5), accounting for 51% of the recommended daily intake from fruit-derived Se. Under similar soil conditions, Wójcik [2023] found comparable Se levels in Burlat sweet cherry fruit following early-season sprays of this nutrient. However, Zhang et al. [2025] reported that despite applying a considerably lower total Se dose in foliar sprays over the season (25.6 g ha^{-1} versus 60 g ha^{-1}), Se levels in Mei Zao sweet cherry fruit reached 21.5 µg per 100 g FW. The reasons for such pronounced differences in the uptake efficiency of exogenously-applied Se by sweet cherry fruit between our study and that of Zhang et al. [2025] are difficult to explain.

In the present study, pre-harvest sprays of I and Se met from 49% to 89% and from 35% to 51% of the daily requirements for fruit-derived I and Se, respectively, with the highest levels recorded in the treatments applying the greatest rates of these nutrients. It is worth noting that pre-harvest I and Se sprays at the highest rates were comparably effective in biofortifying fruit as early-season sprays of I or Se (Table 5). The high efficiency of single pre-harvest spraying using high nutrient rates in increasing their fruit level has also been demonstrated for Ca in apples and pears [Wójcik et al. 2009, 2014], and I and Se in apples [Wójcik and Wójcik 2021, Wójcik et al. 2024].

Fruit levels of I and Se following early-season plus pre-harvest sprays of these nutrients met, on average, 126% and 80% of the daily requirements for fruit-derived I and Se, respectively. This indicates that the above sprays were highly effective in biofortifying fruit and can be considered for recommendation in sweet cherries. However, multiple sprays of I and Se during the season generate production costs. To reduce these costs, it is justified to combine the application of I and Se with pesticides and/or $CaCl_2$ fertiliser, which is commonly used in sprays to decrease rain-induced fruit cracking. Combined I and Se sprays with $CaCl_2$ should be particularly preferred because, as demonstrated by Wójcik [2023, 2024] the addition of this salt improved the uptake of exogenously applied I and Se by apples.

CONCLUSIONS

The results of this study demonstrated that in mature Kordia sweet cherries grown in soil with low levels of I and Se, four sprays of these nutrients, applied alone or combined during fruit growth (up to 6 days before harvest) at rates of 25 g I ha^{-1} and 15 g Se ha^{-1} per spray, did not affect tree vigour and fruit yield, although Se–containing sprays increased leaf chlorophyll concentration and SSC in the fruit. A single combined I and Se spray at rates of 50–100 g ha^{-1} and 30–60 g ha^{-1}, respectively, applied 3 days before harvest, damaged leaves and caused partial defoliation, which, however, did not affect tree growth, yield, and fruit quality. The above sprays with the highest rates of I and Se were as effective in biofortifying fruit with these nutrients as early-season sprays. Thus, given that early-season sprays of Se improved both SSC and the level of this nutrient in fruit, these treatments can be implemented in sweet cherries. To biofortify fruit with I, early-season sprays of Se can be applied combined with I.

In the case of the impact of the tested I and Se spray combinations on fruit enrichment with these nutrients, the most successful strategy was the application of early-season sprays plus an additional spray of I and Se before harvest at rates of 50 g ha^{-1} and 30 g ha^{-1}, respectively. This approach largely covered the target dietary requirements for Se and fully met them for I from fruit-derived sources. Therefore, we also conclude that the proposed schedule of combined I and Se sprays during the early season and shortly before harvest should be recommended in sweet cherry orchards for biofortifying fruit with these nutrients, at least for the Kordia variety and other late-ripening cultivars. However, further research should focus on verifying the above-mentioned strategy for early-ripening sweet cherry varieties, as well as on reducing the costs of Se and I sprays by using Se- and I-containing spray solutions with pesticides, plant growth regulators, and/or stimulants.

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AUTHORS' CONTRIBUTIONS

P.W. – conception and assumptions, analysing the results, writing a manuscript; W.P., A.S. – developing the methods; J.F., Z.B., A.S, W.P. – conducting research.

DECLARATION OF THE USE OF GENERATIVE AI IN THE PROCESS OF PREPARING THE PAPER

We declare that this work has not been prepared using generative artificial intelligence (genAI) tools.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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EFFECTS OF DIFFERENT ORGANIC FERTILIZER APPLICATIONS IN SOILLESS LETTUCE CULTIVATION

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ABSTRACT

The present study was conducted for the purpose of investigating the potential of different organic fertilizers as alternatives to chemical fertilizers. Three organic fertilizers (protein hydrolysate – PH, black soldier fly manure – BSF and wood ash – WA) were compared with a chemical fertilizer (15-15-15+ME) as a control. The fertilizers were applied at a rate of 1.5 dS m^{-1} once a week, at a rate of 1 L $plant^{-1}$. The results show that the highest values for plant height (23.9 cm), leaf number (24.7), crown diameter (35.2 cm), photosynthesis rate (83.2 µmol $m^{-2}s^{-1}$), plant dry weight (16.2 g) and yield (324.8 g $plant^{-1}$) were observed in lettuce plants treated with PH organic fertilizer. The highest leaf chlorophyll content (8.6 CCI) and stomatal conductance (408.7 mmol $m^{-2}s^{-1}$) were recorded in the control, while BSF manure significantly increased root length (36.8 cm) and root dry weight (6.2 g). The potential of organic fertilizers as a viable alternative to chemical fertilizers in the soilless lettuce cultivation has been demonstrated. The utilization of protein hydrolysate has emerged as a pivotal aspect in lettuce cultivation. The nitrogen content of organic fertilizers has been identified as a plant nutrient element affecting success in lettuce cultivation.

Keywords: black soldier fly manure, chemical fertilizer, protein hydrolysate, wood ash

INTRODUCTION

The engagement in agricultural activities is driven by the objective of meeting the population's dietary needs. This socio-economically significant issue has resulted in the emergence of systems in which chemical fertilizers are employed as an alternative to traditional agricultural methods, in parallel with the global population increase. The widespread and indiscriminate use of chemical fertilizers has exerted deleterious effects on soil quality and human health, thus necessitating the exploration of alternative cultivation systems, such as soilless farming. Soilless cultivation has become increasingly common, particularly for fast-growing vegetables such as lettuce, with the objective of enhancing both yield and quality [Savci 2012, Şahin 2016, Saraçoğlu et al. 2022, Durak and Polat 2025].

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Among leafy vegetables, lettuce (*Lactuca sativa* L.) is the most widely cultivated crop, and our country holds a significant position in global lettuce production. Lettuce, generally consumed fresh, is a vegetable rich in minerals, particularly from a nutritional standpoint [Okudur and Ercan 2016, Kilim et al. 2022]. Lettuce is categorized as a cool-season vegetable, showing strong resistance to low temperatures and requiring high humidity. Due to its short vegetation period, this species can be cultivated in all regions of Turkey and possesses considerable economic value [Yıldırım et al. 2015, Kilim et al. 2022].

Soilless agriculture is a cultivation method that is particularly effective in terms of the efficient use of limited agricultural land. It is also posited that the practice has the capacity to reduce environmental risks through the regulated utilization of nutrients. Nevertheless, even within these systems, a reliance on chemical fertilizers remains entrenched. Consequently, there is a compelling need to explore the utilization of alternative organic and biological materials as fertilizers [Durak and Polat 2025].

The present study examined the feasibility of utilizing various organic fertilizers as substitutes in soilless lettuce cultivation. The primary motivation for the research is to reduce the use of chemical fertilizers by promoting plant growth through the utilization of various organic fertilizers, either individually or in combination with chemical fertilizers. To this end, an experiment was conducted to examine the effects of three different organic fertilizers (protein hydrolysate, insect fertilizer, and wood ash) on soilless lettuce cultivation.

Materials and methods

The study was conducted at Ondokuz Mayıs University, Faculty of Agriculture, Department of Horticulture, between December 20, 2024, and February 12, 2025. The study was conducted in a greenhouse (120 m²) using fully automated soilless cultivation. Two climate control fans were installed in the greenhouse to maintain the humidity and temperature during cultivation. Lettuce seedlings (*Lactuca sativa* var. *crispa* Maritima) were planted in the growing pots, which were placed 25 cm apart in rows. The lettuce seedlings used in the study were obtained from a commercial company (Agtohum, Antalya, Turkey). The seedlings were planted in 7-liter plastic pots containing coconut coir (cocopeat) on a 5% sloped gutter, 40 cm above ground level for their growing environment on December 20, 2024.

The process of fertilization was initiated at the moment of sunrise and concluded at sunset, during the period of the growing season. The irrigation schedule was set to irrigate every 30 minutes for 2 minutes each time. Additional fertilizers were applied manually once a week according to the procedure specified in the experimental setup.

Climate data were recorded using data loggers (Hobo, MX2301A and H21 Onset, Bourne, USA) during the greenhouse cultivation period. Temperature values were measured at an average of 19.8 °C. Average relative humidity values were determined to be 40.8%. Measured average light values were 186.2 μmol $m^{-2}s^{-1}$ (PAR).

Experimental setup

For chemical fertilization (control), the compound chemical fertilizer 15–15–15+ME (Gübretaş, Istanbul) was used. The fertilizer's composition is reported as total nitrogen (15%), ammonium nitrogen (9%), urea nitrogen (6%), phosphorus pentoxide (22%), neutral ammonium citrate and phosphorus pentoxide (25%), potassium oxide (10%), sulfur trioxide (10%), and zinc (0.5%).

Table 1. Nutrient content of organic fertilizers

Elements	Protein hydrolysate (PH)	Black soldier fly manure (BSF)	Wood ash (WA)
EC (dS m^{-1})	2.39	2.92	13.6
pH	5.56	8.04	12.2
N (%)	10.5	3.2	0.13
P (ppm)	8808.3	650	7826
K (ppm)	23500	17000	66742
Ca (ppm)	214.1	13.9	66200
Mg (ppm)	454.1	0.4	43000
Zn (ppm)	60	166.6	36
Mn (ppm)	3.3	164.5	20000
Cu (ppm)	3.6	40.3	60
Fe (ppm)	82.5	841.2	1850

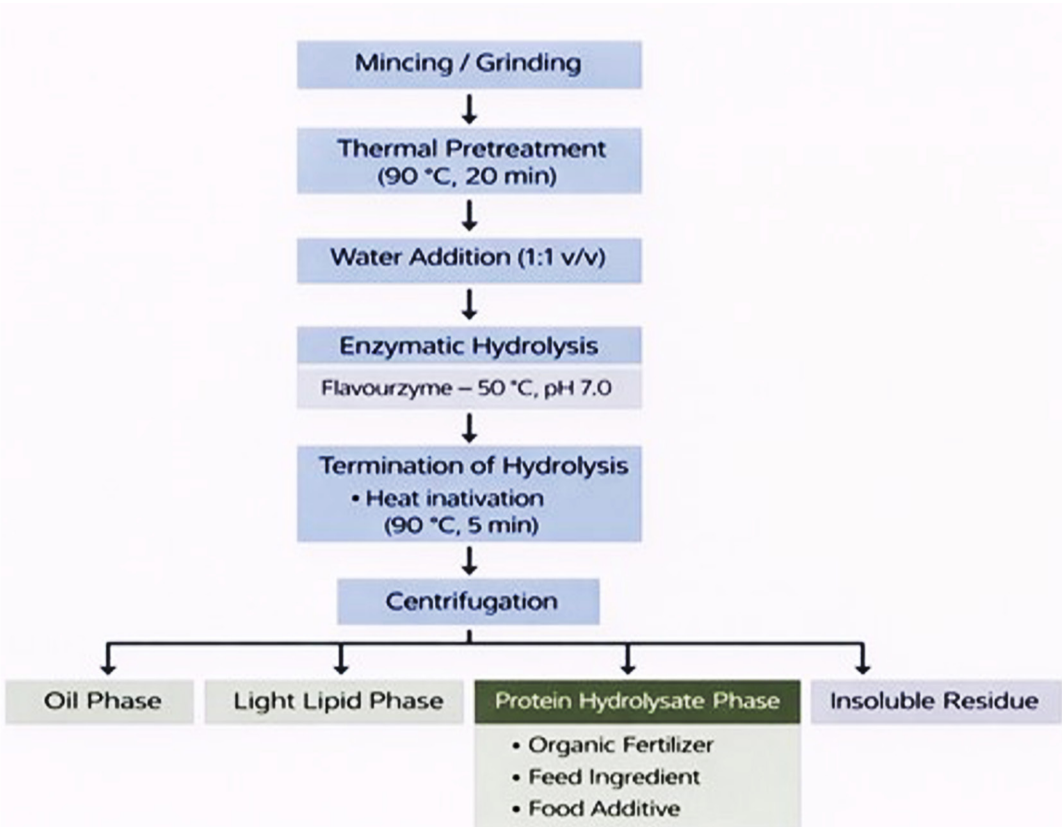
The EC value of the fertilization solution was set at 1.5 dS m^{-1}, in accordance with the findings of Kilim et al. [2022] and Çilingir Tütüncü et al. [2025]. In order to achieve an EC value of 1.5 dS m^{-1} for chemical fertilization, it was necessary to dissolve 2.27 g of fertilizer in 1 L of water. The study utilised three distinct organic fertilizers (protein hydrolysate – PH, black soldier fly manure – BSF, and wood ash – WA), and a chemical fertilizer for the purpose of establishing a control group. The analyses of the obtained fertilizers (PH, BSF, WA) solutions were made according to Kacar and İnal [2008] and are given in Table 1.

Preparation of organic fertilizers

The preparation steps for the organic fertilizers, whose plant nutrient content is detailed above, are described in detail below.

Protein hydrolysate (PH). In the study, anchovy (*Engraulis encrasicolus*) processing waste was obtained from SASTAŞ (Samsun Soğutma Tesisleri A.Ş https://www.sastas.com.tr). SASTAŞ processes fresh and frozen seafood in accordance with HACCP, BRC Food, and IFS Food Safety Standards, Social (Ethical) Standards, and National and International Legislation. The waste obtained from the facility was vacuum-packed in 5 kg packages, placed in foam boxes filled with ice, and transported to the Processing Technology Department laboratory of the Department of Fisheries Technology Engineering, Faculty of Marine Sciences, Fatsa, Ordu University, where it was stored at –40 °C until protein hydrolysate was obtained. The waste was thawed at room temperature using a mincer, then minced and homogenized. The material was subsequently heated at 90 °C for 20 minutes to ensure the inactivation of endogenous enzymes. The minced fish meat, with its enzymes inactivated, was cooled and homogenized by adding pure water at a 1 : 1 ratio. Protein hydrolysate from fish waste was obtained by enzymatic hydrolysis (Figure 1), following the method used by international companies, according to Korkmaz [2018]. When preparing the protein hydrolysate solution, 1 g of PH was added to 1 liter of water to achieve an EC of 1.5 dS m^{-1}.

Figure 1. Process flow diagram of fish protein hydrolysate production from anchovy (*Engraulis encrasicolus*) processing by-products



Black soldier fly manure (BSF). For the purposes of this study, a mixture of waste weighing 50 kilograms was prepared. The mixture was prepared by blending 30% (15 kg) of fish waste, 40% (20 kg) of vegetable and fruit waste, and the remaining 30% (15 kg) of household organic waste. A total of ten plastic containers, each measuring 64 × 40 × 10 cm, were utilised for the purpose of storing the mixed and homogenised waste, with each container containing 5 kg of the aforementioned waste. The experiment involved the use of 50 grams of 5-day-old black soldier fly (*Hermetia illucens*) larvae, which had been freshly hatched from eggs, and these were then weighed and placed into each container. A feeding study was conducted for 20 days until the larvae pupated. Subsequently, the pupae were extracted from the plastic containers, and the residual larval frass was subjected to desiccation and conversion into fertilizer.

Wood ash (WA). Waste ash of oak (*Quercus* sp.) species was used to prepare the wood ash solution. Waste ash was taken from a bakery shop in Samsun province (Yazıcıoğlu Kardeşler Bread Bakery). A solution with an electrical conductivity of 1.5 dS m^{-1} was prepared from wood ash. While creating the solution, 11 g of wood ash was added to 1 liter of water.

Fertilization for all treatments began four days after planting, on December 24, 2024, and was applied weekly at a rate of 1 liter per application. The study concluded on February 12, 2025, the harvest date.

Plant measurements

On the designated harvest date, the stem and crown diameters of the measurement and observation plants were measured using a digital caliper. The number of leaves was also counted. Leaf chlorophyll content (CCI), stomatal conductance (mmol $m^{-2}s^{-1}$), and photosynthesis rate (µmol $m^{-2}s^{-1}$) were measured between 9.00 and 11.00 a.m. on leaves at different levels of the measurement plants (3 leaves per plant). Leaf chlorophyll content was measured using a CCM-200 chlorophyll meter (Opti-Sciences, USA). The assessment of stomatal conductance was performed employing an SC-1 leaf porometer (Decagon Devices, Pullman, USA). The quantitation of photosynthesis rate values was accomplished using a Mini PPM (EARS, Wageningen, Netherlands) device.

Lettuce plants were extracted from the soil and divided into roots and leaves. The rosette mass was determined by weighing the fresh weight of each plant. Roots were washed with tap water to remove all soil particles. The root length was measured in centimeters. The leaves and roots were then placed separately in small paper bags and dried in an oven (Venticell 55, Ecocell, MMM Group, Germany) at 80 °C for 48 hours. The weight change method was used to determine when the drying process was complete. The dried leaf and root samples were weighed using a digital scale.

Statistical analysis

In this study, which was established according to a randomized block design, measurements were taken on a total of 60 plants, with 4 treatments, 3 replications, and 5 measurement and observation of plants in each replication. Differences between groups were determined using Duncan's multiple comparison test ($p > 0.05$). The OMU-licensed SPSS 21 software package was used for data analysis.

Results and discussion

The seedlings of the lettuce *Lactuca sativa* var. *crispa* Maritima (planted in four true-leaf stages), were ready for harvest 54 days after planting. Harvest times in lettuce cultivation vary from 36 to 80 days. Temperature has a significant effect on the harvest date. It has been reported that lettuce grown without soil is harvested earlier than lettuce grown in soil [Maboko and Du Plooy 2007, Okudur and Ercan 2016, Yörük et al. 2021, Kilim et al. 2022].

In our study, different fertilizer (PH, BSF, WA and Control) applications were found to have significant effects ($p < 0.05$) on the plant height, leaf number, crown diameter, chlorophyll content, stomatal conductance, and photosynthesis rate of lettuce grown in a soilless cultivation.

According to the results, the highest plant height (23.9 cm), number of leaves (24.7), crown diameter (35.2 cm), and photosynthesis rate (83.2 µmol $m^{-2}s^{-1}$) were measured with the PH organic fertilizer application, while the highest chlorophyll content (8.6 mmol $m^{-2}s^{-1}$) and stomatal conductance (408.7 mmol $m^{-2}s^{-1}$) were measured with the control application. However, for leaf chlorophyll content, crown diameter, and leaf number values, BSF manure and control applications were similar and measured lower values, while photosynthesis rate values were measured similarly for BSF manure. The WA treatment yielded the lowest values in general (Table 2). It is hypothesized that the low nitrogen content of the WA organic fertilizer may have been a contributing factor (Table 1).

Table 2. The effects of different organic (PH, BSF and WA) and chemical (control) fertilizers on plant height, leaf number, crown diameter, leaf chlorophyll content, stomatal conductance and photosynthesis rate in soilless lettuce cultivation

Fertilizers	Plant height (cm)	Leaf number	Crown diameter (cm)	Chlorophyll content (CCI)	Stomatal conductance (mmol $m^{-2}s^{-1}$)	Photosynthesis rate (µmol $m^{-2}s^{-1}$)
Control	20.8 ±1.84b	23.7 ±1.56a	33.5 ±2.56a	8.6 ±1.11a	408.7 ±6.80a	76.4 ±3.61b
PH	23.9 ±1.61a	24.7 ±1.04a	35.2 ±1.04a	7.3 ±1.18ab	310.8 ±6.05b	83.2 ±6.54a
BSF	22.2 ±0.54ab	24.2 ±1.61a	33.8 ±1.61a	8.5 ±1.16a	282.0 ±8.81b	80.8 ±5.87a
WA	9.8 ±0.57c	16.5 ±1.32b	17.3 ±1.32b	6.9 ±0.31b	189.7 ±4.11c	74.0 ±3.56b

Notes: means in the same column followed by different letters were significantly different ($p < 0.05$)

Plant-derived protein hydrolysates (PH) are attracting global attention due to their sustainability and positive effects on plants under abiotic stresses. The effects of these known effects on yield and quality in lettuce and tomato cultivation were investigated. For this purpose, PH (0 or 3 g L^{-1}) was applied to plants fertilized with four different N levels (2, 5, 10, and 15 mM) via foliar and root application. In the study, root application effectively stimulated yield, photosynthesis, water use efficiency, chlorophyll content, and antioxidant activities and compounds at all nitrogen levels compared to foliar application [Choi et al. 2022]. According to Cilingir Tuttuncu et al. [2025] compost solution, nettle solution, and wood ash solution have been identified as important alternatives to chemical fertilizers in lettuce cultivation. Wood ash applications have been reported to be notable for plant growth parameters. When lettuce was grown in soil, the application of fermented plant water, fermented fruit juice, and commercial organic leaf fertilizer had significant effects on yield and aroma, particularly on plant height (6.48–11.06 cm) and root length (4.19–7.26 cm), compared to the control [Caberoy and Ortuoste 2025]. Earthworm castings alone or in combination with different chemical fertilizers have been found to have significant effects on lettuce growth performance. While the best results were obtained with 100% chemical fertilizer application, 50% control + 20 ml/L earthworm castings-cow manure performed better than all other combinations. Specifically, this was attributed to leaf width, root length, root volume, root weight, leaf weight per plant, and total yield results [Ramos 2026]. The findings of our study are consistent with other studies on soil-less lettuce cultivation [Aruho et al. 2026, Bantis et al. 2026, Ghimire et al. 2026, Parra et al. 2026].

Our study determined that different fertilizers (PH, BSF, WA, and control) had significant ($p < 0.05$) effects on yield, dry weight, root length, and root dry weight. In our study, the highest yield (324.8 g $plant^{-1}$) and dry weight were obtained from the application of PH organic fertilizer, while the highest root length (36.8 cm) and root dry weight (6.2 g) were obtained from the application of BSF manure (Table 3).

Table 3. The effects of different organic (PH, BSF and WA) and chemical (control) fertilizers on yield, dry weight, root length and root dry weight in soilless lettuce cultivation

Fertilizers	Yield (g $plant^{-1}$)	Dry weight (g $plant^{-1}$)	Root length (cm)	Root dry weight (g)
Control	244.5 ±7.16b	14.7 ±0.71b	35.2 ±3.65a	3.7 ±0.78bc
PH	324.8 ±5.33a	16.2 ±1.27a	32.2 ±2.02b	4.3 ±1.05b
BSF	297.7 ±4.41ab	12.0 ±0.18c	36.8 ±2.88a	6.2 ±0.69a
WA	86.7 ±6.53c	8.7 ±0.35d	36.4 ±2.69a	3.1 ±0.57c

Notes: means in the same column followed by different letters were significantly different ($p < 0.05$)

The study found that root application effectively stimulated yield, photosynthesis, water use efficiency, chlorophyll content, and antioxidant activities and compounds at all nitrogen levels compared to foliar application. PH applications significantly increased fresh weight (21%) and dry weight (31%) in lettuce [Choi et al. 2022]. In the study of Çilingir Tütüncü et al. [2025], three different organic fertilizers (compost solution, nettle solution, and wood ash solution) were tested in lettuce cultivation as alternatives to chemical fertilization. The highest yield was obtained from wood ash solution applications (555 g $pant^{-1}$), while the lowest yield was measured in chemical fertilizer applications at 402 g $pant^{-1}$. Unlike our study, this study obtained the highest yield from wood ash application. We believe this situation stems from the high organic matter content (4.36%) of the soil in which the study was conducted. This is because nitrogen is known to be the key nutrient determining success in lettuce cultivation [Choi et al. 2022, 2025]. To this end, the effectiveness of using nitrogen in combination with PH was investigated. Specifically, the application of PH to the roots increased the yield (shoot fresh weight), quality (chlorophylls, carotenoids, flavonoids, and phenols), and nutrient uptake in hydroponically grown lettuce [Choi et al. 2025]. The findings of these studies are consistent with the PH yield values in our findings.

In the study of Turan et al. [2026], six different biostimulants were investigated in lettuce cultivation, including combinations with no fertilizer, chemical fertilizer, and positive control. Total chlorophyll levels increased with biostimulant application, indicating that biostimulants enhance photosynthetic efficiency. Biostimulant applications significantly improved plant and stem diameter, fresh and dry biomass, and yield ($p < 0.01$). The best yield and morphological performance were obtained in samples receiving biostimulant-fertilizer combinations (Kiana Climate® + 75 : 50 : 75 kg ha^{-1} N : P : K) and T7 (Kiana Earth® + 150 : 100 : 150 kg ha^{-1} N : P : K) applications.

In soilless systems, composting and vermicomposting are identified as the primary methods that can be used in for production of biological or organic fertilizers. However, agricultural waste, kitchen waste, biological solids, rumen waste, slaughterhouse waste, market waste, plant leaves, and wastewater are also available. Nutrients, compost teas, vermicompost teas, fermentation (immersion, solid state, co-culture, bokashi), and algae tea can be obtained from these substrates. It is emphasized that the development of the most suitable liquid biological fertilizer formulations depends on the optimal level of nutrients and the presence of beneficial microorganisms being higher than that of harmful pathogens [Zirebwa et al. 2026]. Similar to this study, our study has shown that the nutrient content of PH and BSF organic fertilizers can be an important alternative in soilless lettuce cultivation.

CONCLUSION

Thanks to its many cultivation and marketing advantages, lettuce can be grown year-round using soilless methods. In soilless lettuce cultivation, the use of fertilizers can be reduced by using natural and sustainable resources. Furthermore, this approach can have significant environmental impacts through improved waste management. Considering all these characteristics, it has been demonstrated that PH and BSF manure applications in soilless lettuce cultivation can serve as alternatives to chemical fertilization. The main factor limiting WA fertilizer is the nitrogen content of the waste. However, we believe that the high potassium and phosphorus content of WA waste will increase efficiency when used in combination with other organic fertilizers.

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Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DECLARATION OF THE USE OF GENERATIVE AI IN THE PROCESS OF PREPARING THE PAPER

Deepl AI was employed as an assistive proofreading instrument for the article.

AUTHORS' CONTRIBUTIONS

A.Ç.T, A.Ç. – investigation, data curation and visualization; S.D. – plant nutrient analysis, writing, investigation, data curation, and visualization; İ.C.D., O.Y. – preparation of black soldier fly fertilizer, investigation, writing, editing; K.K. – preparation of protein hydrolysate fertilizer, investigation, writing, editing; H.Ö. – writing, reviewing, supervision, investigation, methodology, conceptualization, data curation, project administration.

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FERTILIZATION-DRIVEN CHANGES IN BIOCHEMICAL PROFILE AND STORAGE STABILITY OF DRIED TOMATOES

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ABSTRACT

This study evaluated the effects of different fertilization strategies on the biochemical composition and storage stability of dried tomatoes. Tomatoes were grown under ten fertilization treatments and analyzed in two periods: immediately after drying (Period 1) and after six months of storage (Period 2). Total phenolic content (TPC), antioxidant capacity, and organic acid composition (oxalic, citric, malic, succinic, and fumaric acids) were determined. Fertilization treatment, drying period, and their interaction significantly affected TPC and organic acid composition ($p < 0.0001$). Antioxidant capacity was significantly influenced by fertilization and drying period ($p < 0.05$). In Period 1, TPC values ranged from approximately 31.40 to 35.96 mg GAE g^{-1}, while antioxidant capacity varied between 175.69 and 373.38 Trolox µmol TE g^{-1}, depending on fertilization treatment. After six months of storage (Period 2), both TPC (32.14–40.86 mg GAE g^{-1}) and antioxidant capacity (563.19–781.25 Trolox µmol TE g^{-1}) generally increased. Heatmap analyses revealed distinct clustering patterns between periods, with a more coherent association among malic acid, succinic acid, and TPC observed in Period 2. These findings indicate that fertilization strategies strongly influence both the initial biochemical composition and the long-term stability of dried tomatoes.

Keywords: dried tomato, fertilization strategy, antioxidant capacity, organic acids

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and consumed vegetables worldwide, not only for its culinary value but also for its rich composition of bioactive compounds, such as phenolic substances and antioxidants, which are associated with human health benefits. Phenolic compounds in tomatoes are important contributors to antioxidant capacity and are influenced by both genetic and environmental factors as well as cultivation practices. Studies have shown that phenolic content and antioxidant activity can vary significantly across tomato varieties and cultivation methods, with organic and fertilization regimes playing a key role in modulating these traits [Perea-Domínguez et al. 2018].

Fertilization management, including the type and rate of nutrient application, has been demonstrated to affect the accumulation of phenolic compounds and other quality parameters in tomatoes. For example, tomatoes fertigated with different nitrogen levels exhibited variations in total phenolic content and antioxidant capacity, indicating that nutrient supply influences secondary metabolite synthesis in fruit tissues [Jorge et al. 2017]. Moreover, differentiated organic fertilization strategies have been reported to affect phenolic composition and antioxidant potential across tomato varieties, suggesting that both nitrogen and carbon availability can alter the profile of bioactive molecules [González-Coria et al. 2022].

In addition to fertilization, post-harvest processes such as drying and storage can significantly alter the biochemical composition of tomato products. Drying is widely used to extend shelf life and concentrate nutrients;

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however, it also induces structural and metabolic changes that influence the stability and extractability of bioactive compounds. Recent studies have demonstrated that drying treatments can significantly affect the global metabolome and health-related compounds in tomatoes, leading to variations in phenolic content, organic acids, and antioxidant capacity [Bakir et al. 2023]. Furthermore, different drying conditions and processing parameters have been shown to influence the retention and degradation of bioactive compounds and structural properties of tomatoes [Fterich et al. 2023, Bayana et al. 2024]. In addition, comprehensive reviews have emphasized that advances in drying technologies play a critical role in determining the stability and functional quality of plant-based products, including tomatoes [Belwal et al. 2022].

Previous studies have shown that storage can influence the phenolic composition and antioxidant capacity of processed tomato products, although the direction and magnitude of these changes depend on product type and processing conditions. While reductions in phenolic content have frequently been reported for liquid tomato products such as juices and sauces during storage, dried tomato products often exhibit different behavior due to their low water activity and reduced enzymatic activity. In dried matrices, phenolic compounds may remain relatively stable or become more extractable over time, resulting in variable antioxidant responses during storage [Dewanto et al. 2002, Chang et al. 2006].

Despite the increasing number of studies on drying technologies and fertilization practices, limited information is available regarding their combined effects on the biochemical stability of dried tomatoes during storage. In particular, the interaction between pre-harvest fertilization strategies and post-harvest metabolic changes remains insufficiently understood.

In this study, it was hypothesized that different pre-harvest fertilization strategies (chemical, vermicompost, and organomineral) would not only determine the initial biochemical profile of dried tomatoes immediately after drying, but also significantly modify the metabolic reorganization and biochemical stability processes occurring during six months of storage. Specifically, it is anticipated that different nutrient source combinations will have a decisive impact on the dynamic changes (accumulation or reduction) of organic acids during storage, as well as on the long-term preservation of phenolic compounds and antioxidant capacity.

MATERIAL AND METHODS

Field experimental design

Seedlings of the Nergis F1 tomato cultivar were used as plant material for the experiment. The study was conducted in 30 plots arranged according to a randomized complete block design with three replications. Each plot was separated by a 1-m buffer zone, while blocks were spaced 2 m apart. The field trial was carried out under open-field conditions at Van Yüzüncü Yıl University, Turkey (38°34'25"N, 43°17'40"E). The experimental layout and field views of the study are presented in Figure 1.

Transplanting was performed on 11 June 2021 using a spacing of 100 cm between rows and 50 cm between plants within rows, resulting in a total of 600 plants, with 20 tomato seedlings assigned to each plot. To minimize border effects, six centrally located plants in each plot were selected for sampling and permanently tagged at the early flowering stage to ensure uniformity in fruit selection.

Tomato fruits were harvested at the red-ripening stage from the mid-canopy region of the tagged plants to reduce variation related to fruit position and light exposure. Only fruits that were uniform in size and maturity and free from visible defects were included in the analyses, ensuring a standardized and unbiased sampling procedure for all biochemical and quality measurements.

Pre-plant soil analysis indicated that the experimental site had slightly alkaline soil (pH 7.83), low organic matter content (0.93%), high lime content (10.84%), and an electrical conductivity of 0.01%, indicating no salinity problems. Throughout the growing season, plants were irrigated using a drip irrigation system to maintain consistent soil moisture. Detailed soil characteristics and climatic conditions during the 2021 growing season are presented in Tables 1 and 2, respectively.

Fertilization program

The fertilizer treatments were established to represent practical nutrient management approaches commonly used to improve both crop performance and soil quality. The different ratios of chemical fertilizer (CF), organo-mineral (OMF), and vermicompost (VC) were selected to take advantage of their complementary nutrient

Figure 1. Experimental layout and field views of the study

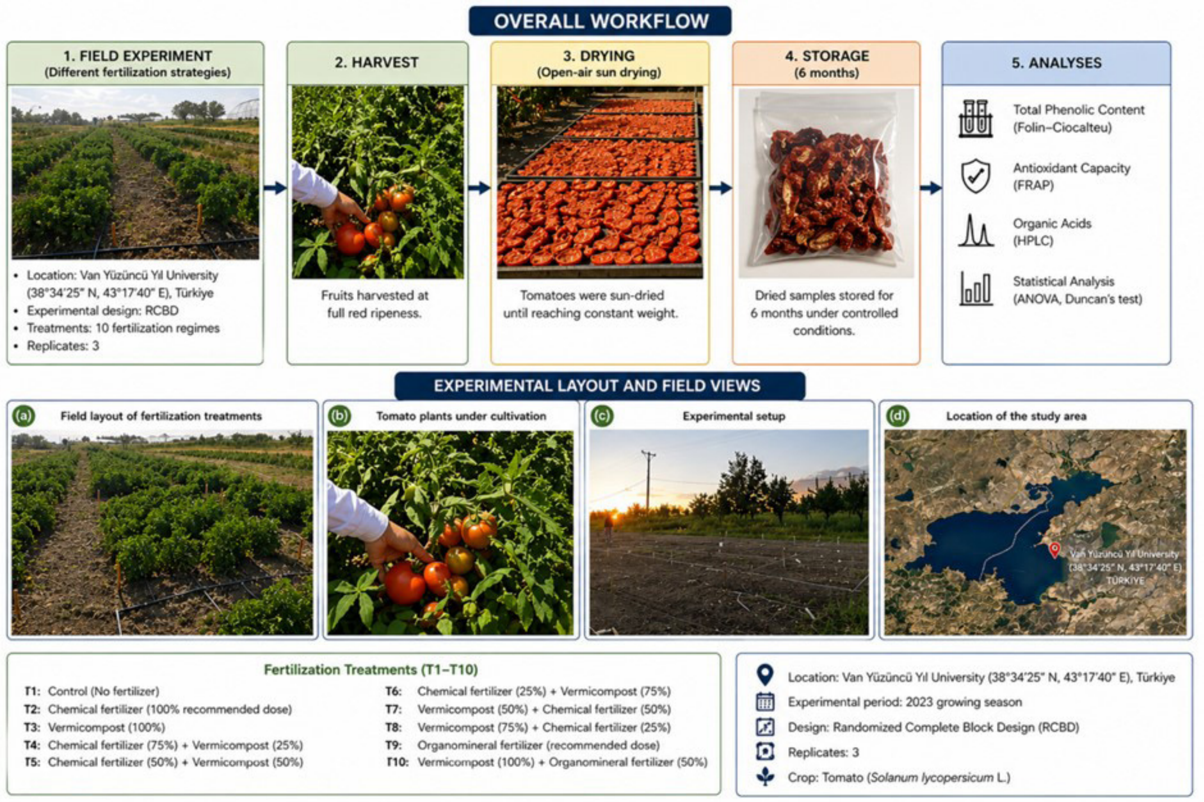


Table 1. Soil properties of the experimental site

Parameter	Value
P (mg kg^{-1})	5.50
K (mg kg^{-1})	11.04
Organic matter (%)	0.93
Lime (%)	10.84
pH (%)	7.83
Electrical conductivity/salt (%)	0.01
Structure (%)	sand: 37, clay: 24.3, silt: 38.6
N (%)	0.17
Mn (mg kg^{-1})	0.98
Na (mg kg^{-1})	86.35
Fe (mg kg^{-1})	1.12
Cu (mg kg^{-1})	0.53
Zn (mg kg^{-1})	0.46
Ca (mg kg^{-1})	1025
Mg (mg kg^{-1})	134

Table 2. Climatic conditions during the 2021 growing season

Parameter	May	June	July	August	September
Average temperature (°C)	19.2	21.1	24.5	26.4	22.8
Minimum temperature (°C)	10.5	14.3	17.1	19.6	16.5
Maximum temperature (°C)	27.8	27.9	31.8	33.2	29.1
Sunshine duration (h)	11.9	12.7	12.5	12.6	11.8
Precipitation (mm)	8.1	14.7	12.7	15.5	1.1
Wind (m s^{-1})	3.1	2.7	2.1	2.3	2.1
Relative humidity (%)	41.8	48.3	34.2	30.9	35.8
Evaporation (mm)	65.2	188.5	199.4	213.4	25.0

contributions. Chemical fertilizers supplying readily available nutrients, while vermicompost and organo-mineral fertilizers support longer-term improvements in soil organic matter content and microbial activity. This integrated approach aligns with sustainable agriculture principles by enhancing nutrient-use efficiency and reducing potential environmental impacts, which is particularly relevant for regions challenged by soil degradation and rising agricultural input costs.

Table 3. Composition of the fertilizers used during plant growth

Chemical fertilizer (CF) (20:20:20) (%)	Triple super phosphate (TSP)	46% nitrogen fertilizer	Organomineral fertilizer (OMF) 11:11:11 (%)	Vermicompost (VC) (%)
Total nitrogen: 20 Ammonium nitrogen: 3.9 Nitrate nitrogen: 5.9 Urea (N-NH_2): 10.2 Water soluble phosphorus pentoxide: 20 Water soluble potassium oxide: 20 Water soluble iron: 0.05 Water soluble copper: 0.02 Water soluble zinc: 0.02 Water soluble manganese: 0.02	Water soluble phosphorus pentoxide: 39 Phosphorus pentoxide soluble in neutral ammonium citrate (P_2O_5): 42	Urea: 46	Organic matter: 18.00 Total nitrogen: 11.00 Ammonium nitrogen: 5.00 Urea nitrogen: 6.00 Water soluble potassium oxide: 11.00 Total phosphorus pentoxide: 11.00 Water soluble phosphorus pentoxide: 9.00 Water soluble sulfur trioxide: 7.00 Total sulfur trioxide: 11.00 Total zinc: 0.10 Total humic acid + fulvic acid: 5.00	Organic matter: 35.00 Total nitrogen: 2.00 Organic nitrogen: 1.00 Water soluble potassium oxide: 2.00 Total phosphorus pentoxide: 0.40 Total humic acid + fulvic acid: 20.00 C/N: 10.04 Max. EC (dS m^{-1}): 2.00 Max. moisture: 35.00 pH: 6–8

C/N – carbon-to-nitrogen ratio, EC – electrical conductivity

Table 4. Fertilizer application rates used during the plant growing period

Treatment	Fertilizer combinations	Fertilizer doses
T1	control	–
T2	$CF_{(\%100)}$	19.8 kg da^{-1} N:P:K (20:20:20) + 14.21 kg da^{-1} TSP + 23.63 kg da^{-1} 46% urea
T3	$VC_{(\%100)}$	741.5 kg da^{-1}
T4	$OMF_{(\%100)}$	134.82 kg da^{-1}
T5	$CF_{(\%25)}+VC_{(\%75)}$	4.75 kg da^{-1} N:P:K (20:20:20) + 3.55 kg da^{-1} TSP + 5.91 kg da^{-1} 46% urea + 556.13 kg da^{-1} VC
T6	$CF_{(\%50)}+VC_{(\%50)}$	9.9 kg da^{-1} N:P:K (20:20:20) + 7.11 kg da^{-1} TSP + 11.82 kg da^{-1} 46% urea + 370.75 kg da^{-1} VC
T7	$CF_{(\%75)}+VC_{(\%25)}$	14.85 kg da^{-1} N:P:K (20:20:20) + 10.66 kg da^{-1} TSP + 17.73 kg da^{-1} 46% urea + 185.38 kg da^{-1} VC
T8	$OMF_{(\%25)}+VC_{(\%75)}$	33.71 kg da^{-1} OMF + 556.13 kg da^{-1} VC
T9	$OMF_{(\%50)}+VC_{(\%50)}$	67.41 kg da^{-1} OMF + 370.75 kg da^{-1} VC
T10	$OMF_{(\%75)}+VC_{(\%25)}$	101.12 kg da^{-1} OMF + 185.38 kg da^{-1} VC

CF – chemical fertilizer, VC – vermicompost, OMF – organo-mineral fertilizer, N:P:K – nitrogen:phosphorus:potassium, TSP – triple super phosphate

The experiment included three main fertilizer sources: chemical fertilizer supplied by Gübretaş, vermicompost sourced from Yaşa Tarım, and organo-mineral fertilizer produced by Hektaş (Table 3). These inputs were blended in different proportions to generate ten fertilizer treatments, as outlined in Table 4. Throughout the growing period, urea applications were made twice approximately four weeks apart to boost nitrogen supply in treatments receiving chemical fertilizer alone or in combination.

Drying procedure

Tomatoes harvested at the red-ripening stage were used as plant material in the study. Fruits were visually inspected, and those with uniform size and free from physical damage or defects were selected for further analysis. Selected samples were gently washed with distilled water to remove surface impurities and allowed to dry at room conditions. After preparation, tomatoes were sliced uniformly and arranged in a single layer on drying surfaces. Drying was carried out under natural sun-drying conditions by exposing the samples directly to sunlight under ambient environmental conditions. During the drying period, the average daytime temperature ranged between approximately 25–35 °C, and relative humidity ranged between 30–50%. The drying process lasted approximately 5–7 days, depending on environmental conditions. Samples were exposed to natural air circulation throughout the drying period. The drying process was monitored periodically and continued until constant weight was achieved. Following drying, samples were cooled to room temperature and stored in airtight containers protected from light. Samples analyzed immediately after drying were defined as Period 1 (P1), while the remaining samples were stored under ambient conditions (approximately 20–25 °C and 40–60% relative humidity) for six months with limited oxygen exposure due to sealed storage conditions, and subsequently analyzed as Period 2 (P2). A storage period of six months was selected to represent a typical long-term storage duration under ambient conditions, allowing sufficient time for biochemical transformations, stabilization of metabolites, and assessment of storage-related changes in dried tomato quality.

Extraction and HPLC–DAD determination of organic acids

Organic acids were determined following a modified protocol based on Bevilacqua and Califano [1989], adapted specifically for tomato tissue. Four fresh tomato fruits were homogenized, and 1 g of the homogenate was extracted with 20 mL of 0.009 N sulfuric acid (H_2SO_4). The mixture was homogenized thoroughly and shaken for 1 hour to facilitate extraction, then centrifuged at 15,000 rpm for 15 minutes. The supernatant was clarified through sequential filtration using coarse filter paper, a 0.45 µm membrane, and a SEP-PAK C18 cartridge to remove particulate matter and impurities.

Analysis was performed on an Agilent 1100 HPLC system equipped with an Aminex HPX-87H column, with detection carried out by a diode-array detector at 214 and 280 nm. Organic acid concentrations were quantified by comparison with external standards prepared under the same chromatographic conditions, ensuring accurate and reproducible measurements.

Total antioxidant capacity (TAC) and total phenol (TP)

Total phenols (TP) and total antioxidant capacity (TAC) were measured following the methods of Swain and Hillis [1959], and Benzie and Strain [1996], respectively. For TP, 150 µL of sample was reacted with Folin–Ciocâlteu reagent and sodium carbonate, incubated for 60 min, and absorbance was measured at 760 nm using a UV–Vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA). Results were expressed as gallic acid equivalents (GAE/100 mg FW). TAC was determined using the ferric reducing antioxidant power (FRAP) assay. Briefly, 150 µL of sample was mixed with FRAP reagent, incubated for 30 min, and absorbance was measured at 593 nm using a UV–Vis spectrophotometer mentioned above. Results were expressed as Trolox equivalents (TE/100 mg FW).

Statistical analysis

All experimental data were subjected to statistical analysis using a factorial experimental design. The effects of fertilization treatment (F), post-drying period (P), and their interaction (F × P) on organic acid composition, total phenolic content, and antioxidant capacity of dried tomato samples were evaluated. Data were analyzed by two-way analysis of variance (ANOVA), followed by Duncan's multiple range test, using IBM SPSS Statistics software (Version 26, IBM Corp., Armonk, NY, USA) to determine the significance of main effects and interaction terms. Results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Fertilization treatments (F), post-drying period (P), and their interaction (F × P) had a highly significant effect on organic acid composition, total phenolic content, and antioxidant capacity ($p < 0.0001$), indicating that both pre-harvest nutrient management and post-drying processes jointly regulate the biochemical profile of dried tomatoes (Tables 5 and 6).

Table 5. Organic acid (μg g^{-1}) profile, total phenolics (mg GAE 100 g^{-1}), and antioxidant capacity (Trolox μmol TE g^{-1}) of dried tomatoes as affected by fertilization and storage period

Period	Treatment	Organic acid					Antioxidant capacity	Total phenolics
		oxalic	citric	malic	succinic	fumaric		
P1	T1	331.02 ±14.86de	3406.95 ±265.21a	38334.41 ±2595.44g	86936.92 ±4744.53j	1659.83 ±199.55d	233.57 ±29.87cd	35.96 ±1.11c
	T2	315.94 ±0.97f–h	3542.23 ±34.24a	35548.05 ±2797.66g–ı	85236.91 ±666.54j	1503.53 ±87.87d	213.66 ±7.90cd	33.19 ±2.03d–g
	T3	332.53 ±15.78de	2968.52 ±865.50ab	32866.42 ±6445.09ı–k	85131.72 ±4879.21j	1449.66 ±3.36d	210.42 ±36.32cd	32.83 ±1.33e–g
	T4	299.75 ±1.94ıj	3099.55 ±96.33ab	35679.99 ±3160.32g–ı	75100.84 ±1689.06k	1751.99 ±185.55d	373.38 ±252.19c	32.60 ±0.67fg
	T5	293.89 ±0.50j	3074.18 ±81.56ab	39163.68 ±3271.55g	93588.20 ±1334.19ı	1883.72 ±184.47cd	178.94 ±45.16d	31.40 ±1.19g
	T6	303.04 ±12.86h–j	2663.86 ±754.07bc	30126.40 ±3904.22jk	65827.56 ±4801.94l	1472.21 ±178.36d	217.82 ±9.45cd	32.95 ±1.31e–g
	T7	307.70 ±17.32g–j	2371.72 ±839.10c	28739.14 ±4926.29k	69373.44 ±6385.16l	1505.16 ±196.02d	175.69 ±52.21d	34.01 ±1.04d–f
	T8	590.48 ±1.09a	119.83 ±0.51d	37648.24 ±86.36gh	88616.08 ±135.82j	1890.64 ±14.18cd	194.68 ±48.93d	31.59 ±1.18g
	T9	602.05 ±4.22a	68.26 ±4.87d	34638.14 ±314.73g–j	77662.47 ±1247.14k	1747.57 ±6.19d	259.03 ±58.55cd	34.94 ±1.20cd
	T10	320.71 ±17.01e–g	3168.80 ±27.08ab	32987.27 ±638.96h–k	76078.37 ±743.57k	1533.75 ±80.90d	235.42 ±6.37cd	33.25 ±0.20d–g
P2	T1	386.92 ±3.49b	351.23 ±4.69d	79369.46 ±1465.03b	206615.53 ±2534.66bc	5966.84 ±603.28a	712.50 ±93.03ab	38.01 ±0.46b
	T2	325.67 ±0.23ef	419.68 ±0.95d	75695.07 ±243.35bc	203046.91 ±144.90c	2851.52 ±15.63b	611.81 ±36.14b	38.50 ±0.18b
	T3	178.22 ±1.26k	369.47 ±0.81d	78913.86 ±231.04b	231026.30 ±640.75a	2913.95 ±26.44b	591.67 ±142.92b	40.82 ±0.86a
	T4	389.31 ±7.12b	144.87 ±1.49d	83588.05 ±2259.11a	164542.54 ±3879.87f	5565.52 ±1119.03a	781.25 ±93.20a	39.01 ±1.48b
	T5	315.86 ±2.82f–h	432.93 ±5.41d	73412.26 ±773.23cd	197062.19 ±1561.15d	3268.35 ±963.34b	600.69 ±44.70b	40.86 ±0.31a
	T6	313.97 ±2.39f–ı	321.65 ±2.04d	69587.89 ±713.37d	192149.08 ±1738.29e	2750.54 ±56.15b	595.83 ±39.11b	38.60 ±0.41b
	T7	311.87 ±4.86f–ı	444.64 ±1.26d	75124.45 ±1573.87bc	208089.16 ±254.33b	3268.31 ±1044.96b	563.19 ±66.97b	38.05 ±0.71b
	T8	365.38 ±1.21c	156.19 ±0.12d	45273.73 ±498.37f	138969.21 ±893.84h	1364.06 ±128.65d	637.50 ±140.41ab	36.26 ±0.70c
	T9	393.59 ±0.55b	153.61 ±0.38d	50126.30 ±63.85e	150567.11 ±119.55g	2602.15 ±27.71bc	720.83 ±109.57ab	34.70 ±0.98c–e
	T10	345.06 ±2.05d	159.65 ±0.48d	44145.60 ±1421.86f	135012.90 ±1831.69h	1417.83 ±219.09d	597.22 ±55.55b	32.14 ±1.32fg

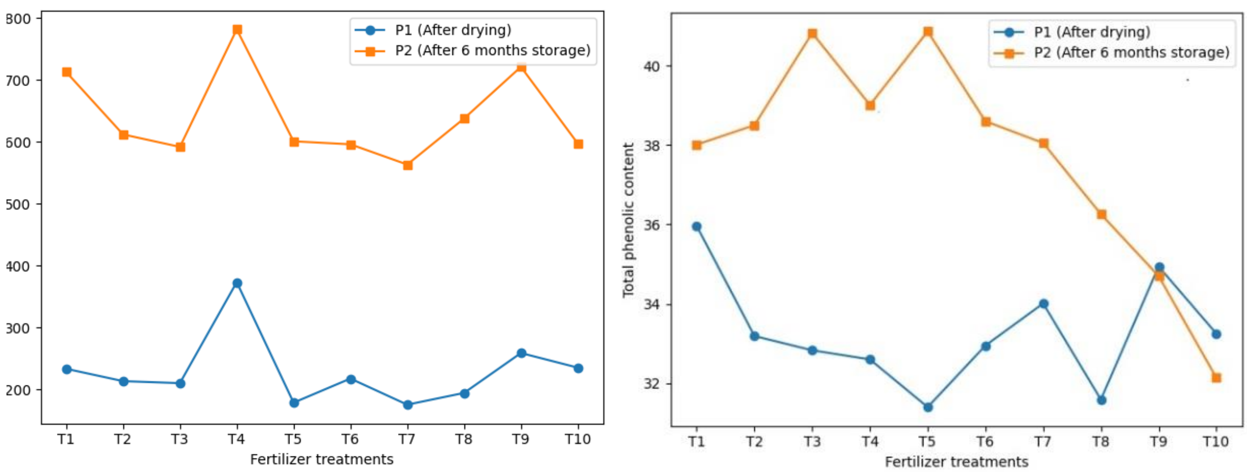
Different lowercase letters within the same column indicate statistically significant differences (Duncan's multiple range test, $p < 0.05$).

Table 6. Mean effects of fertilization treatments and storage period on the organic acid profile (μg g^{-1}), total phenolic content (mg GAE 100 g^{-1}), and antioxidant capacity (Trolox μmol TE g^{-1}) of dried tomatoes

Treatment	Organic acid					Antioxidant	Total phenolics
	oxalic	citric	malic	succinic	fumaric		
T1	358.97 ±32.10c	1879.09 ±1682.08ab	58851.94 ±22554.72ab	146776.23 ±65638.90b	3813.33 ±2393.04a	473.03 ±269.51ab	36.98 ±1.36a
T2	320.80 ±5.36f	1980.96 ±1710.42a	55621.56 ±22061.04c	144141.91 ±64528.64b	2177.53 ±740.48b	412.73 ±219.33b	35.85 ±3.18ab
T3	255.37 ±81.11h	1668.99 ±1525.18a–c	55890.14 ±25548.91bc	158079.01 ±79970.34a	2181.80 ±802.20b	401.04 ±228.70b	36.82 ±4.49a
T4	344.53 ±49.28d	1622.21 ±1619.49a–c	59634.02 ±26355.10a	119821.69 ±49062.29e	3658.76 ±2208.52a	577.32 ±280.76a	35.81 ±3.65ab
T5	304.88 ±12.17g	1753.55 ±1447.59a–c	56287.97 ±18878.83bc	145325.20 ±56689.92b	2576.04 ±979.78b	389.82 ±234.48b	36.13 ±5.23ab
T6	308.51 ±10.22g	1492.75 ±1368.66bc	49857.15 ±21759.21d	128988.32 ±69264.50d	2111.38 ±710.08bc	406.83 ±208.60b	35.77 ±3.22ab
T7	309.79 ±11.61g	1408.18 ±1181.41c	51931.79 ±25615.96d	138731.30 ±76085.14c	2386.74 ±1176.75b	369.44 ±218.93b	36.03 ±2.35ab
T8	477.93 ±123.30b	138.01 ±19.92d	41460.98 ±4188.89ef	113792.64 ±27585.47f	1627.35 ±299.81cd	416.09 ±260.14b	33.93 ±2.70c
T9	497.82 ±114.21a	110.93 ±46.85d	42382.22 ±8485.64e	114114.79 ±39939.37f	2174.86 ±468.42b	489.93 ±264.86ab	34.82 ±0.99bc
T10	332.88 ±17.19e	1664.23 ±1648.27a–c	38566.43 ±6190.68f	105545.64 ±32303.97g	1475.79 ±160.78d	416.32 ±201.30b	32.70 ±1.04d
Mean periods							
Period 1	369.71 ±116.26A	2448.39 ±1297.21A	34573.17 ±4362.53B	80355.25 ±9076.43B	1639.81 ±200.32B	229.26 ±91.03B	33.27 ±1.68B
Period 2	332.58 ±61.13B	295.39 ±123.12B	67523.67 ±14543.68A	182708.09 ±31890.88A	3196.91 ±1543.85A	641.25 ±102.06A	37.69 ±2.69A
P values							
Fertilization (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.010	<0.0001
Period (P)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F × P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.976	<0.0001

The significant interaction effect further demonstrates that temporal changes in biochemical parameters are strongly dependent on fertilization strategy rather than being uniform across treatments. Organic acids exhibited compound-specific responses during the post-drying period, reflecting differences in their metabolic roles. Oxalic acid decreased after drying, although the magnitude of reduction varied among treatments, suggesting that its behavior is governed by both degradation processes and fertilization-dependent metabolic regulation. This indicates that post-drying changes in oxalic acid cannot be explained solely by time, but are closely linked to pre-harvest nutrient status. Citric acid showed a pronounced decline from Period 1 to Period 2, indicating high sensitivity to post-drying processes (Tables 5–6). The reduction in citric acid may be associated with continued metabolic activity or transformation during storage [Agius et al. 2018, Paolo et al. 2019, Kıralan and Gündoğdu 2021, Kaygısız et al. 2025]. The variability observed among fertilization treatments further suggests that nutrient availability prior to harvest modulates the rate and extent of these transformations. In contrast to citric acid, malic, succinic, and fumaric acids showed substantial increases during the second period. These increases can be attributed to a combination of concentration effects resulting from water loss and ongoing metabolic conversions within organic acid pathways. Drying-induced reduction in moisture content may enhance the apparent concentration of these acids, while residual metabolic activity during storage may contribute to their accumulation [Osunde and Musa Makama 2007, Hussein et al. 2016, Qiu et al. 2018, Omorodion and Obiobu 2025]. The strong fertilization-dependent variation observed in these acids indicates that pre-harvest nutrient management influences both initial metabolite levels and their subsequent evolution during storage. The observed variability in organic acids is consistent with previous reports indicating that cultivation practices, particularly organic and organomineral fertilization systems, significantly influence carbon allocation and organic acid metabolism in tomato fruits [Oliveira et al. 2013, Paolo et al. 2019, Fibiani et al. 2022]. These findings suggest that fertilization strategies modulate the metabolic framework of the fruit, thereby determining its biochemical response during post-drying storage. Antioxidant capacity was significantly affected by fertilization and post-drying period, while the interaction effect was not significant, indicating that these factors independently contributed to antioxidant behavior. The marked increase in antioxidant capacity during storage suggests enhanced extractability and stabilization of antioxidant compounds in the dried matrix. This response is likely associated with structural changes induced by drying, including cell wall disruption and reduced water activity, which limit enzymatic degradation and facilitate the release of bound antioxidants [Dewanto et al. 2002]. Consistent with this interpretation, previous studies have reported higher antioxidant activity in dried tomato products compared to fresh fruits [Chang et al. 2006, Ayan 2010]. Total phenolic content was highly sensitive to both fertilization and storage period, as well as their interaction, indicating a strong dependence on both pre- and post-harvest factors. The increase observed during storage suggests that phenolic compounds were either preserved or became more extractable over time. This may be explained by drying-induced disruption of cellular structures, which enhances the release of bound phenolics, combined with reduced oxidative degradation under low moisture conditions [Jorge et al. 2018]. The higher phenolic content observed in organic-based fertilization treatments further confirms that organic nutrient sources stimulate phenolic metabolism, likely through their effects on plant stress responses and carbon allocation. Overall, the results demonstrate that the biochemical evolution of dried tomatoes is governed by a complex interaction between fertilization strategy, drying-induced structural changes, and storage-related metabolic processes. These findings emphasize that fertilization practices not only determine the initial biochemical composition of tomato fruits, but also play a critical role in regulating their stability and transformation during post-drying storage.

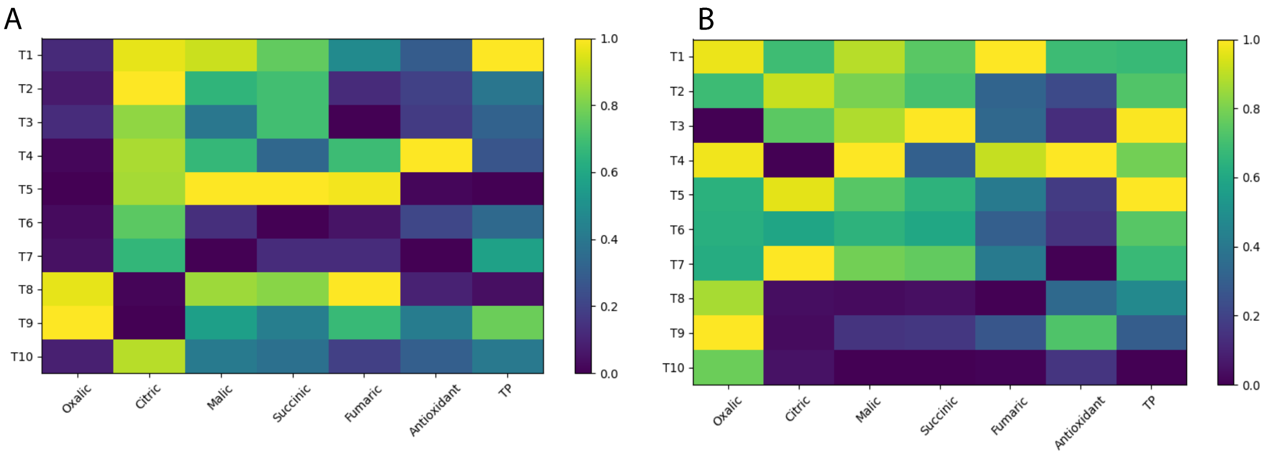
Figure 2. Interaction effects of fertilization regime and storage period on total phenolic content and antioxidant capacity of dried tomato samples



As illustrated in Figure 2, the direction and magnitude of changes in total phenolic content and antioxidant capacity during storage varied depending on the fertilization regime. These graphical trends support the statistical results presented in Tables 5 and 6, demonstrating that certain fertilization treatments were more effective in preserving or enhancing phenolic compounds and antioxidant capacity during the storage period. To evaluate these trends from a more integrative perspective, heatmap analyses constructed for P1 and P2 (Figure 3) revealed treatment-dependent variation patterns across biochemical parameters. In the P1 heatmap, organic acids exhibited noticeable variability among treatments, whereas total phenolic content and antioxidant capacity did not show a consistent association with these compounds. This observation suggests that the biochemical system had not yet reached equilibrium immediately after drying, and that time-dependent metabolic adjustments were still ongoing. Similar dynamic changes in phenolic and antioxidant components during post-processing, influenced by cultivation practices, have been reported previously [Toor and Savage 2006, Vallverdú-Queralt et al. 2011].

As storage progressed, the P2 heatmap showed more coordinated variation patterns among certain parameters, particularly total phenolic content, malic acid, and succinic acid. This indicates that prolonged storage promoted metabolic reorganization and strengthened the influence of fertilization strategies on the biochemical profile of dried tomatoes. Previous studies have also reported that during storage and processing, phenolic compounds may stabilize and antioxidant capacity may increase over time [Dewanto et al. 2002].

Figure 3. Heatmaps showing variation in organic acids, total phenolic content, and antioxidant capacity of dried tomatoes under different fertilization treatments in A) Period 1 and B) Period 2



The six-month post-harvest storage period generally resulted in increased levels of malic acid, fumaric acid, and succinic acid, as well as total phenolic content and antioxidant capacity in dried tomatoes. Previous studies have indicated that extended storage periods can influence the stability and transformation of biochemical compounds in tomato products [Bakir et al. 2023]. In contrast, reductions in polyphenol content and antioxidant activity have been reported during shorter storage durations (7–21–35 days), particularly under accelerated storage conditions involving elevated temperature and oxygen exposure [Nyawo et al. 2025]. Under the drying and storage conditions applied in the present study, reduced water activity, slowed enzymatic reactions, and concentration effects may have contributed to the relative stability of phenolic compounds and antioxidant capacity. In conclusion, the behavior of total phenolic content and antioxidant capacity in dried tomatoes during storage was shown to depend not only on storage duration, but also on pre-harvest conditions, particularly fertilization strategy, as well as the combined effects of drying and storage processes. Among fertilization treatments, T3 (vermicompost) was most effective in enhancing succinic acid and phenolic compounds, whereas T4 (organomineral fertilizer) was associated with higher malic acid content and antioxidant capacity. These findings demonstrate that soil nutrient management can modulate fruit biochemical composition in a multidimensional manner.

CONCLUSION

This study demonstrated that fertilization strategies play a decisive role in shaping the biochemical composition, and long-term stability of dried tomatoes. Total phenolic content, antioxidant capacity, and organic acid profiles were significantly influenced by fertilization treatments, drying period, and their interaction, indicating that both pre-harvest management and post-drying processes contribute to the final quality of the product. The results showed that immediately after drying (P1), organic acids responded sensitively to fertilization regimes. However, antioxidant activity and total phenolic content did not consistently align with organic acid patterns, suggesting that biochemical stabilization was not fully established at this stage. In contrast, after six months of storage (P2), a more coherent biochemical profile emerged, particularly among malic acid, succinic acid, and total phenolic content. Heatmap analyses further confirmed that prolonged storage promoted metabolic reorganization, and enhanced the expression of fertilization-dependent biochemical traits. These findings indicate that fertilization strategies not only affect the initial accumulation of bioactive compounds, but also influence their stability during long-term storage. The observed fertilization and period interaction highlights that the response of biochemical parameters to storage is treatment-specific rather than uniform. Therefore, optimizing fertilization practices before harvest may be as critical as post-harvest handling in preserving the nutritional and functional quality of dried tomatoes. Overall, this study emphasizes that the biochemical stability of dried tomatoes is determined by an integrated effect of fertilization management and drying-related processes. The results provide valuable insights for producers and processors aiming to improve the quality, stability, and functional properties of dried tomato products through targeted fertilization strategies. Despite these findings, some limitations should be acknowledged. The study was conducted under specific environmental and storage conditions, and therefore the results may vary under different climatic conditions or storage environments. In addition, the evaluation was limited to selected biochemical parameters, and other quality attributes such as sensory properties and volatile compounds were not considered. Future research should focus on evaluating different drying techniques and storage conditions in combination with fertilization strategies, as well as incorporating sensory and metabolomic analyses to achieve a more comprehensive understanding of quality changes in dried tomatoes.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

DECLARATION OF THE USE OF GENERATIVE AI IN THE PROCESS OF PREPARING THE PAPER

No generative AI tools were used in the preparation of this manuscript.

AUTHORS CONTRIBUTION

The author solely conceived and designed the study, conducted the experiments, performed the analyses, interpreted the data, and wrote and approved the final manuscript.

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RESEARCH PAPER

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CONSUMPTION PATTERNS AND INFLUENCING FACTORS OF POTTED HERBS: A STUDY ON THE POLISH MARKET

Wioletta Wróblewska[1], Eugenia Czernyszewicz[2]

ABSTRACT

The objective of this study is to identify consumption patterns and the factors influencing the selection of potted herbs in Poland. The research was conducted on a sample of n = 1000 using the survey method with the CAWI technique. The data underwent quantitative analysis, including descriptive statistics (percentages of responses), Pearson correlation analysis, and factor analysis using the principal component method. To assess the significance of relationships between socio-demographic variables and the study variables, the non-parametric Kruskal-Wallis test, as well as the Mann-Whitney U, Wilcoxon W, and Z tests, were used. Over 80% of Poles buy fresh potted herbs, most often in discount supermarkets and less often in health food shops, small grocery stores, greengrocers, and market stalls. The most frequently purchased species are parsley, mint, marjoram, coriander, and lovage. The primary reasons for purchasing potted herbs are flavor and seasoning qualities, health considerations, and nutritional value. The most important factors in the purchasing decision are aroma and taste, attractive appearance and freshness, health benefits, and nutritional value. Factors cited as encouraging consumers to consume fresh potted herbs more frequently included chemical-free cultivation and the use of organic methods, a wider range of products, lower prices, and better flavor. The socio-demographic characteristics of consumers had a significant impact on their purchasing behavior. These characteristics also differentiated the importance of factors influencing purchasing decisions, encouraged more frequent consumption of potted herbs, explained the reasons for a lack of interest in potted herbs among those who do not buy them, and identified factors that could encourage these people to consume fresh herbs. This study focuses specifically on consumption patterns of potted herbs in Poland, a segment influenced by sustainability trends and health-conscious behaviors. The study's findings may be relevant to producers and distributors of potted herbs, particularly for planning production and distribution to meet actual consumer requirements and preferences.

Keywords: potted herbs, preferences, purchase motives, consumer behavior

INTRODUCTION

Purchase decisions are a key aspect of the study of consumer behavior. For this reason, it is important to understand the factors influencing buyer decisions. As Kotler and Keller [2015] point out, a purchase decision is a process in which consumers choose and decide whether to buy, what to buy, when, where, and how to buy. This decision is influenced by many internal and external factors, such as product quality, brand, price, experience, and the consumer's socio-economic characteristics. Consumer preferences, shaped by product features and other factors considered in the purchasing decision, play a crucial role in the decision-making process [Suryaningrum et al. 2024]. Buying behavior refers to the choices and actions of consumers when purchasing goods. Although many scholars have studied buying behavior across various products, it has not yet been thoroughly examined in the context of potted herbs.

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The literature on the subject reveals a lack of research on the consumption of potted herbs in Poland, both quantitatively and behaviourally. Existing studies focus mainly on the general category of herbs, primarily in the markets of selected countries in Western Europe, Asia, and North America, whilst overlooking the specific nature of potted products and consumer conditions in Central and Eastern Europe. It is therefore appropriate to conduct a study identifying consumption patterns and the determinants of pot herb selection on the Polish market. In pursuit of the research objective, the following research problem was set:

RQ1. Where and how often do consumers purchase potted herbs in Poland?

RQ2. What motives and factors influence potted herb purchases?

RQ3. What factors encourage and foster more frequent consumption of fresh potted herbs?

RQ4. What are the reasons for the lack of interest in potted herbs?

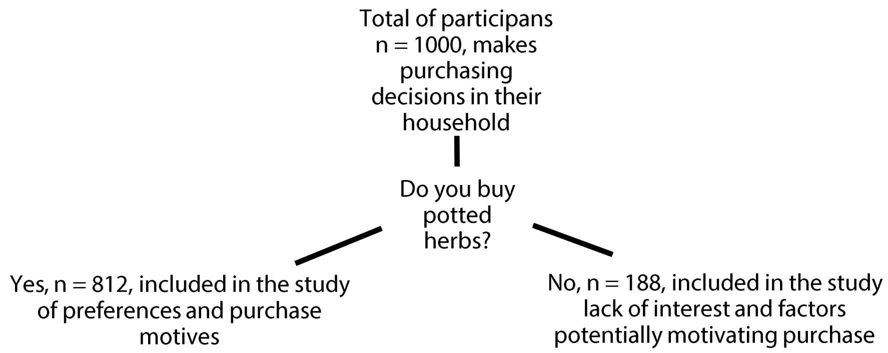
RQ5. Do consumers' socio-demographic characteristics influence their motives and behavior in the fresh potted herb market?

Herbs and spices have been known and used since the dawn of history, serving as a source of food for humans, as well as preservatives and medicinal agents [Jiang 2019, Chaudhari et al. 2021, Wiluk 2024, Grigore-Gurgu et al. 2025]. Many species of herbal plants have found use as spices, and their aromatic and medicinal properties have a beneficial effect on the process of digestion and the absorption of nutrients [Nurzyńska-Wierdak 2012]. An example is basil, one of the few herbs that was incorporated into cuisine specifically because it tastes good, that is, due to its very pleasant aromatic flavor profile [Spence 2024]. Herbs constitute an important group of horticultural products and have long been recognized as key elements in the culinary arts [Jiang 2019]. Research among plant buyers has shown that herbs are a popular category of household purchases [Knuth et al. 2023]. Fresh herbs used as spices or additives to dishes are utilized in many different cuisines and food products, but they are consumed in relatively small quantities [Awulachew 2024], especially compared to other horticultural plant-based food products [Kucharski et al. 2022]. However, according to the aforementioned authors, the popularity of herbal plants worldwide is on the rise. The value of the global fresh herbs market in 2025 was USD 6.56 billion. It is projected that this market will grow from USD 7.18 billion in 2026 to USD 14.86 billion in 2034, showing an average annual growth rate of 9.52% during the forecast period [Fresh Herbs Market size…]. Therefore, a growth rate of 10% per year is a strong indicator of the spices market's potential [Newerli-Guz 2017]. In recent years, potted herbs have represented a growing segment of both the horticultural and food markets in Europe and Poland. The observed growth results, among other things, from changes in consumption trends, with a key driver being the increasing demand for fresh and natural ingredients, as consumers become more aware of the health benefits of eating fresh herbs. Globalization of culinary practices also plays a significant role in market development, as consumers, discovering international cuisines, frequently use fresh herbs. Convenience is becoming an important factor as well, since herbs help save time and effort in meal preparation. Hence, this trend reflects a desire for kitchen convenience while also enjoying the flavor of fresh herbs and their health benefits. The correlation between health benefits and culinary uses often leads to an increased interest in organic herbs from local farms [Fresh Herbs Market 2025–2030]. According to Wiluk [2024], changes in consumer awareness make them more likely to seek traditional and organic methods of nutrition and health care. Widyaningtyas et al. [2023] state that health awareness and attitudes are important predictors of consumers' intentions toward herbal products. Abdullah and Salleh [2010], by identifying consumer preferences and opinions on health and natural resources in the context of the use of herbs and herbal products, showed that factors influencing consumer behavior include product commercialization, consumer preferences, and media influence. Consumers expect herbal products to be produced organically or at least in line with the principles of sustainable agriculture. In this context, product quality plays an important role in shaping consumers' purchasing behavior: when a consumer perceives a product as high quality, they are more likely to buy it. In the herb sector, much attention is directed towards farmers, who are the first and most important link in the supply chain [Kucharski et al. 2022]. Philips et al. [2024] point to potential implications of herbs' fragrance in the retail environment for consumers' health and well-being. These researchers emphasize the role of the retail environment in influencing food choices and sales: exposure to herbs activates associations with cooking and homemade meals, which in turn motivates consumers to choose healthier ingredients for preparing meals at home.

MATERIALS AND METHODS

The study was conducted as part of the TANGO project "Natural elicitors in the production of herbs of increased pro-health and consumer quality", carried out at the University of Life Sciences in Lublin. To achieve the article's goal, a portion of the empirical research was used. The research tool used to identify the demand side of the potted herb market in Poland was a structured questionnaire, and the data collection method was a CAWI (computer-assisted web interviewing) survey. The primary data, based on a questionnaire developed by the researchers, was collected by an external contractor, namely the BIOSTAT Research and Development Center. Participants were informed that they had consented to participate in the study and could withdraw if any question proved too sensitive. The study's target group was adult Poles. The research sample was limited to individuals who make purchasing decisions in their households. This criterion was verified based on a qualifying question included in the research questionnaire. The study was conducted on a research group of n = 1000 respondents. A stratified random sampling method was used, with the place of residence (province) serving as the stratum. Proportions by respondents' gender and age were also taken into account. The survey question "Do you buy potted herbs?" made it possible to identify the group of actual buyers, totalling n = 872 individuals. However, non-buyers were not disqualified from participation. This was based on the understanding that, as product markets mature, demand expansion can occur by converting non-buyers into buyers. Therefore, in the group of non-buyers of potted herbs (n = 188), respondents were asked about the motivators that could influence their purchasing decisions. Consequently, the study of purchasing preferences and motivations for buying potted herbs covered the group of actual buyers, while the study of lack of interest and potential motivation for purchasing included those who do not currently buy these products (Figure 1).

Figure 1. Selection of participants



The survey questions used a 5-point Likert scale, most commonly used in psychometrics in social sciences [Joshi et al. 2015]. For buyers of potted herbs, the survey questions covered, among other things, the types and frequency of purchases, including places of purchase, annual spending levels, reasons for buying, the importance of factors influencing purchasing decisions, and motivators for increased consumption of potted herbs. Those who do not currently buy these products were asked about reasons for their lack of interest in potted herbs and potential motivators encouraging them to consume these products.

The research model assumes that potted herb consumption patterns are determined by demographic factors and product characteristics. These factors influence consumers' purchasing motives, which then shape their consumer behavior, such as purchase frequency and product selection.

In the statistical analysis of the data, descriptive statistics (% of indications), Pearson correlation analysis, and factor analysis using the principal components method with Varimax rotation and Kaiser normalization were applied, following preliminary Kaiser-Meyer-Olkin and Bartlett's tests. To examine the significance of relationships between socio-demographic variables and the study variables, the nonparametric Kruskal-Wallis test, Mann-Whitney U test, Wilcoxon W test, and Z tests were used. Inference was conducted at a significance level of $\alpha < 0.05$. Statistical analysis was performed using IBM SPSS 29.

CHARACTERISTICS OF THE RESPONDENT SAMPLE

In the sample of purchasing respondents, there were slightly more women than men, while in the non-purchasing group, men predominated (Table 1). In both groups, people over the age of 60 were the majority. Individuals under 18 years of age were excluded from the study. Nearly 35% of all respondents had completed secondary education, and 29% held higher education degrees. Every tenth respondent had only primary or lower secondary education. Among the respondents, 60% were professionally active, while 28% were retirees or pensioners. Nearly

61% of respondents had a disposable income per family member between 1,001 and 3,000 PLN, and slightly more than one in five (21.9%) reported 3,001–5,000 PLN. Twelve percent of respondents were in the lowest income group, with up to 1,000 PLN per family member. The sample was dominated by people from two-person families (30.4%) and three-person families (27.4%). More than one in ten respondents belonged to single-person households (11.8%), and an equal proportion came from five-person or larger families. All respondents reported making purchasing decisions for their households.

Table 1. Characteristics of the sample of respondents (%)

Characteristics of respondents		The entire sample n = 1000	Buyers n = 812	Non-acquiring n = 188
Overall		100.0	81.2	18.8
Gender	woman	52.4	55.9	37.2
	man	47.6	44.1	62.8
Age	under 18 years old	0.0	0.0	0.0
	18–29 years old	16.3	17.9	9.6 10.6 15.4 17.0 47.3
	30–39 years old	19.3	21.3	10.6
	40–49 years old	18.5	19,.2	15.4
	50–59 years old	14.7	14.2	17.0
	60 years and older	31.2	27.5	47.3
Education	primary/junior high	10.2	6.5	26.1
	vocational education	26.0	23.8	35.6
	secondary	34.9	37.3	24.5
	higher	28.9	32.4	13.8
Social status	unemployed	3.0	2.8	3.7
	pupil/student	3.4	3.6	2.7
	employed professionally	59.7	63.2	44.7
	pensioner/retiree	28.0	25.0	41.8
	I take care of the house, without official status	5.9	5.4	8.0
Disposable income in the household / per person	Up to 1000 PLN	12.0	10.1	20.2
	1001–3000 PLN	60.6	61.6	56.4
	3001–5000 PLN	21.9	22.9	17.6
	>5000 PLN	5.5	5.4	5.6
Number of people in the household	1	11.8	9.7	20.7
	2	30.4	29.3	35.1
	3	27.4	28.8	21.3
	4	18.9	19.7	15.4
	5	7.8	8.4	5.3
	6 or more people	3.7	4.1	2.1

RESULTS

The results of the conducted research indicate that 81.2% of Poles buy herbs in pots. The most frequently purchased species are parsley, mint, and marjoram, with coriander and lovage also commonly bought. A significant percentage of respondents also buy rosemary, thyme, and oregano rarely or occasionally (Table 2; RQ1). Almost one in five respondents very often buys potted herbs at discount stores, and nearly 14% grow them for their own use (Table 2). Kucharski [2022] also emphasizes that both in rural and small-town settings, as well as increasingly in urban environments, residents have access to both home-grown culinary herbs and ready-to-use fresh herbs widely available in many stores. Never purchase potted herbs: 48.4% directly from the producer, 38.2% from health food stores, and 34% from small grocery stores. Respondents rarely buy potted herbs in health food stores (26.6%), in small grocery stores (26.5%), at greengrocers (24.1%), and at marketplaces (23.8%). This may indicate a lack of or limited selection of fresh herbs in these distribution outlets (RQ1).

Table 2. Frequency and place of purchasing potted herbs (%)

	Never	Rarely	Sometimes	Quite often	Very often
		Species			
Basil	1.1	22.0	44.2	26.5	6.2
Rosemary	13.7	35.1	36.1	13.1	2.1
Mint	9.0	18.3	31.0	28.6	13.1
Coriander	23.9	28.7	31.4	12.2	3.8
Parsley	10.5	13.3	19.3	32.1	24.8
Lovage	22.2	25.5	25.6	17.0	9.7
Oregano	12.8	25.9	30.8	21.8	8.7
Thyme	18.0	26.8	32.4	17.1	5.7
Marjoram	17.0	20.7	25.1	23.8	13.4
		Place of purchase			
Supermarket/hypermarket	10.2	21.4	34.7	24.5	9.1
Discount stores	4.8	12.2	31.7	32.3	19.1
Small grocery stores	34.0	26.5	24.3	10.5	4.8
Greengrocers	19.0	24.3	28.3	19.5	9.0
Health food stores	38.2	26.6	20.9	8.9	5.4
Marketplace	20.9	23.8	30.8	16.3	8.3
Directly from the manufacturer	48.4	20.2	18.6	8.1	4.7
I grow it myself	25.5	18.6	25.6	16.4	13.9

Every third respondent spends 51–100 PLN per year on purchasing potted herbs, every fourth spends 101–200 PLN, and every fifth spends 21–50 PLN. More than 200 PLN per year is spent on potted herbs by 14.5% of respondents, while up to 20 PLN is spent by 7%. Over the years, changes have occurred in the consumption of potted herbs. Nearly 70% of Polish consumers of potted herbs have noticed these changes, with almost 60% reporting an increase in consumption and only 8% a decrease. The most important reasons for purchasing potted herbs are primarily their flavor and seasoning qualities (91.6% rating them as important or very important), health benefits (87.2%), and nutritional value (82.3%), as in Table 3 (RQ2).

Table 3. Importance of reasons and factors for purchase when buying potted herbs (%)

	Doesn't matter	Not important	Indifferent	Important	Very important
		Reasons for purchase			
Flavor qualities – spicy	0.5	2.0	5.9	46.8	44.8
Nutritional value	1.0	4.7	12.1	46.7	35.6
Aesthetic values (meals)	1.6	10.0	22.2	48.5	17.7
Matters of taking care of health	1.0	2.1	9.7	47.2	40.0
Applied diet	7.3	9.4	26.8	36.9	19.6
		Factors			
Attractive appearance, freshness	0.6	3.0	8.5	38.5	49.4
Nutritional values	0.9	2.7	13.9	43.0	39.5
Scent, taste	0.1	1.8	5.8	37.7	54.6
Health benefits	0.5	2.7	11.7	45.0	40.1
Brand (producer)	10.7	13.8	38.1	28.0	9.5
Price	1.5	5.9	18.1	53.8	20.7
Country of origin	2.5	9.1	21.7	44.1	22.7
Packaging method	2.2	7.1	27.8	50.5	12.3
Complete information on the packaging	2.1	6.5	20.6	48.0	22.8

The most important factors when purchasing fresh potted herbs are their aroma and taste (92.3% of respondents indicated very important or important), attractive appearance and freshness (87.9%), health-promoting properties (85.1%), and nutritional values (82.5%). Brand is important or very important to 37.5% of respondents, price to 74.5%, country of origin to 66.8%, packaging method to 62.8%, and complete information on the packaging to 70.8% (Table 3). The information regarding the brand (producer) was frequently deemed to be of minimal significance or unimportant (RQ2).

Production of herbs without the use of chemicals and organic methods (82.5% of responses), a wider assortment (82.2%), lower price (81.1%), and better taste qualities (80.1%) would be the main incentives for more frequent consumption of fresh potted herbs (RQ3). According to Wiluk [2024], consumer awareness is changing, with people increasingly seeking traditional and organic methods of nutrition and health care. The needs of family members are also an important factor determining more frequent purchases of potted herbs (80.8%). As Durmaz and Durmaz [2014] demonstrated, family members such as a spouse, children, and parents can exert a strong influence on a consumer's purchasing behavior. Greater availability of herbs for sale and higher incomes were indicated as "rather yes" or "definitely yes" by 78.0% and 67.2% of respondents, respectively (Figure 2).

Respondents uninterested in potted herbs mainly indicated the use of dried herbs (62.1% of responses as important and very important), as in Figure 3. For more than one in three respondents, the lack of interest in potted herbs is associated with high prices (38.3%) and limited availability (34.5%) (RQ4).

People who do not buy potted herbs would be encouraged to consume them, "rather yes" or "definitely yes" by a lower price (69.7%), organic production (65.4%), and the needs of family members (61.2%), as in Figure 4.

Figure 2. Factors encouraging more frequent consumption of potted herbs (percentage of responses)

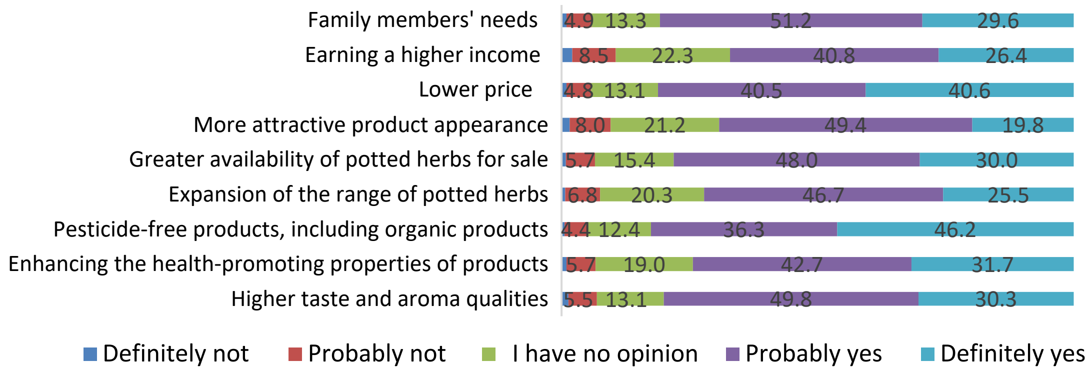
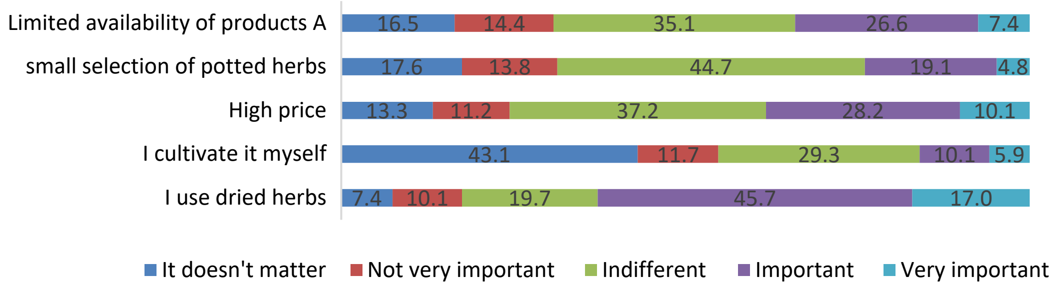
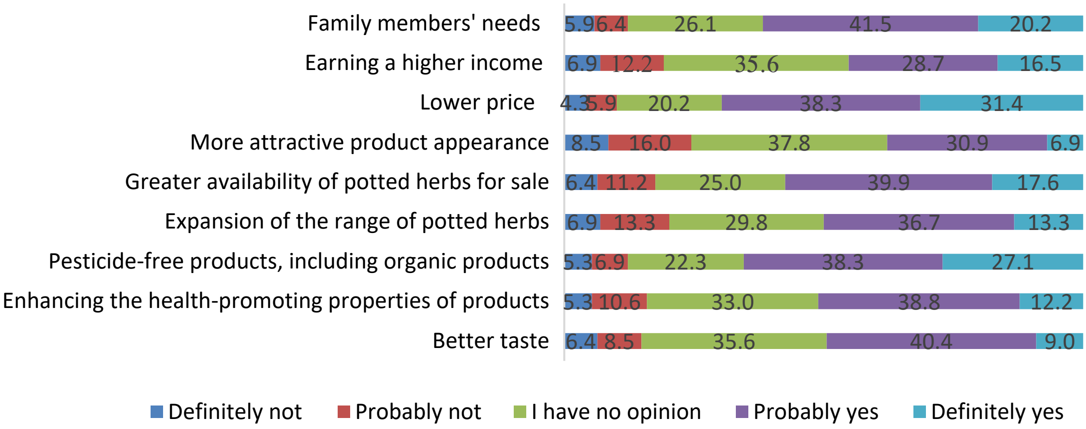


Figure 3. Reasons for lack of interest in potted herbs (percentage of responses)



The socio-demographic characteristics of respondents significantly differentiated their opinions and behaviors, including the frequency of purchasing various types of potted herbs, the places where they bought them, the average annual expenditure on purchasing potted herbs, as well as the motives for their purchase and the importance of various factors influencing purchases. They also differentiated the factors encouraging more frequent consumption of potted herbs, the reasons for lack of interest in potted herbs among people who do not buy them, and the factors that could encourage these individuals to consume fresh herbs (RQ5).

Figure 4. Factors encouraging consumers to consume fresh potted herbs (percentage of responses)



The frequency of purchasing various species of potted herbs was significantly influenced by socio-demographic characteristics, especially age (in terms of purchasing basil, rosemary, mint, coriander, oregano, and thyme), as well as gender (in terms of purchasing basil, coriander, and lovage), education (in terms of purchasing mint, lovage, and marjoram), social status (in terms of purchasing basil, rosemary, mint, and oregano), number of people in the family (in terms of purchasing basil, mint, and coriander), and income (when it comes to purchasing coriander and thyme). The frequency of purchasing potted herbs in various locations was primarily differentiated by age, social status, and the number of people in the household, and less frequently by education (in terms of buying potted herbs at a greengrocer's, at a marketplace, and directly from the producer) and income (when it comes to growing herbs independently). Annual expenditures on purchasing potted herbs differed significantly depending on the respondents' age, social status, income, and the number of people in the household (Table 4).

The motives for purchasing herbs in pots significantly differentiated primarily by the respondents' gender, as well as age, social status, and income (in terms of health consciousness). The importance of various factors influencing purchases varied significantly depending on the demographic characteristics of the surveyed population (Table 2). Most often, significant differences in evaluation concerned factors such as attractive appearance/freshness, aroma, taste, country of origin, and type of packaging. Based on factor analysis, two groups of factors influencing the purchase of potted herbs were identified (Table 5). These factors explained 52.7% of the variability in the assessment of these factors. The first component included factors such as brand, country of origin, type of packaging, and information on the packaging, while the second consisted of features directly related to the product, such as attractive appearance/freshness, nutritional value, aroma, taste, and health-promoting properties.

The significance of factors encouraging more frequent consumption of potted herbs depended on all the socio-demographic characteristics studied, with different factors being of varying importance to different groups of respondents. Most often, the significance of these factors varied according to gender, household size, and income. The socio-demographic characteristics of the respondents most frequently influenced opinions regarding the production of herbs without pesticides, including organic ones, as a factor encouraging more frequent consumption of potted herbs. Based on factor analysis, two groups of factors encouraging greater consumption of potted herbs were identified (Table 6). These factors explained 54.5% of the variation in the assessment of these factors. The first component included factors related to the characteristics of the products—potted herbs—and the needs of family members, while the second included economic factors such as lower product prices and achieving higher incomes.

The indicated reasons for the lack of interest in potted herbs among people who do not purchase them depended on gender (regarding the use of dried herbs and high prices), age and education (regarding the use of dried herbs instead of fresh ones), income (due to the limited availability of products), and family size (because of growing them themselves). The significance of factors encouraging people who do not buy fresh herbs in pots to consume them varied significantly depending on gender (in terms of the more attractive appearance of the products), disposable income (depending on the possibility of earning a higher income), and household size (according to the needs of family members). Significant interdependencies between the studied variables were also confirmed in the Pearson correlation analysis.

Table 4. Value and significance of nonparametric tests for grouping variables: gender, age, education, social/professional status, disposable income per person in the household, and number of people in the family

Tests	P1.	P2.	P3.01.	P3.02.	P3.03.	P3.04.	P3.05.	P3.06.	P3.07.	P3.08.	P3.09.	P4.01.	P4.02.	P4.03.	P4.04.	P4.05.	P4.06.	P4.07.	P4.08.
Grouping variable: M1 Gender																			
U	124712.000	110456.000	70839.000	80012.000	75477.000	72566.500	75780.500	74692.500	75368.500	80201.500	80672.500	79727.500	71963.500	77074.500	78494.500	75887.000	75212.500	80089.000	69405.500
W	238238.000	248006.000	135100.000	144273.000	139738.000	175851.500	140041.500	138953.500	139629.500	183486.500	144933.500	183012.500	136224.500	180359.500	142755.500	179172.000	139473.500	183374.000	133666.500
Z	0.000	–4.618	–3.342	–0.397	–1.802	–2.719	–1.704	–2.033	–1.833	–0.332	–0.183	–0.480	–2.913	–1.312	–0.858	–1.694	–1.878	–0.380	–3.661
α	1.000	0.000	0.001	0.691	0.072	0.007	0.088	0.042	0.067	0.740	0.855	0.631	0.004	0.189	0.391	0.090	0.060	0.704	0.000
Grouping variable: M2. Age																			
H	0.000	36.752	17.039	9.476	25.572	18.607	7.569	2.552	11.810	10.204	2.124	36.718	11.184	67.424	23.641	13.877	7.388	15.508	1.872
α	1.000	0.000	0.002	0.050	0.000	0.001	0.109	0.635	0.019	0.037	0.713	0.000	0.025	0.000	0.000	0.008	0.117	0.004	0.759
Grouping variable: M3. Education																			
H	0.000	90.719	1.398	3.768	1.048	3.076	18.762	22.262	3.503	2.369	14.278	4.904	2.982	2.930	12.580	7.376	11.899	10.271	3.078
α	1.000	0.000	0.706	0.288	0.790	0.380	0.000	0.000	0.320	0.499	0.003	0.179	0.394	0.403	0.006	0.061	0.008	0.016	0.380
Grouping variable: M4. Social/professional status																			
H	0.000	25.079	24.955	21.671	19.056	18.801	5.471	7.815	17.003	8.903	4.793	28.743	2.390	37.188	17.233	16.284	11.867	12.864	6.068
α	1.000	0.000	0.000	0.000	0.001	0.001	0.242	0.099	0.002	0.064	0.309	0.000	0.664	0.000	0.002	0.003	0.018	0.012	0.194
Grouping variable: M5. Number of people in the household																			
H	0.000	25.381	19.348	6.121	24.389	23.584	13.491	14.080	24.559	20.052	11.922	23.178	4.342	42.939	31.454	25.009	12.996	30.895	11.707
α	1.000	<0.001	0.002	0.295	<0.001	<0.001	0.019	0.015	<0.001	0.001	0.036	<0.001	0.501	<0.001	<0.001	<0.001	0.023	<0.001	0.039
Grouping variable: M6. Disposable income in the household / per person																			
H	0.000	15.727	3.528	11.857	7.562	11.622	1.232	2.303	2.313	10.948	3.316	6.190	1.333	6.928	2.817	7.155	3.870	5.852	9.897
α	1.000	0.001	0.317	0.008	0.056	0.009	0.745	0.512	0.510	0.012	0.345	0.103	0.721	0.074	0.421	0.067	0.276	0.119	0.019

	P5.	P6.	P7.01.	P7.02.	P7.03.	P7.04.	P7.05.	P8.01.	P8.02.	P8.03.	P8.04.	P8.05.	P8.06.	P8.07.	P8.08.	P8.09.	P9.01.	P9.02.	P9.03.	P9.04.
Grouping variable: M1 Gender																				
U	75819.500	74676.000	71688.000	59636.000	68087.500	66301.000	71504.000	67665.500	63215.000	71770.500	69504.000	76587.500	76730.000	69817.000	70877.000	68271.500	72730.500	71673.000	70368.000	74486.500
W	179104.500	177961.000	135949.000	123897.000	132348.500	130562.000	135765.000	131926.500	127476.000	136031.500	133765.000	140848.500	140991.000	134078.000	135138.000	132532.500	136991.500	135934.000	134629.000	138747.500
Z	–1.697	–2.290	–3.213	–7.064	–4.262	–4.950	–3.065	–4.522	–5.879	–3.232	–3.861	–1.471	–1.501	–3.654	–3.401	–4.202	–2.795	–3.076	–3.559	–2.187
α	0.090	0.022	0.001	0.000	0.000	0.000	0.002	0.000	0.000	0.001	0.000	0.141	0.133	0.000	0.001	0.000	0.005	0.002	0.000	0.029
Grouping variable: M2. Age																				
H	11.716	2.973	11.095	18.726	18.873	19.562	3.490	24.494	12.044	17.402	15.095	1.546	4.184	46.644	8.304	6.288	9.407	3.463	25.065	1.378
α	0.020	0.562	0.026	0.001	0.001	0.001	0.479	0.000	0.017	0.002	0.005	0.819	0.382	0.000	0.081	0.179	0.052	0.484	0.000	0.848
Grouping variable: M3. Education																				
H	1.343	5.041	3.403	1.545	7.506	6.313	2.152	1.572	4.316	1.726	2.249	11.847	5.300	11.949	6.068	5.269	0.783	1.608	3.480	3.777
α	0.719	0.169	0.334	0.672	0.057	0.097	0.542	0.666	0.229	0.631	0.522	0.008	0.151	0.008	0.108	0.153	0.853	0.658	0.323	0.287

Grouping variable: M4. Social/professional status																				
H	31.310	6.853	4.160	10.992	10.667	11.852	8.148	20.668	8.864	9.843	9.147	5.832	1.053	34.899	5.309	5.150	6.105	4.191	21.751	7.139
α	0.000	0.144	0.385	0.027	0.031	0.018	0.086	0.000	0.065	0.043	0.058	0.212	0.902	0.000	0.257	0.272	0.191	0.381	0.000	0.129
Grouping variable: M5. Number of people in the household																				
H	27.696	1.879	5.922	4.520	10.016	3.456	5.742	12.515	10.011	5.172	6.485	16.871	1.815	5.793	12.950	6.731	26.452	13.400	20.616	14.054
α	<0.001	0.866	0.314	0.477	0.075	0.630	0.332	0.028	0.075	0.395	0.262	0.005	0.874	0.327	0.024	0.241	<0.001	0.020	<0.001	0.015
Grouping variable: M6. Disposable income in the household / per person																				
H	25.568	6.114	7.545	7.535	6.110	9.732	1.713	9.952	7.240	7.981	6.791	2.993	14.654	5.876	8.540	4.395	5.993	4.746	9.939	2.647
α	0.000	0.106	0.056	0.057	0.106	0.021	0.634	0.019	0.065	0.046	0.079	0.393	0.002	0.118	0.036	0.222	0.112	0.191	0.019	0.449
	P9.05.	**P9.06.**	**P9.07.**	**P9.08.**	**P9.09.**	**P10.**	**P11.01.**	**P11.02.**	**P11.03.**	**P11.04.**	**P11.05.**	**P12.01.**	**P12.02.**	**P12.03.**	**P12.04.**	**P12.05.**	**P12.06.**	**P12.07.**	**P12.08.**	**P12.09.**
Grouping variable: M1 Gender																				
H	67983.000	69287.000	78304.500	71819.000	75778.000	66325.000	3245.000	3810.500	3302.000	3615.500	4110.000	3944.500	4091.000	4021.000	3981.500	3708.000	3390.500	3618.000	3546.500	3733.000
W	132244.000	133548.00 0	142565.500	136080.000	140039.000	130586.000	10266.000	10831.500	10323.000	10636.500	6595.000	6429.500	6576.000	11042.000	6466.500	10729.000	10411.500	10639.000	10567.500	10754.000
Z	−4.321	−3.889	−0.960	−2.997	−1.807	−4.868	−2.600	−0.938	−2.392	−1.507	−0.057	−0.546	−0.114	−0.316	−0.429	−1.224	−2.147	−1.493	−1.683	−1.159
α	0.000	0.000	0.337	0.003	0.071	0.000	0.009	0.348	0.017	0.132	0.954	0.585	0.909	0.752	0.668	0.221	0.032	0.136	0.092	0.247
Grouping variable: M2. Age																				
H	2.777	3.928	6.303	1.672	3.204	33.595	9.490	0.934	8.720	4.226	0.966	2.409	2.096	6.417	3.143	1.205	2.945	5.796	2.982	2.465
α	0.596	0.416	0.178	0.796	0.524	0.000	0.050	0.920	0.068	0.376	0.915	0.661	0.718	0.170	0.534	0.877	0.567	0.215	0.561	0.651
Grouping variable: M3. Education																				
H	6.961	2.719	1.830	9.789	3.047	2.321	10.675	3.859	5.591	2.490	1.083	7.378	0.493	2.546	1.362	1.645	4.135	0.668	0.336	1.468
α	0.073	0.437	0.608	0.020	0.384	0.509	0.014	0.277	0.133	0.477	0.781	0.061	0.920	0.467	0.715	0.649	0.247	0.881	0.953	0.690
Grouping variable: M4. Social/professional status																				
H	5.083	3.274	5.157	2.394	7.421	28.024	3.092	0.526	3.955	1.923	2.794	7.961	3.520	7.687	4.225	1.920	1.943	1.892	1.877	3.826
α	0.279	0.513	0.272	0.664	0.115	0.000	0.543	0.971	0.412	0.750	0.593	0.093	0.475	0.104	0.376	0.750	0.746	0.756	0.758	0.430
Grouping variable: M5. Number of people in the household																				
H	12.144	4.776	11.542	5.852	10.365	15.275	4.204	11.090	4.136	1.616	4.397	5.580	6.620	1.669	2.548	0.690	1.770	5.081	0.965	14.470
α	0.033	0.444	0.042	0.321	0.066	0.009	0.520	0.050	0.530	0.899	0.494	0.349	0.250	0.893	0.769	0.984	0.880	0.406	0.965	0.013
Grouping variable: M6. Disposable income in the household/ per person																				
H	6.072	7.155	2.878	14.085	4.770	6.395	0.839	4.343	5.029	3.316	8.130	2.017	2.768	1.884	5.126	6.116	1.855	5.867	7.877	2.239
α	0.108	0.067	0.411	0.003	0.189	0.094	0.840	0.227	0.170	0.345	0.043	0.569	0.429	0.597	0.163	0.106	0.603	0.118	0.049	0.524

U – Mann-Whitney test; W – Wilcoxon test; Z – Z tests; H – Kruskal-Wallis test; $α < 0.05$; P1. Do you make purchasing decisions in your household? P2. Do you buy potted herbs, e.g., basil, rosemary, etc.? P3. How often do you buy potted herbs? P3.1. Basil; P3.2. Rosemary; P3.3. Mint; P3.4. Coriander; P3.5. Parsley; P3.6. Lovage; P3.7. Oregano; P3.8. Thyme; P3.9. Marjoram; P4. Where and how often do you buy fresh potted herbs? P4.1. Supermarket/hypermarket; P4.2. Discount stores; P4.3. Small grocery stores; P4.4. Greengrocers; P4.5. Health food stores; P4.6. Marketplace; P4.7. Directly from the producer; P4.8. Home cultivation; P5. How much do you spend on average per year on potted herbs? P6. Has your consumption of potted herbs changed over the past years? P7. Reasons for purchasing potted herbs; P7.1. Taste – seasoning qualities; P7.2. Nutritional value; P7.3. Aesthetic value (of meals); P7.4. Health concerns; P7.5. Dietary reasons; P8. How important are the following factors/elements to you when purchasing this type of food? P8.1. Attractive appearance, freshness; P8.2. Nutritional value; P8.3. Smell, taste; P8.4. Health-promoting properties; P8.5. Brand (producer); P8.6. Price; P8.7. Country of origin; P8.8. Packaging method; P8.9. Complete information on the packaging; P9. What would encourage you to consume fresh potted herbs more often? P9.1. Better taste and aroma; P9.2. Enhanced health-promoting properties of products; P9.3. Products grown without pesticides, including organic products; P9.4. Broader selection of potted herbs; P9.5. Greater availability of potted herbs for sale; P9.6. More attractive appearance of products; P9.7. Lower price; P9.8. Higher income; P9.9. Needs of family members; P10. Would you purchase potted herbs produced using natural production methods? P11. Please indicate and assess the reasons for lack of interest in potted herbs; P11.1. I use dried herbs; P11.2. I grow them myself; P11.3. High price; P11.4. Limited selection of potted herbs; P11.5. Limited product availability; P12. What would encourage you to consume fresh potted herbs? P12.1. Better taste and aroma; P12.2. Enhanced health-promoting properties of products; P12.3. Products grown without pesticides, including organic products; P12.4. Broader selection of potted herbs; P12.5. Greater availability of potted herbs for sale; P12.6. More attractive appearance of products; P12.7. Lower price; P12.8. Higher income; P12.9. Needs of family members

Table 5. Rotated component matrix regarding factors influencing the purchase of potted herbs

Variables (factors)	Component	
	1	2
Attractive appearance, freshness	0.018	0.776
Nutritional values	0.359	0.655
Scent, taste	0.021	0.822
Health benefits	0.434	0.588
Brand (producer)	0.770	-0.054
Price	0.255	0.293
Country of origin	0.694	0.211
Packaging method	0.719	0.154
Complete information on the packaging	0.688	0.293

Factor extraction method – principal components. Rotation method – Varimax with Kaiser normalization

Table 6. Rotated component matrix regarding factors encouraging more frequent consumption of potted herbs

Variables (factors)	Component	
	1	2
Higher taste and aroma qualities	0.733	0.125
Improving the health-promoting properties of products	0.754	0.117
Products from pesticide-free production, including organic ones	0.734	0.145
Expansion of the range of potted herbs	0.690	0.105
Greater availability of potted herbs for sale	0.695	0.244
More attractive product appearance	0.567	0.309
Lower price	0.184	0.800
Earning a higher income	0.123	0.850
Family Members' Needs	0.463	0.377

Factor extraction method – principal components. Rotation method – Varimax with Kaiser normalization

DISCUSSION

This research focuses on identifying the demand side of the potted herb market and determining the main factors influencing purchasing decisions in the Polish market. The results indicate that 81.2% of Poles buy potted herbs. The lack of data on demand size in other countries' markets makes it impossible to compare Poland's situation, but as Behe et al. [2013] point out, there is a market segment in the United States and Canada called "herbivores," which focuses mainly on herbs, as 100% of this segment buys herbs. These consumers prefer basil, lower prices, water-saving production, and recyclable containers. Meanwhile, culinary herb consumers in South Africa prefer fresh herbs (about 96%), followed by dried and ground herbs [Mphafi et al. 2023]. In the USA, chefs mainly buy fresh basil, parsley, and coriander, and are also interested in potted herbs as an additional offering from local farmers [Goss et al. 2007]. A study among adult Australians showed that 64.3% use herbs and spices 1–2 times a day. Herbs and spices are commonly used in Australian households, mostly during lunch and dinner, with basil, pepper, and garlic being the most popular [Wilson et al. 2025]. In Poland, people most often buy herbs in discount stores, mainly parsley, mint, and marjoram. The main reasons for buying potted herbs are their flavor and seasoning qualities, a concern for health, and their nutritional value. Polish consumers are willing to increase the frequency of consumption, mainly thanks to the availability of herbs grown without chemicals and organically. In their research, Brijesh and Soni [2019] also found that naturalness and quality are the two main factors influencing customers' decisions to buy herbal products. According to Jiang [2019], consumers increasingly turn to naturally grown herbs that can offer various health benefits, and with this awareness, the demand for organic herbs is also growing. Syafrizal and Vafaei-Zadeh [2024] demonstrated that health awareness and health value play an important role in shaping customer attitudes and purchase intentions regarding herbal products and supplements. Ramli and Arumugam [2021] found a relationship between age and customer awareness and attitudes toward purchasing herbal products. According to these researchers, variables such as gender, education, occupation, and income do not have a significant relationship with customers' awareness and attitudes regarding herbal product purchases. The analyses carried out showed that the socio-demographic characteristics of the respondents significantly differentiated their opinions and purchasing behaviors in the potted herb market. This concerned the frequency of purchasing particular species, places of purchase, spending levels, as well as motives and factors influencing purchasing decisions. The frequency of purchasing specific herb species was particularly strongly dependent on respondents' age, which was also the most important factor differentiating species preferences (e.g., basil, rosemary, mint, coriander, oregano, thyme), while gender mainly differentiated purchases of basil, coriander, and lovage. Places of potted herb purchase varied primarily by age, social status, and household size. Income mainly differentiated the inclination to grow herbs independently.

The primary motivations for purchasing potted herbs include their flavor and seasoning properties, health considerations, and nutritional benefits. These motivations vary significantly based on respondents' gender, age, social status, and income level, particularly concerning health-related concerns. The significance of factors influencing purchasing decisions exhibits demographic variations, most notably in the evaluation of attributes such as visual appeal and freshness, aroma and taste, country of origin, and packaging method. Consumers' purchasing decisions are influenced by both image- and information-related elements, as well as the sensory and health attributes of the product, with the importance of these factors differing according to the socio-demographic profile of buyers. In the context of South African consumers, preferences regarding image- and information-related factors are shaped by labels, attractive packaging, and availability, alongside socio-economic factors such as income. While many consumers do not prioritize the origin of products, segments with higher material status may exhibit greater diversity in preferences [Mphafi et al. 2023]. Health is a predominant concern that drives consumer interest in herbal products, with health being the most frequently cited motive for purchasing natural products [Wee et al. 2014]. The naturalness of food is of paramount importance to most consumers [Romana et al. 2017], and health awareness significantly influences consumers' intentions regarding the use of herbal products [Widyaningtyas et al. 2023]. According to Ismail and Mokhtar [2015], factors such as attitude, social influence, product safety, and cultural beliefs also impact purchase intentions for herbal products. In the study by Kalra et al. [2023], safety emerged as the most critical factor in purchasing herbal products, followed by quality.

The significance of factors promoting increased consumption of potted herbs was markedly influenced by all examined socio-demographic characteristics, with the most pronounced differences observed in gender, household size, and income. The importance of herb production without pesticides, including organic methods, was particularly distinct. The reasons for disinterest in potted herbs also varied significantly according to gender, age, education, income, and household size. The most frequently cited reasons included the preference for dried herbs, high prices, limited availability, and home cultivation. Market factors, such as brand recognition, appear to be of lesser significance to Polish consumers, potentially due to the relatively nascent stage of the potted herb market in Poland. Comparable findings have been reported in studies of the Mexican herbal market [National Market… 1996]. Looking ahead, there is a possibility of customer segment diversification, with a preference for organic and high-quality potted herbs. Such herbal products are also gaining traction among urban Malaysian consumers, thereby creating opportunities for segmentation across various price ranges, distribution models, and consumer profiles [Malaysia Culinary Herbs and Spices Market Size 2026-2033, 2026].

CONCLUSIONS

Over 80% of Poles buy fresh potted herbs, most often at discount stores, and rarely at health food shops, small grocery stores, greengrocers and market stalls. Nearly half of consumers never buy potted herbs directly from the producer. Respondents cited the herbs' flavour and seasoning qualities, health benefits and nutritional value as the most important reasons for purchasing potted herbs. The most important factors when making a purchase are aroma and taste, attractive appearance and freshness, health-promoting properties, and nutritional value. Factors cited as encouraging consumers to consume fresh potted herbs more frequently included the cultivation of herbs without the use of chemicals and the use of organic methods, a wider range of products, lower prices and better flavour. The lack of interest in purchasing fresh potted herbs stems primarily from the use of dried herbs, but also from the high prices of fresh herbs and their limited availability. The socio-demographic characteristics of consumers significantly influenced behaviour regarding the frequency, place of purchase, and expenditure on potted herbs. They also differentiated the importance of factors influencing purchasing decisions and factors encouraging more frequent consumption of potted herbs, the reasons for the lack of interest in potted herbs among those who do not buy them, and factors that could encourage these people to consume fresh herbs.

The research conducted has facilitated the identification of patterns in the consumption of potted herbs in Poland, a subject that has not been thoroughly analyzed until now. Due to the representativeness of the study's results, these findings can be generalized to the Polish population. The insights gained from this research will aid in optimizing the assortment of potted herbs in alignment with consumer preferences concerning species and quality attributes.

These results can be leveraged to implement more effective sales and promotional strategies, enabling companies to concentrate their marketing efforts on appropriate channels and thereby enhance sales. Furthermore, an understanding of consumer preferences can stimulate innovation and the development of new products, as well as simplify market segmentation and the customization of marketing communication. Consequently, this supports the promotion and enhancement of public health, increases consumer ecological awareness, and contributes to the overall well-being of society.

The limitations of this study stem from the adopted set of motives and factors influencing consumer purchasing decisions and consumption of potted herbs. Future research should expand the scope of motives for using herbs with health, aesthetic, therapeutic, and cosmetic applications. The influence of other factors, such as place of residence, family diets, and knowledge of the medicinal properties of herbs, could also be expanded.

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AUTHORS' CONTRIBUTION

W.W. – working on the conception and assumptions; W.W. – developing the methods and conducting the study; E.C., W.W. – data analysis; W.W., E.C. – manuscript writing, review, and editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF THE USE OF GENERATIVE AI IN THE PROCESS OF PREPARING THE PAPER

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

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RESEARCH PAPER

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EFFECTS OF FOLIAR-APPLIED FULVIC ACIDS ON YIELD, BIOMASS DISTRIBUTION, AND NITRATE ACCUMULATION IN HYDROPONICALLY GROWN RADISH (*Raphanus sativus* L.)

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ABSTRACT

Fulvic acids are humic-derived biostimulants that can modulate nutrient uptake, carbon metabolism, and biomass allocation in horticultural crops; however, their effect on assimilate partitioning and nitrate accumulation in hydroponically grown root vegetables remains poorly understood. This study evaluated the effect of weekly foliar application of a fulvic acid-based biostimulant (K-TIONIC®, 4 mL L^{-1}) on yield, biomass distribution, nitrate content, and quality of radish (*Raphanus sativus* L. Crimson Giant) cultivated in an ebb-and-flow hydroponic system under greenhouse conditions in Chihuahua, Mexico. A completely randomized design with two treatments (control and fulvic acids) and 36 experimental units per treatment was used. Fulvic acid application increased equatorial diameter by 8.50%, fresh storage root weight by 28%, and total yield by 41.66%. In contrast, vegetative growth parameters and photosynthetic pigments were unaffected. Regarding quality attributes, nitrate concentration in edible tissues decreased by 4.96% and water content by 0.57%, pH, total soluble solids, color index were not significantly affected, and visual quality index improved by 98%. These results indicate that foliar-applied fulvic acids improve radish productivity and commercial quality in soilless systems primarily by promoting biomass distribution to the storage organ.

Keywords: biostimulants, soilless culture, root vegetables, storage organ, vegetative growth parameters

INTRODUCTION

Radish (*Raphanus sativus* L. var. *sativus*) is an economically important horticultural crop due to its rapid production cycle, high adaptability to protected environments, and relevance in fresh vegetable markets worldwide [Gamba et al. 2021, Dos Santos et al. 2023]. Its short growth cycle and high responsiveness to nutrient availability make it a suitable model crop for evaluating agronomic strategies under controlled conditions. However, intensive production systems often face challenges related to nitrate accumulation in edible tissues, negatively affecting nutritional quality and market acceptance [Colla et al. 2018]. This issue is particularly relevant in root vegetables

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such as radish, where nitrate accumulation is directly associated with nitrogen uptake and assimilation processes [Di Gioia et al. 2017].

Hydroponic systems have emerged as efficient alternatives to conventional agriculture due to their efficient use of water and nutrients supply, reduced environmental impact, and ability to optimize crop management [Kechasov et al. 2021, Saldinger et al. 2023]. Among these, ebb-and-flow systems allow periodic root-zone aeration and stable nutrient availability, which are critical for short-cycle species such as radish. Nevertheless, even under optimal nutrient conditions, crop productivity and quality largely depend on internal physiological processes, including nitrogen assimilation and the allocation of assimilates toward storage organs [Tegeder et al. 2018].

In this context, plant biostimulants have gained increasing attention as tools to enhance crop performance without increasing fertilizer inputs [Colla et al. 2017, Conversa et al. 2021]. Fulvic acids, a fraction of humic substances, are characterized by their low molecular weight and high solubility, allowing them to interact with plant physiological processes such as nutrient uptake, enzyme activation, and carbon metabolism [Cataldo et al. 2022, Asif et al. 2023]. Previous studies have reported that fulvic acid-based biostimulants can improve growth and yield in horticultural crops, mainly through enhanced nutrient availability and metabolic activity [Zhang et al. 2021, Barzegar et al. 2022, Keskin et al. 2025]. Nevertheless, their effectiveness may vary depending on plant species, application rate, and method of application [Sun and Shahrajabian 2023]. However, most of these studies have focused on leafy vegetables or aboveground biomass, while limited attention has been given to root crops cultivated under hydroponic conditions.

In root crops such as radish, yield is closely associated with plant's capacity to allocate assimilates toward the storage organ, whereas quality is influenced by factors such as nitrate concentration and water content. A reduction in nitrate levels may reflect a more efficient assimilation of nitrogen into organic compounds, while changes in biomass distribution may indicate modifications in source-sink relationships [Xu et al. 2012]. Despite the increasing use of biostimulants in horticulture, there is limited information regarding their effect on nitrate accumulation and assimilate partitioning in hydroponically grown root vegetables, particularly under ebb-and-flow systems.

Therefore, the objective of this study was to evaluate the effect of foliar application of fulvic acids on yield, nitrate content, and biomass distribution in radish cultivated in an ebb-and-flow hydroponic system. The hypothesis was that foliar application of fulvic acids reduces nitrate accumulation, suggesting improved nitrogen assimilation, and assimilate partitioning toward the storage organ, resulting in increased yield and improved commercial quality.

MATERIAL AND METHODS

The experiment was conducted during the 2024 growing season in a greenhouse at the Faculty of Agrotechnological Sciences, Autonomous University of Chihuahua (28.6434°N, 106.0765°W), Chihuahua, Mexico. *Raphanus sativus* L. var. *sativus* Crimson Giant was grown in four-ounce expanded polystyrene containers filled with perlite as substrate in an ebb-and-flow hydroponic system.

The system operated with flooding cycles of 10 min every 2 h during daylight and every 4 h at night, ensuring adequate nutrient supply and root-zone aeration.

The Steiner nutrient solution (Soluponic®, Inverfarms México, San Pablo, Querétaro, Mexico) was used as the nutrient source. This solution is widely used in commercial hydroponic production systems due to its balanced composition and adaptability to a wide range of horticultural crops. The nutrient concentrations were as follows: $N\text{-}NO_3$ 15.7 mmol L^{-1}, $N\text{-}NH_4$ 1.3 mmol L^{-1}, P 1.3 mmol L^{-1}, K 1.3 mmol L^{-1}, Ca 4.7 mmol L^{-1}, Mg 3.0 mmol L^{-1}, S 3.3 mmol L^{-1}, B 13.9 µmol L^{-1}, Fe-EDTA 28.0 µmol L^{-1}, Zn-EDTA 3.3 µmol L^{-1}, CU-EDTA 1.7 µmol L^{-1}, Mn-EDTA 15.2 µmol L^{-1}, and Mo 0.7 µmol L^{-1}. The nutrient solution pH was maintained between 6.0-6.5, adjusted with HNO_3 (10% *v/v*), and electrical conductivity was kept at 2.3 mS cm^{-1} [Morgan 2000].

The experiment followed a completely randomized design with two treatments: (1) control (distilled water spray) and (2) fulvic acid-based biostimulant application. Each treatment consisted of 36 experimental units, with one plant per experimental unit. Each container thus constituted an independent experimental unit, providing physical independence of the root environment. Plants were arranged in groups of nine per tray, with each tray connected to an independent nutrient solution reservoir. For destructive measurements, 20 plants were randomly selected per treatment.

The fulvic acid-based biostimulant (K-TIONIC®) contained 100 g L^{-1} of total humic carbon extract (10% humic acid and 90% fulvic acids), 6 g L^{-1} nitrogen, and 38 g L^{-1} K_2O. It was applied weekly at a concentration of 4 mL L^{-1}

by foliar spraying using a hand-held atomizer (Specialized Plastic and Packaging, Chihuahua, Mexico), to the point of incipient runoff. This concentration was selected based on the manufacturer's recommended dose and represents the standard application rate established by the manufacturer for horticultural crops, serving as a baseline for evaluating its agronomic efficacy under the conditions of this study. Application started at the appearance of the second true leaf stage and continued until one week before harvesting. All applications were performed at 07:30 a.m.

Vegetative parameters were determined 45 days after sowing. From each treatment, 20 plants were randomly selected for destructive measurement and determine number of leaves, leaf area (cm^2), root length (mm), neck diameter (mm), and polar and equatorial diameters (mm). Fresh and dry biomass were recorded and expressed in grams (g). Dry biomass was obtained by drying the samples at 65°C until constant weight (approximately 72 h) and weighing them using a digital balance (Ohaus, Adventure Pro AV264C, USA). Diameters were measured using a Vernier caliper (Starrett®, EC799A-6/150, USA). Measurements were performed using a digital balance, a digital caliper, and leaf area was determined using ImageJ software. Shape index was calculated as longitudinal diameter / equatorial diameter [Wei et al. 2022].

Quality parameters were determined from fresh tissue extracts. pH was measured using 5 g of macerated tissue, and the extracted juice was used for pH determination using a digital potentiometer (HI98103 pH Tester, Hanna Instruments, Woonsocket, RI, USA). Total soluble solids (TSS) content was determined using a digital refractometer, and expressed as °Brix. Color was evaluated using the CIE Lab* system (Minolta Chromameter CR-310, Konica Minolta, Osaka, Japan), and the color index (CI) was calculated as $CI = (1000 \times a) / (L \times b)$ [Cordoba-Novoa et al. 2018]. Moisture content was calculated as [(fresh weight – dry weight) / fresh weight] × 100 [Jamil et al. 2007].

Photosynthetic pigments were determined according to Lichtenthaler and Wellburn [1983]. Leaf samples (0.1 g) were extracted with 4 mL of 80% (v/v) acetone, centrifuged at 3000 rpm for 5 min, and absorbance was measured at 663, 645, and 470 nm.

Nitrate concentration was determined following Toscano et al. [2021]. Fresh tissue (1 g) was homogenized in 3 mL of distilled water, centrifuged at 4,000 rpm for 15 min. An aliquot of 20 µL of supernatant was reacted with 80 µL of sulphury acid-salicylic solution, followed by neutralization with 3 mL of 1.5 N NaOH. Absorbance was measured at 410 nm, and nitrate concentration was calculated using a KNO_3 standard curve (0–10 mM; $R^2 = 0.9918$). Result was expressed as mg NO_3^- kg^{-1} fresh weight.

Visual quality was evaluated using a five-point scale based on color, shape, texture, and size. Each attribute was scored from 1 (non-commercial quality) to 5 (optimal market quality). The visual quality index was calculated as the mean score. Yield was calculated as fresh storage root weight per plant multiplied by the planting density, determined from the footprint area of each individual four-ounce expanded polystyrene container (21.5 cm^2), and expressed as kg m^{-2}.

The differences between the treatment and control groups were determined using Student's t-test ($p \le 0.05$) after verifying normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Statistical analyses were performed using Jamovi software v. 2.4.8 [The Jamovi Project 2023].

RESULTS

No significant differences were observed in vegetative parameters such as number of leaves, leaf area, root length, or neck diameter between treatments (Table 1). Likewise, chlorophyll *a*, chlorophyll *b*, and carotenoid contents did not differ significantly between treatments (Table 2).

Table 1. Vegetative parameters of radish under fulvic acid application

Parameter	Control	Fulvic acids
Number of leaves	7.80 ±1.50 a	7.50 ±1.40 a
Leaf area (cm2)	462.88 ±128.70 a	483.33 ±291.90 a
Root length (mm)	21.88 ±5.20 a	18.38 ±6.36 a
Neck diameter (mm)	7.64 ±1.42 a	8.29 ±1.70 a
Polar diameter (mm)	30.22 ±4.96 a	32.80 ±4.39 a
Equatorial diameter (mm)	28.00 ±4.23 a	30.98 ±3.14 b
Shape index	0.94 ±0.13 a	0.96 ±0.13 a

Values represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test ($p \le 0.05$).

Table 2. Physiological and photosynthetic parameters of radish under fulvic acid application

Parameter		Control	Fulvic acids
pH		6.32 ±0.10 a	6.25 ±0.14 a
Total soluble solids (°Brix)		3.74 ±0.35 a	3.75 ±0.63 a
Color index		163.26 ±11.20 a	180.69 ±14.54 a
Water content (%)		97.46 ±0.87 b	96.89 ±0.70 a
Photosynthetic pigments (mg g^{-1} FW)	chlorophyll a	0.51 ±0.17 a	0.52 ±0.23 a
	chlorophyll b	0.21 ±0.12 a	0.20 ±0.19 a
	carotenoids	0.01 ±0.00 a	0.01 ±0.00 a

Values represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$).

However, the equatorial diameter of radish roots increased by 8.50% in plants treated with fulvic acids compared to the control (30.98 mm to 28.00 mm respectively; Table 1). In addition, the weight of fresh storage roots increased by 28% with the application of fulvic acid compared to the control, indicating greater biomass accumulation in the storage organ (14.34 g to 11.20 g respectively; Table 3).

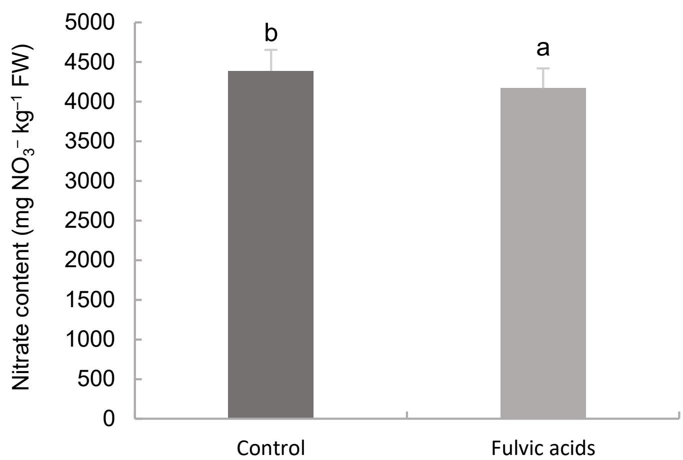
Physiological quality parameters including pH, TSS, and color index were not significantly affected by fulvic acid application (Table 3). In contrast, water content of the storage root decreased by 0.57% in treated radishes (Table 3). Treatment with fulvic acids resulted in a 4.96% reduction in nitrate concentration in radish roots compared to the control (Figure 1).

Table 3. Biomass parameters of radish under fulvic acid application

Parameter	Control	Fulvic acids
Fresh weight of leaves (g)	29.50 ±11.54 a	32.88 ±7.62 a
Fresh weight of storage root (g)	11.20 ±4.25 b	14.34 ±5.28 a
Fresh weight of fibrous root (g)	3.60 ±2.42 a	3.37 ±1.70 a
Dry weight of leaves (g)	1.47 ±0.60 a	1.75 ±0.55 a
Dry weight of storage root (g)	0.31 ±0.23 a	0.44 ±0.17 a
Dry weight of fibrous root (g)	0.21 ±0.10 a	0.20 ±0.11 a

Values represent mean ±SE (n= 20). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$).

Figure 1. Nitrate content of radish roots under fulvic acid application. Bars represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$)



Visual quality also improved markedly, with a 98% increase in the visual quality index compared to the control (Figure 2A, Table 4). Treated radishes exhibited uniform red coloration, smooth surface texture, and optimal commercial size (Figure 2B, Table 4).

Finally, fulvic acid application resulted in a 41.66% increase in total yield compared to untreated plants (Figure 3).

Figure 2. Visual quality of radish under fulvic acid application. (A) Visual quality index based on five-point hedonic scale. (B) Representative images of radish roots at harvest. Bars represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test (p ≤ 0.05)

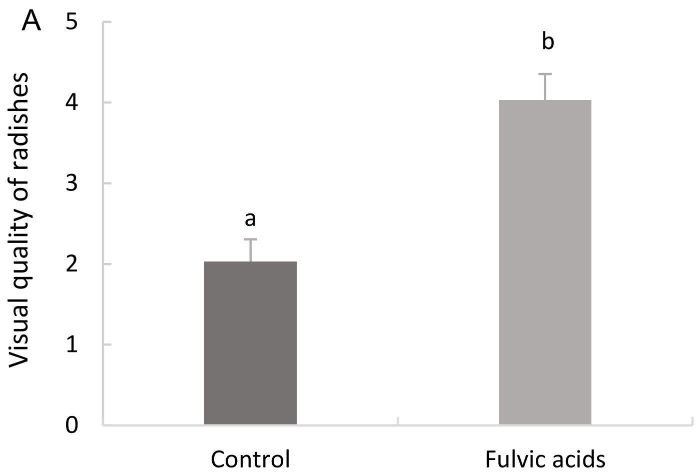


Table 4. Visual assessment scale for quality of hydroponically grown radishes treated with fulvic acid

Score	Description	Visual quality
5	The product is essentially free of defects. • Intense and uniform red color without discoloration. • Symmetrical, cylindrical, or conical root representative of the variety. • Smooth and clean surface. • Optimal size (diameter 3.0–4.0 cm; length 5–6.5 cm).	excellent
4	Minor defects, not objectionable. • Uniform red color with slight variations (<10%). • Slightly irregular but acceptable shape. • Mostly smooth surface. • Commercially acceptable size (2.5–3.0 cm diameter).	good
3	Moderate defects, acceptable in the local markets. • Pink to red color with variability. • Slight curvature or asymmetry. • Slightly rough surface. • Acceptable size (2.0–2.5 cm diameter or >4.5 cm length).	fair
2	Severe defects, low marketability. • Pale or uneven color. • Irregular or curved shape. • Rough surface with visible damage. • Size outside optimal range (<0.8 in. or >2 in.).	poor
1	Non-commercial quality. • Whitish or greenish/brown discoloration. • Severely deformed or bifurcated roots. • Rough surface with cracks. • Unacceptable size.	very poor

Figure 3. Yield of radish under fulvic acid application. Bars represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test (p ≤ 0.05)

DISCUSSION

The results indicate that foliar application of fulvic acids did not modify early vegetative growth of photosynthetic pigment content under the evaluated hydroponic conditions. The absence of significant changes in leaf traits and pigment concentrations suggests that plants in both treatments operate under optimal nutritional and environmental conditions, which tend to stabilize these parameters in soilless systems.

Despite the lack of response in vegetative traits, fulvic acid application promoted significant increase in root diameter, suggesting preferential biomass distribution toward the storage organ. This response is directly associated with commercial quality in root crops, and suggests a modification in source-sink relationships. Similar increases in fruit or root diameter following fulvic or humic acid application have been reported in watermelon [Barzegar et al. 2022], cucumber [Yassin and Al-Zubaidi 2025], and guava [Mosa et al. 2021], and have been attributed to enhanced physiological activity, nutrient uptake, and carbohydrate accumulation.

The observed increase in fresh root weight agrees with previous reports in radish, and other crops treated with humic-based biostimulants. Šlosár et al. [2025] reported increased root weight in radish following humic biostimulant application, while Kaya [2025] observed similar responses under saline stress conditions. Although cultivation systems and stress factors differ, these studies consistently indicate that fulvic-acid-based biostimulants promote biomass accumulation by enhancing activity and resource allocation efficiency. The significant decrease in water content (Table 2) indicates a higher proportion of dry matter in treated roots, aligning with the observed pattern of enhanced biomass allocation toward the storage organ.

The preferential accumulation of biomass in the storage root observed in this study may be explained, at least in part, by the auxin-like activity attributed to fulvic acids. Humic substances, including fulvic acids, contain bioactive fragments that mimic auxin action by interacting with hormonal receptors and activating plasma membrane H^{+-}ATPase proton pumps [Canellas et al. 2015, Nardi et al. 2018]. This proton pump activation generates an electrochemical gradient that energizes secondary transporters involved in nutrient and sugar uptake at the sink level [Canellas et al. 2015]. Furthermore, auxin-mediate signaling has been shown to directly regulate the expression of sucrose transporters, thereby promoting phloem unloading and increasing carbohydrate import into sink organs [Zhang et al. 2022]. In addition, fulvic acid application has been reported to increase endogenous auxin and zeatin concentration in leaves, which may further stimulate assimilate mobilization from source tissues toward developing storage organs [Ampong et al. 2022]. Together, these mechanisms provide a plausible physiological basis for the enhanced assimilate partitioning toward the storage root observed in the present study, although direct evidence from enzyme activity or hormone quantification was not obtained and further studies are warranted.

Fulvic acid application did not significantly affect pH, TSS, or color index, indicating that internal quality attributes associated with basic biochemical composition were preserved. However, the slight reduction in water content suggests an increase in dry matter proportion, which may contribute to improved postharvest quality. Previous studies have shown that higher dry matter content is associated with improved tissue integrity and reduced susceptibility to mechanical damage in root crops [Lockley et al. 2021, Lupu et al. 2024].

The observed reduction in nitrate concentration in treated radishes aligns with the hypothesized role of fulvic acids in enhancing nitrogen assimilation efficiency and regulating nitrate accumulation in edible tissues. This suggests a metabolic redirection of inorganic nitrogen toward organic compounds, a phenomenon consistent with similar reductions reported in radish microgreens treated with plant-derived biostimulants [Toscano et al. 2023]. Internationally, the Joint FAO/WHO Expert Committee on Food Additives has established an Acceptable Daily Intake (ADI) of 3.7 mg nitrate kg^{-1} body weight day^{-1}, which serves as the reference value underlying most national risk assessments; however, no global or food-specific maximum level has been established for nitrate in radish. In its risk-benefit assessment of nitrate from vegetables, the EFSA Panel on Contaminants in the Food Chain [CONTAM 2008] concluded that high-level consumers of vegetables grown under unfavorable production conditions may exceed the ADI by approximately twofold – a concern particularly relevant to crops prone to elevated nitrate accumulation, such as radish. Uddin et al. [2021] reported that, among the root and tuber vegetables analyzed, radish was the only one whose Health Risk Index exceeded the acceptable threshold for both adults and children. Taken together, these findings underscore the practical importance of agronomic strategies, such as foliar fulvic acid application, for mitigating nitrate accumulation in hydroponically grown radish.

Improvements in visual quality further reinforce the positive effect of fulvic acids on commercial performance. Enhanced coloration, surface uniformity, and market size have also been reported in hydroponically grown tomato and cucumber following fulvic acid application [Zhang et al. 2021, Liu et al. 2022, Song et al. 2025], and are commonly linked to optimized nutrient uptake and biomass partitioning.

In terms of yield, the 41.66% increase observed with the application of fulvic acid in this study is consistent with the improvements reported for radishes in other biostimulant-based strategies. Šlosár et al. [2025] observed a 34.8% increase in radish yield following the application of a humic biostimulant, while De Guzman et al. [2026] reported that increasing the application frequency of the hydroponic nutrient solution improved radish yield, reaching a maximum of 6.60 kg m^{-2}.

Overall, the findings demonstrate that foliar application of fulvic acids in an ebb-and-flow hydroponic system enhances radish productivity and commercial quality without altering key physiological quality parameters. The increases in root diameter, fresh biomass, and yield, together with reduced nitrate concentration and water content, suggest a more efficient biomass distribution toward the storage organ. These results support the use of fulvic-acid-based biostimulants as a sustainable agronomic strategy for radish production in soilless systems, while highlighting the need for future studies to elucidate underlying physiological mechanisms and validate these responses under different environmental conditions. Given that the concentration used in this study corresponded to the manufacturer's recommended dose for horticultural crops, future research could evaluate additional concentrations to expand our understanding of crop response, and optimize the agronomic use of the biostimulant in hydroponic systems. Additionally, although the potential contribution of the nitrogen and potassium (1.71 mmol L^{-1} N, 1.61 mmol L^{-1} K, 10%) supplied by biostimulant per foliar application cannot be fully excluded, the observed responses are consistent with the bioactive mechanisms of humic substances documented independently of their mineral content [Canellas et al. 2015, Nardi et al. 2016]. Future studies using purified fulvic acid formulations are recommended to allow more precise attribution of the effect to the humic fraction specifically.

CONCLUSIONS

Foliar application of fulvic acids in an ebb-and-flow hydroponic system improved radish productivity and commercial quality without altering vegetative growth parameters, photosynthetic pigments, or internal quality attributes such as pH, total soluble solids, and color index. The increase in equatorial diameter, fresh root weight, and total yield suggests enhanced biomass distribution toward the storage organ, suggesting a modification in source-sink relationships. The reduction in nitrate concentration within edible tissues indicates a lower accumulation of inorganic nitrogen under the evaluated conditions. Furthermore, visual quality improved markedly, with treated radishes showing enhanced uniformity in coloration, surface texture, and commercial size. The slight decrease in water content may indicate a higher dry matter proportion, which could be advantageous for postharvest quality. Overall, fulvic acids act primarily by promoting biomass distribution toward the storage organ rather than modifying vegetative growth, supporting their use as a sustainable agronomic strategy for hydroponic radish production. Future studies should incorporate direct measurements of nitrogen assimilation activity and evaluate dose-response effects across multiple growing seasons to validate and extend these findings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DECLARATION OF THE USE OF GENERATIVE AI IN THE PROCESS OF PREPARING THE PAPER

The following generative artificial intelligence tools were used in the process of preparing this manuscript: Consensus and Perplexity AI: used to support the systematic search of scientific literature on the topics covered in the article. DeepL: used for translation support between English and Spanish in the preparation of the manuscript text. Claude: used for language editing, grammar checking, and structural revision of the English manuscript text. Following their use, the authors assumed full responsibility for the publication content, reviewed and edited it as necessary.

ATHORS CONTRIBUTIONS

R.P. – methodologhy; M.R. – project administration and supervision; J.H. – formal analysis, data curation, writing-original draft; A.G. – validation and visualization; K.Q. – investigation; A.A. – conceptualization, writing-review and editing.

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RESEARCH PAPER

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EFFECT OF DIFFERENT FERTILIZATION SYSTEMS ON WEED FLORA IN A TOMATO CROP IN SERBIA

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ABSTRACT

This study evaluated weed community composition and diversity in tomato crops under five fertilization regimes, including mineral fertilization (NPK) applied alone or in combination with well-decomposed cattle manure, retort beech charcoal, and their combinations.

A total of 23 weed species were recorded across all treatments. Species richness ranged from 10 to 23 species (T1 = 17, T2 = 12, T3 = 15, T4 = 23, T5 = 10). Total weed density ranged from 70.67 to 187.99 individuals per m^2. The highest Shannon diversity value was recorded in T4 (2.439), while the lowest was in T5 (1.965). Simpson's dominance index ranged from 0.116 to 0.182, with consistently high evenness across treatments. Jaccard's similarity index ranged from 31.82% to 73.91%, while Steinhaus coefficients ranged from 0.412 to 0.718.

PERMANOVA indicated significant differences in weed community composition among treatments ($p = 0.001$). NMDS ordination (stress ≈ 0.16; $R^2 = 0.973$) suggested partial separation among treatments, with T3 and T4 appearing more similar, while T5 was separated from other treatments and characterized by comparatively reduced community complexity. *Chenopodium album* and *Amaranthus retroflexus* were present in all treatments (100% frequency). Therophytes represented the dominant life form across treatments (78.26%). The results showed that the fertilization strategy influenced the frequency and abundance of the weed population in tomato crops.

Key words: NMDS, NPK, PERMANOVA, retort beech charcoal, tomato, weeds

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) ranks among the most extensively grown vegetable crops worldwide and plays an important role in human nutrition. In the Republic of Serbia, tomato is grown on an area of 7,782 ha, with an average yield of 14.6 t/ha and an annual production of 113,913 t [Statistical Yearbook of the Republic of Serbia 2024]. Favorable climatic and agroecological conditions, together with the use of modern cultivars and hybrids, provide Serbia with significant potential for successful tomato production.

Organic tomato production in the Republic of Serbia is becoming increasingly important each year. Chemical weed control in tomato production is limited, even in conventional farming. Although there are clear guidelines for managing organic crops, it is often difficult to keep weed populations below the tolerance threshold. Maintaining a weed-free crop is challenging, as organic production excludes the use of herbicides for weed control

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[Edesi et al. 2012]. Weed management in organic production is based on the application of preventive (indirect) measures, unlike conventional production, which relies on a combination of agronomic, chemical, and biological measures (direct measures) aimed at eliminating weeds from the field due to their harmful effects on the cultivated plants [Awan et al. 2018, López-Correa et al. 2022].

Organic farming combines tradition, innovation, and scientific knowledge to produce healthy products while protecting the habitat. The application of activated carbons in organic farming is a strategy to preserve and enhance soil quality, thereby contributing to increased crop yields [Baronti et al. 2010, Tian et al. 2018, Yadav et al. 2018, Živković et al. 2020a, 2020b, 2023].

Activated carbon and biochar are pyrogenic carbonaceous materials (PCM), produced through the thermochemical conversion of carbon-rich feedstocks (pyrolysis and/or activation). Today, both biochar and activated carbon are employed for soil remediation, whereas in the past this practice was largely restricted to activated carbon. When activated carbon remains in the soil after application and is produced from renewable feedstocks while meeting additional specifications, it can be considered biochar [Baronti et al. 2010, Yadav et al. 2018].

Numerous studies have confirmed that soils amended with biochar can improve plant growth [Yadav et al. 2018, Tian et al. 2018, Živković et al. 2020a, 2020b, 2023]. According to Tian et al. [2018], biochar incorporation induces soil alkalization, which can increase soil nitrification and nitrogen (N) levels. Increases in soil pH are also likely to affect electrical conductivity (EC), cation exchange capacity (CEC), and the content of alkaline metal oxides (Mg^{2+}, Ca^{2+}, and K^+). Additionally, biochar reduces soluble forms of aluminum, which has been identified as a key factor influencing phosphorus (P) solubility [Yadav et al. 2018, Tian et al. 2018].

Previous studies conducted in Serbia demonstrated that retort beech charcoal significantly increased tomato and pepper yields in organic production systems [Živković et al. 2020a, 2020b]. In addition, improved vegetative growth and rooting of blueberry plants were observed following retort beech charcoal application [Živković et al. 2023]. However, weed infestation remained a major production challenge, indicating the need for further investigation of weed dynamics under different fertilization systems.

This study investigated the diversity of weed species in tomato crops subjected to five different fertilization treatments, each with three replicates, to assess how these treatments influence weed community composition. The composition of weeds in crops depends on numerous factors, including crop species, weed control measures, environmental conditions, soil fertility, and climatic characteristics [Roschewitz et al. 2005, Barroso et al. 2015, Pinke et al. 2016, Travlos et al. 2018]. Understanding the influence of crop fertilization on weed composition is a key step toward shifting from reactive to predictive weed management [Barroso et al. 2015]. In addition, climate change may alter weed composition, with certain species potentially becoming invasive [Scott et al. 2014]. Due to the dynamic nature of weed occurrence, continuous monitoring and identification of dominant weeds in tomato production, as well as an understanding of their biology and ecology, are essential for establishing effective management strategies [Ljevnaić-Mašić et al. 2022, Božić et al. 2025].

For these reasons, this study continued with the aim of comparing weed flora in tomato crops under different fertilization systems. The analysis focused on weed composition (species richness, floristic diversity, evenness, and dominance) and weed abundance (occurrence and frequency). The findings of this study are expected to aid in the development of effective strategies for weed management.

MATERIALS AND METHODS

The experiment was conducted during the 2024 growing season in the locality of Trmčare, Kruševac municipality, Serbia (latitude: 43.50555°N; longitude: 21.35571°E; elevation: 300 m) under a continental climate. The study was carried out in field conditions using five treatments, each covering 50 m² (total experimental area 2,500 m²). Each treatment was replicated three times, with 25 plants per replication. Row spacing was 70 cm, and plant spacing within a row was 40 cm. Tomato seedlings of the Optima genotype were started in a greenhouse on 20 March 2024 and transplanted to the field on 30 May 2024. Tomatoes were grown by a support (pillar) on a single tree on Vertisol soil. For many years, no herbicides have been used in the experimental field. Prior to sowing, the soil was prepared by applying burnt cow manure at a rate of 250 kg per 50 m² in the sowing layer. The selection of fertilization treatments and application rates was based on previous experimental studies demonstrating significant effects of retort beech charcoal on plant growth and yield parameters across different crop production systems [Živković et al. 2020a, 2020b, 2023]. Mineral fertilization (NPK 15 : 15 : 15) was included as a conventional reference treatment to enable comparison with organic and combined fertilization systems.

Seven days before planting, all five fields were further prepared according to the following treatments:
Treatment 1 (T1): 250 g of retort beech charcoal + 200 g of burnt cow manure per plant;
Treatment 2 (T2): 20 g of NPK 15 : 15 : 15 + 200 g of burnt cow manure per plant;
Treatment 3 (T3): 200 g of retort beech charcoal + 200 g of burnt cow manure per plant;
Treatment 4 (T4): 300 g of retort beech charcoal per plant;
Treatment 5 (T5): 20 g of NPK 15 : 15 : 15 per plant.

The activated charcoal used in this study was produced from natural beech wood and obtained through carbonation under strictly defined technical conditions, followed by steam activation in a static furnace.

Due to its organic origin and production process, the charcoal exhibits a defined level of physicochemical activity, with an iodine number of 233–750 mg/g, enabling effective water retention and providing the moisture required for plant uptake. The material supplies macronutrients (N, P, K), essential micronutrients (mg/kg: Ca – 8590, Mg – 1260, K – 7400, P – 380, S – 350, Mn – 32, Fe – 230, Zn – 20, Cu – 23), as well as organic compounds, humic acids, and amino acids that enhance physiological processes and improve plant health. The granulometry of 0–2.5 mm maintains soil porosity and structure, as the charcoal is chemically stable and does not dissolve in the soil. Its ash fraction is enriched with oxides of potassium, calcium, and magnesium and contains phosphates, providing an excellent source of this nutrient for plants. Due to the content of alkali metals, the material exhibits an alkaline pH ranging from 9 to 11.

Species nomenclature follows Josifović [1970a, 1970b, 1972a, 1972b, 1973, 1974, 1975, 1976, 1977] and Sarić [1986]. The invasiveness status of weeds in Europe and Serbia, as well as globally significant invasive species, was determined based on DAISIE [2018] and GISD [2019]. Taxonomic classification into families, life forms, and flowering periods was adopted from Landolt et al. [1977] and Takhtajan [2009].

Weed density (number of individuals per square meter, m^2 was assessed using the quantitative quadrat method with completely random sampling. All individual plants of each species were counted within 1 m^2 plots in three replicates for each of the five experimental tomato fields, after which the mean density was calculated (Formula 1).

Total weed density per treatment was subsequently computed according to Formula 2 [Ljevnaić-Mašić et al. 2022].

To evaluate floristic similarities and differences in weed composition, abundance, and dominance across the five tomato treatments, the following metrics were calculated: total number of species, i.e., species richness (*S*), weed density, frequency, Shannon–Weaver diversity index (*H'*), Shannon–Weaver evenness index (*E*), Simpson's dominance index (*D*), Jaccard similarity index (S_J), and Steinhaus coefficient (S_A), according to Formulas 3–8 [Shannon and Weaver 1964, Magurran 2004, Nkoa et al. 2015, Ljevnaić-Mašić et al. 2022].

$$\bar{x} = \frac{n_i/m^2}{3} \quad (1)$$

$$Weediness = \frac{\sum N}{m^2} \quad (2)$$

$$Frequency = \frac{\sum Z_i}{Z} \times 100\ (\%) \quad (3)$$

$$H' = -\sum_{i=1}^{S_t} p_i ln\, p_i, \text{ for } p_i = \frac{n_i}{N} \quad (4)$$

$$E = \frac{H'}{ln\, S} \quad (5)$$

$$D = \sum \frac{n_i(n_i-1)}{N(N-1)} \quad (6)$$

$$S_J = \frac{c}{a+b+c} \times 100\ (\%) \quad (7)$$

$$S_A = \frac{2W}{A+B} \quad (8)$$

where
$\bar{x}$ – the average number of individual plants of species i in plot measured in 3 repetitions,
n_i – number of the i^{th} species,
N – the total abundance of all species within the plot,
Z_i – number of sampling plots with species i present,
Z – total number of sampling plots surveyed,
p_i – a proportional abundance of i^{th} species,
ln – natural logarithm,
S – species richness (total number of species),
a – total number of species in the first population,
b – total number of species in the second population,
c – number of common species,
A – total number of individuals in population A,
B – total number of individuals in population B,
W – the sum of the lesser abundance of each species across the populations.

Statistical analysis

For performing statistical analysis we used the R software [R Core Team 2023]. We prepared data for analyses with the R packages readxl [Wickham and Bryan 2023] and dplyr [Wickham et al. 2023]. For manipulation of Microsoft Word document we used the R package officer [Gohel 2023a] and for tabular reporting we used the R package flextable [Gohel 2023b]. Before the analysis we transformed abundances with square-root. First, we used the function *metaMDS* from the R package vegan [Oksanen et al. 2020] for visualizing the differences between the fields in two-dimensional settings according to the non-metric multidimensional scaling (NMDS). Second, we calculated the dissimilarity matrix by using the Bray-Curtis dissimilarity coefficients [Bray and Curtis 1957, Faith et al. 1987].

$$d_{jk} = \frac{\sum_{i=1}^{23} |x_{ij} - {}_{ik}|}{\sum_{i=1}^{23} (x_{ij} + x_{ik})}, \text{where } j = 1,2, \ldots ,15;\ k = 1, 2, \ldots , 15.$$

Multivariate homogeneity of group dispersions described in Anderson [2006] was checked with the function *betadisper* from the R package vegan [Oksanen et al. 2020]. Afterwards, following Anderson [2001] we used permutational multivariate analysis of variance (PERMANOVA), which brings the best of both worlds of non-parametric statistical tests and classical MANOVA, to test the differences among the treatments. It was performed with the function *adonis2* from the R package vegan [Oksanen et al. 2020]. Finally, for pairwise comparison between the treatments, the SIMPER procedure, also called the "Similarity percentages method", was used. It was described as a part of the general framework for non-parametric multivariate analysis of community data in Clarke [1993] and according to the suggestion given in Oksanen et al. [2020] it is sometimes hard to interpret. We used it to single out the species for which the difference among the treatments is an important ingredient for their contribution to dissimilarities. The function *simper* from the R package vegan Oksanen et al. [2020] was used with the default threshold for showing species that contribute at least 70% to the dissimilarity between the treatments according to the Bray-Curtis dissimilarity coefficients mentioned above and 999 permutations.

For further processing and analysis of our data, ChatGPT (OpenAI, GPT-5, accessed May 27, 2026) was used to assist in selecting appropriate statistical methods and their implementation. ChatGPT (OpenAI) was also used exclusively to assist with the English translation of the manuscript and grammatical corrections, as well as text editing. The authors reviewed and verified all content and assume full responsibility for the final version of the manuscript.

RESULTS

A total of 23 weed species were recorded across all fertilization treatments. Species richness varied considerably, ranging from 10 species in T5 to 23 species in T4 (T1 = 17, T2 = 12, T3 = 15, T4 = 23, T5 = 10). Mineral NPK fertilization (T5) was used as the conventional control treatment. Of the total flora, 78.26% of species belonged to *Magnoliopsida* and 21.74% to *Liliopsida*. The families *Asteraceae* and *Poaceae* each included five species, representing 21.73% for each family. The family *Polygonaceae* comprises three species, accounting for 13.04%. The *Amaran-*

thaceae and *Chenopodiaceae* families each included two weed species, representing 8.70% per family. The families *Brassicaceae*, *Convolvulaceae*, *Geraniaceae*, *Malvaceae*, *Portulacaceae*, and *Rubiaceae* were each represented by a single weed species, accounting for 4.35% each (Table 1). Therophytes were the dominant life form (78.26%) across all treatments, followed by geophytes (21.74%). The most frequent species were *Chenopodium album* and *Amaranthus retroflexus*, both present in all treatments (Table 1).

Table 1. Floristic composition of weeds in five treatments

Plant species	Family	LF	LC	Weediness (average No. of individuals per m^2) in tomato crop					Total indiviuals per m^2	Weed abundance	
				T1	T2	T3	T4	T5		Average individuals per m^2	Frequency (%)
Chenopodium album L.	*Chenopodiaceae*	Th4	A	41.67	40.33	23.33	26	7.33	138.66	27.73	100
Amaranthus retroflexus L.	*Amaranthaceae*	Th4	A	33.67	13.33	20.67	15.33	15.67	98.67	19.73	100
Chenopodium polyspermum L.	*Chenopodiaceae*	Th4	A	–	11.67	8.33	11.67	6	37.67	7.53	80
Amaranthus hybridus L.	*Amaranthaceae*	Th4	A	28.00	–	12.33	7	2,67	50	10	80
Galinsoga parviflora Cav.	*Asteraceae*	Th4	A	–	16.67	9.67	8	–	34.34	6.9	60
Setaria glauca (L.) P. B.	*Poaceae*	Th4	A	16.67	5.00	–	6.67	7,67	36.01	7.20	80
Cirsium arvense (L.) Scop.	*Asteraceae*	G3	P	8.33	–	8.33	4.33	11	31.99	6.4	80
Ambrosia artemiisifolia L.	*Asteraceae*	Th4	A	3.33	–	–	4.33	–	7.66	1.53	40
Hibiscus trionum L.	*Malvaceae*	Th4	A	11.67	–	7.67	3	6	28.34	5.67	80
Agropyrum repens Beauv.	*Poaceae*	G1	P	–	4.67	4.67	5.33	5.33	20.00	4.00	80
Geranium dissectum Jusl.	*Geraniaceae*	Th2	B	8.33	6	–	5.33	–	19.66	3.93	60
Setaria viridis (L.) P. B.	*Poaceae*	Th4	A	9	11.3	–	0.67	–	20.97	4.19	60
Cynodon dactylon (L.) Pers.	*Poaceae*	G1	P	2.67	4.3	–	2	4	12.97	2.60	80
Sorghum halepence L.	*Poaceae*	G1	P	3.33	–	4	5	–	12.33	2.47	60
Polygonum convolvulus L.	*Polygonaceae*	Th4	A	2	–	7.67	5.6	–	15.27	3.05	60
Capsella bursa–pastoris (L.) Medic.	*Brassicaceae*	Th1	B	–	4	–	2.33	–	6.33	1.27	40
Portulaca oleracea L.	*Portulaceae*	Th4	A	–	–	6.33	1.67	–	8	1.6	40
Erigeron annuus (L.) Perss.	*Asteraceae*	Th4	A	–	1.67	3	2	–	6.67	1.33	60
Polygonum persicaria L.	*Polygonaceae*	Th4	A	5	6	4.67	0.67	–	16.34	3.3	80
Polygonum lapathifolium L.	*Polygonaceae*	Th4	A	3.66	–	7.33	2.67	–	13.66	2.73	60
Convolvulus arvensis L.	*Convolvulaceae*	G3	P	4	–	–	3.67	–	7.67	1.53	20
Senecio vulgaris L.	*Asteraceae*	Th1	A	3.33	–	6.33	3.67	5	18.33	3.67	80
Galium aparine L.	*Rubiaceae*	Th2	A	3.33	–	–	1.33	–	4.66	0.93	40
Species richness (S)				17	12	15	23	10	23		
Number of annual plants				12	9	12	16	7	16		
Number of biennial plants				2	1	–	2	–	2		
Number of perennial plants				5	2	3	5	3	5		
Weediness (total number sof individuals per m^2)				187.99	124.94	134.33	128.27	70.67	646.20		
H′				2.191	2.082	2.210	2.439	1.965			
E				0.892	0.892	0.823	0.890	0.911			
D				0.136	0.182	0.134	0.116	0.170			

LF – life form; LC – life cycle; T1–T5 – treatments 1–5; A – annual plants; B – biennial plants; P – perennial plants; Th1 – annual plants; germinate in autumn, fruiting in early spring; Th2 – annual plants; germinate in autumn and early spring, fruiting in early summer; Th4 – annual plants; germinate in spring, bring seeds in late summer; G1 – perennial plants with thin underground shoots (rhizomes); G3 – perennial plants with root shoots (i.e. with adventitious buds on the root); *H′* – Shannon-Weaver's diversity index; *E* – evenness; *D* – Simpson's dominance index

Total weed density varied markedly among treatments, ranging from 70.67 individuals per m^2 in T5 to 187.99 individuals per m^2 in T1 (Table 1). Diversity indices showed clear treatment-related differences. Shannon diversity (*H'*) ranged from 1.965 to 2.439, with the highest values in T3 and T4 and the lowest in T5. Evenness (*E*) ranged from 0.823 to 0.911, with the highest value in T5. Simpson's dominance index (*D*) ranged from 0.116 to 0.182, with highest dominance in T2 and T5 (Table 1). Jaccard similarity showed the highest floristic similarity between T1 and T4 (73.91%) and between T3 and T4 (65.22%), while the lowest similarity was between T1 and T2 (31.82%) – as shown in Figure 1. Steinhaus similarity indicated the highest quantitative similarity between T3 and T4 (SA = 0.718) and between T2 and T4 (SA = 0.642), while the lowest values occurred between T2 and T5 (SA = 0.412) and T1 and T5 (SA = 0.415) – as shown in Figure 2.

Perennial species such as *Cirsium arvense* and *Sorghum halepense* were widely distributed across treatments, while *Agropyron repens* and *Cynodon dactylon* were also recorded. Among annual species, *Amaranthus hybridus*, *Hibiscus trionum*, *Polygonum convolvulus*, *Polygonum lapathifolium*, and *Senecio vulgaris* were more frequent in treatments amended with retort beech charcoal.

Figure 1. Jaccard's similarity index (SJ, %). T1 – Treatment 1; T2 – Treatment 2; T3 – Treatment 3; T4 –Treatment 4; T5 – Treatment 5

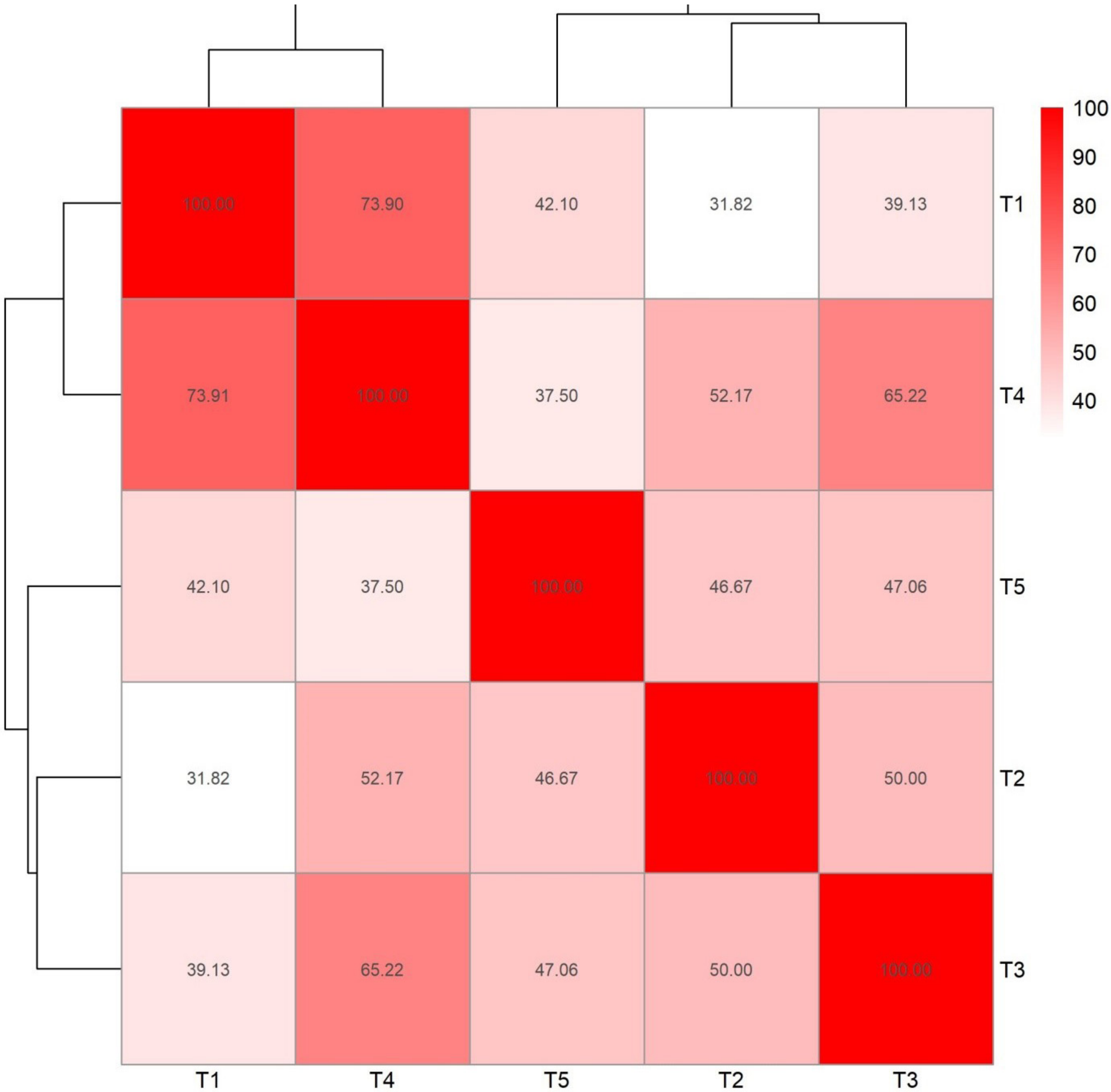
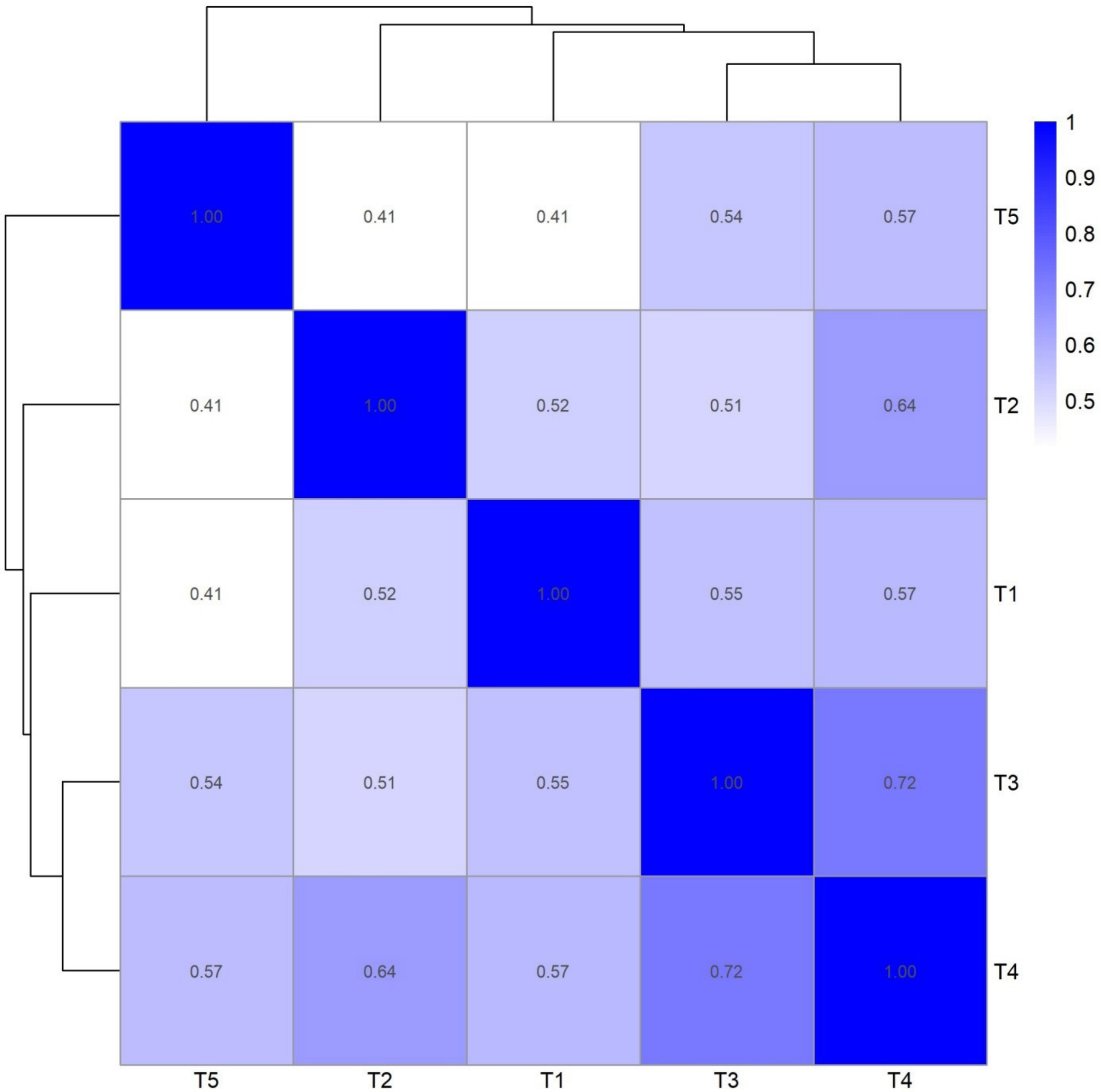


Figure 2. Steinhaus coefficient index (SA). T1 – Treatment 1; T2 – Treatment 2; T3 – Treatment 3; T4 –Treatment 4; T5 – Treatment 5



In contrast, in mineral fertilization treatments (T2 and T5), *Sorghum halepense* and *Convolvulus arvensis* were absent. Several annual species, including *Ambrosia artemisiifolia*, *Polygonum convolvulus*, *Portulaca oleracea*, *Polygonum lapathifolium*, and *Galium aparine*, were not recorded in these treatments.

Statistical analysis

NMDS ordination (Bray–Curtis, stress ≈ 0.16) indicated differentiation among treatments in two-dimensional space. T3 and T4 were positioned closer in ordination space, while T1 and T2 occupied intermediate positions and T5 was separated along the ordination axes. The ordination had a high goodness-of-fit (non-metric $R^2 = 0.973$) – as shown in Figures 1, 2, 3, 4.

Homogeneity of multivariate dispersions was not significant ($p = 0.286$), indicating no evidence of differences in dispersion among treatments. PERMANOVA revealed significant differences in weed community composition among treatments ($p = 0.001$). SIMPER analysis identified species contributing most to observed dissimilarities (Table S1 – available on the journal's website).

Figure 3. Visualization of community composition based on the NMDS in two dimensions. T1 – Treatment 1; T2 – Treatment 2; T3 – Treatment 3; T4 –Treatment 4; T5 – Treatment 5

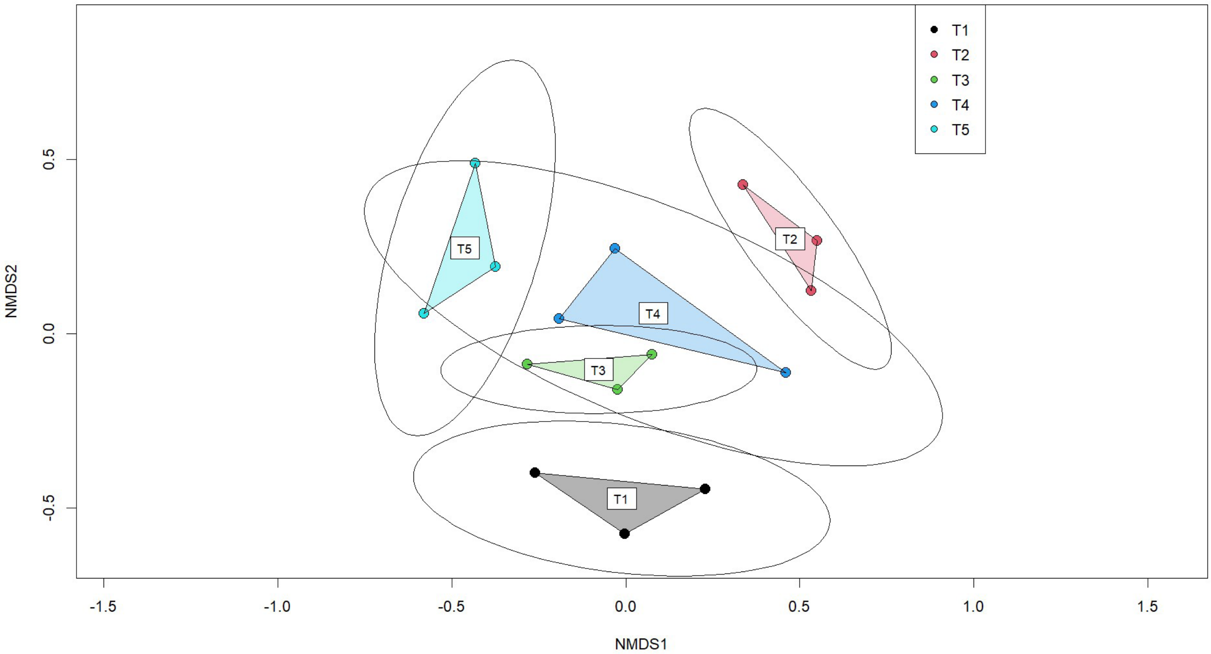
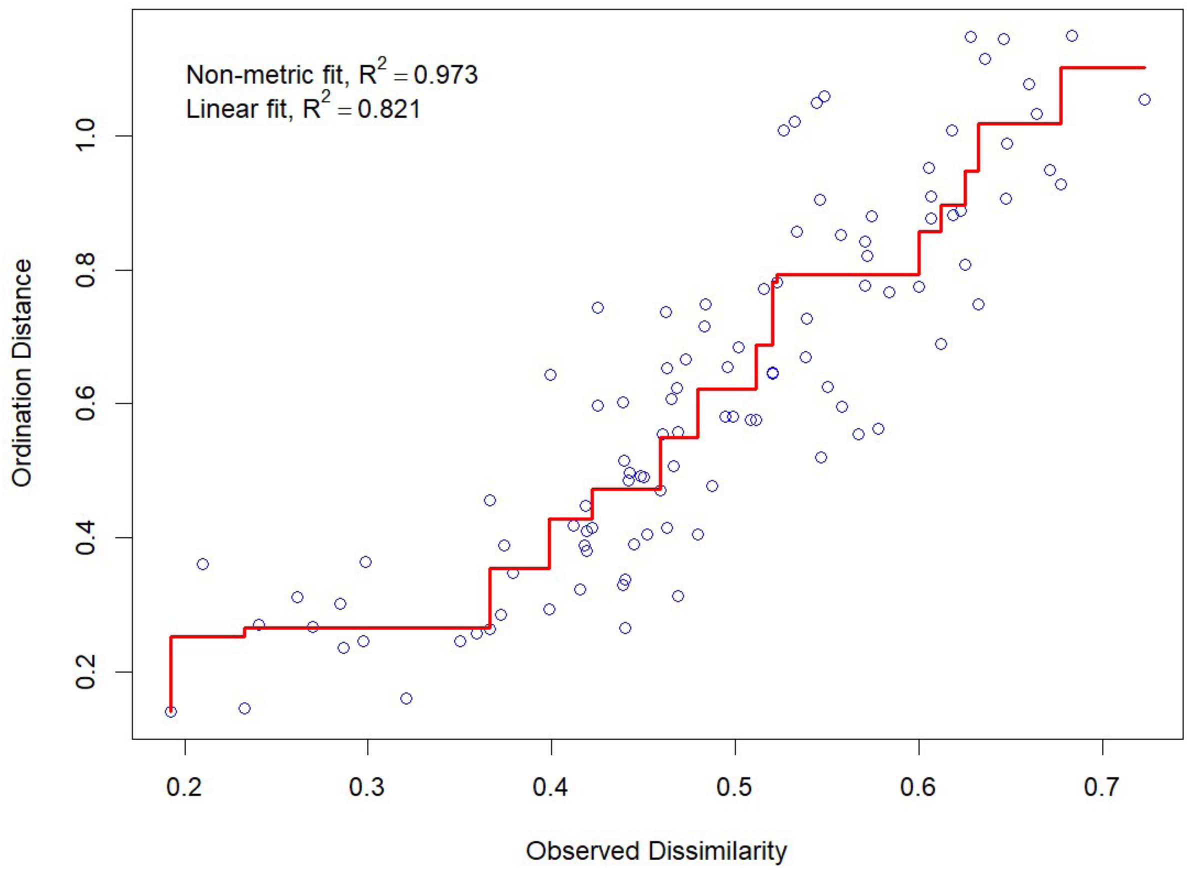


Figure 4. Shepard diagram, i.e., scatter plot of all ordination distances from the NMDS of Figure 1 (y-axis) against the corresponding observed dissimilarity (x-axis) with the fitted monotonic regression line



DISCUSSION

In this study, the fertilization regime significantly influenced weed community structure in tomato agroecosystems, as confirmed by PERMANOVA analysis (p = 0.001). Mineral NPK

fertilization (T5) was used as the control treatment, representing conventional fertilization practice in intensive crop production. Compared with this treatment, organic amendments induced changes in weed community composition and diversity [Roschewitz et al. 2005, MacLaren et al. 2020, Nyawade et al. 2021, Kousta et al. 2023]. Organic amendments, including retort beech charcoal alone or in combination with well-decomposed cattle manure, altered weed species composition and diversity compared with mineral fertilization. Similar effects of organic inputs have been reported in previous studies, mainly related to changes in soil fertility and micro-environmental conditions [Roschewitz et al. 2005, MacLaren et al. 2020, Nyawade et al. 2021, Kousta et al. 2023]. However, responses differed among diversity indices, suggesting context-dependent effects influenced by soil and management conditions [Jeffery et al. 2011, Lehmann and Joseph 2015].

Biochar treatments generally increased species richness and diversity compared with mineral fertilization. This may be associated with improved soil heterogeneity and changes in resource distribution, which can favor establishment of additional weed species. Biochar effects, however, depend on feedstock, pyrolysis conditions, application rate, and soil properties. Temporary reductions in plant-available nitrogen due to adsorption processes may also influence early plant development and competition [Clough and Condron 2010, Spokas et al. 2012].

The dominance of *Chenopodium album* and *Amaranthus retroflexus* across all treatments indicates that these species are well adapted to intensively managed systems. Their persistence is associated with high competitiveness and strong response to nitrogen availability, which allows them to dominate under different fertilization regimes [Blackshaw and Brandt 2008, Storkey and Neve 2018, Allen et al. 2024].

Organic amendments also influenced the presence of several perennial and structurally more complex weed species, including *Cirsium arvense*, *Sorghum halepense*, and species of the genus *Polygonum*. In contrast, mineral fertilization was associated with lower species richness and greater dominance of a limited number of competitive species, indicating a simplified weed community structure under conventional fertilization [Baker et al. 2018, Travlos et al. 2018, Kousta et al. 2023].

Therophytes were dominant in all treatments, reflecting the disturbed conditions typical of intensive agricultural systems. Their dominance is related to life-history traits such as rapid growth, short life cycles, and high reproductive capacity, which allow them to persist under frequent soil disturbance [Storkey and Neve 2018, Travlos et al. 2018, Ljevnaić-Mašić et al. 2022, Božić et al. 2025]

Multivariate analyses provided broadly consistent indications of treatment-related differences. Jaccard similarity suggested moderate to relatively high similarity among selected treatment pairs (notably T1–T4 and T3–T4), while Steinhaus coefficients (SA = 0.412–0.718) indicated quantitative variation in community structure. NMDS ordination (stress ≈ 0.16; $R^2 = 0.973$) suggested partial separation among treatments, with T3 and T4 appearing more similar, while T5 was separated from other treatments and characterized by comparatively reduced community complexity. PERMANOVA indicated statistically significant differences among treatments; however, interpretation of these results should consider both statistical and ecological relevance. SIMPER analysis was used descriptively to identify taxa contributing to observed dissimilarities. Overall, diversity indices, similarity measures, and ordination results showed consistent patterns, supporting the reliability of the findings.

Several methodological limitations should be considered when interpreting the present results. The experiment was conducted under field conditions with three replicates per treatment (each covering 50 m^2, total experimental area 2,500 m^2), which may reduce statistical power and increase sensitivity to spatial heterogeneity typical of agroecosystems. In addition, the study did not include a non-fertilized control treatment, as mineral NPK fertilization was used as a conventional reference for comparison among fertilization systems. Consequently, the observed patterns should be interpreted as relative differences among fertilization regimes rather than absolute fertilization effects under non-fertilized conditions. These limitations should be considered when interpreting and extrapolating the results to broader agroecological contexts. In conclusion, organic amendments modified weed community structure compared with mineral fertilization, particularly by increasing species richness and altering community composition [Roschewitz et al. 2005, MacLaren et al. 2020, Nyawade et al. 2021, Kousta et al. 2023]. Dominant nitrophilous species remained stable across treatments, while changes were mainly observed in secondary species [Blackshaw and Brandt 2008, Barroso et al. 2015, Travlos et al. 2018, Kousta, et al. 2023]. These results highlight the importance of fertilization strategy in shaping weed communities in tomato production systems.

CONCLUSION

The results of this study demonstrate that fertilization regime significantly affects weed community structure in tomato agroecosystems. Mineral NPK fertilization produced a more simplified weed community, while organic amendments resulted in increased species richness and altered community composition. Retort beech charcoal, applied alone or in combination with well decomposed cattle manure, contributed to higher weed diversity compared with mineral fertilization. However, the response of weed communities varied depending on diversity measures and environmental context, indicating that the effects of organic amendments are not uniform. Dominant species such as *Chenopodium album* and *Amaranthus retroflexus* were present across all treatments, suggesting strong ecological adaptation to intensive agricultural conditions and stability under different fertilization regimes. In contrast, organic amendments influenced the occurrence of less competitive and structurally more diverse weed species. Multivariate analyses confirmed consistent differences among fertilization treatments, supporting the reliability of the observed patterns. However, interpretation of the results should consider the field-based experimental design, limited replication, and absence of an unfertilized control, which restricts conclusions to relative differences among treatments.

In conclusion, fertilization strategy is an important driver of weed community structure in tomato production systems. Organic amendments may increase weed diversity and modify species composition compared with conventional mineral fertilization, but their effects remain context-dependent and influenced by environmental conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this work.

DECLARATION OF THE USE OF GENERATIVE AI IN THE PROCESS OF PREPARING THE PAPER

For further processing and analysis of our data, ChatGPT (OpenAI, GPT-5, accessed May 27, 2026) was used to assist in selecting appropriate statistical methods and their implementation. ChatGPT (OpenAI) was also used exclusively to assist with the English translation of the manuscript and grammatical corrections, as well as text editing. The authors reviewed and verified all content and assume full responsibility for the final version of the manuscript.

AUTHORS' CONTRIBUTIONS

S.P.Ž. – conceptualization and study design; S.P.Ž., T.P.V. – methodology development; S.P.Ž., T.P. V., D.R.J. – investigation; M.Z.P. – statistical analysis.

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INFLUENCE OF CULTIVAR AND ROOSTOCK ON FRUIT CRACKING INDEX OF SWEET CHERRY (*Prunus avium* L.)

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ABSTRACT

This study investigated the influence of cultivar and rootstock on the fruit cracking index and harvest date of sweet cherry under the agro-ecological conditions of Trebinje (Bosnia and Herzegovina). Four Italian sweet cherry cultivars (Marysa, Sweet Gabriel, Sweet Valina, and Sweet Stephany) grafted onto two vegetative rootstocks (Gisela 5 and Gisela 6) were evaluated during the 2025 growing season. The results showed statistically significant differences among combinations cultivar/rootstock in both harvest date and fruit cracking index. The lowest cracking index was recorded for Sweet Stephany grafted onto Gisela 6, while the highest susceptibility was observed for Marysa grafted onto the same rootstock. Overall, cultivars grafted onto 'Gisela 6' exhibited higher cracking susceptibility compared to those grafted onto Gisela 5. A strong and highly significant negative correlation ($r = -0.91^{**}$) was established between harvest date and fruit cracking index, indicating that earlier-ripening cultivars were more prone to cracking. The findings highlight the importance of selecting appropriate cultivars and rootstocks in reducing the risk of fruit cracking. However, in order to provide more valuable information for the introduction of new sweet cherry cultivars and the establishment of sustainable orchards under similar agroecological conditions, it is necessary to extend the study over two or even three years, as only in this way can reliable and relevant data be obtained.

Keywords: sweet cherry, cultivar, rootstock, fruit cracking index, harvest date, correlation

INTRODUCTION

Fruit cracking significantly affects fruit quality and leads to a considerable reduction in the commercial yield of sweet cherry. According to Simon [2006] and Khadivi-Khub [2015], fruit cracking—specifically skin cracking—represents a major source of economic losses in sweet cherry production. A large number of authors have addressed this problem in sweet cherry research [Measham et al. 2009, Balbontín et al. 2014, Koumanov 2015]. Rain-induced fruit cracking is considered one of the main limitations of sweet cherry production and a significant threat to its economic profitability. This physiological disorder manifests as cracks in the fruit skin, which may extend deep into the flesh and occur in different regions of the fruit, including the stem cavity, calyx end, and fruit cheeks (side cracks) [Balbotín et al. 2013]. Cultivar genetics play an important role, as some varieties are naturally more resistant due to skin structure and physiological traits. Also, climate change may further intensify sweet cherry fruit crack-

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ing due to the increased frequency of heavy rainfall events and higher air humidity during the ripening period [Huang et al. 2019]. However, the mechanisms involved in fruit cracking have not yet been fully elucidated.

According to Schuller et al. [2016], the phenotypic characteristics of fruits, such as weight, fruit-to-pit ratio, skin thickness, and fruit firmness, vary significantly among different sweet cherry cultivars. Similar findings are confirmed by Kaya et al. [2026]. Numerous studies have confirmed that fruit cracking is influenced by cultivar and rootstock characteristics [Simon et al. 2004, Granger 2005, Duralija et al. 2007]. In recent years, vegetative (clonal) rootstocks such as Gisela 5, Gisela 6, and Colt have become widely used in intensive sweet cherry production systems, as they enable high-density planting, earlier bearing, and improved orchard management. The most important characteristics of the Gisela 5 rootstock are: low–vigorous, propagated by micropropagation, requires more fertile soils, sensitive to drought, resistant to winter frosts, good compatibility with cultivars, early and regular yield, requires support, fertilization, strong and regular pruning. On the other hand, the Gisela 6 rootstock is characterized by traits such as: moderately vigorous, propagated by micropropagation, tolerates drought, heavy, and clay soils, not suitable for very hot climates, good compatibility with cultivars, early and good yield, and fruit size, suitable for plantings with a density of 800–1200 trees/ha. Late-ripening cultivars exhibit better pomological traits, such as fruit size, color, and flavor, as well as lower susceptibility to fruit cracking [Sansavini and Lugli 2008].

The aim of the research was to determine the ripening dates of different sweet cherry cultivars, as well as the influence of cultivar and rootstock on this trait and on the fruit cracking index. The significance of this study lies in providing recommendations for new Italian sweet cherry cultivars with regard to this economically important trait, as well as recommendations for establishing new orchards by selecting cultivars that are less prone to fruit cracking.

MATERIALS AND METHODS

The research was conducted in a sweet cherry orchard located in the area of the city of Trebinje (Bosnia and Herzegovina). The orchard was established in 2024. Four new sweet cherry cultivars of Italian origin were examined: Marysa, Sweet Gabriel, Sweet Valina and Sweet Stephany. Each cultivar was grafted onto two rootstocks, Gisela 5 and Gisela 6. A total of eight cultivar/rootstock combinations were included in the orchard, with each combination represented by nine trees (three trees in three replicates). The training system was slender spindle, and the planting density was 3.0 × 1.2 m. The fruit cracking index was determined using the method described by Christensen [1996]. For each cultivar/rootstock combination, 150 uniform and well-developed fruits were sampled and divided into three replicates (50 fruits per replicate). Fruits were collected from different parts of the tree canopy. The sampled fruits were then immersed in 2 L of distilled water at a temperature of approximately 20 °C. Fruit assessments were performed after 2, 4, and 6 hours of immersion, during which cracked fruits were counted and separated. The fruit cracking index was calculated using the formula:

$$FCI = \frac{(5a + 3b + c) \times 100}{250}$$

Where *a* represents the number of cracked fruits after 2 h, *b* the number of cracked fruits after 4 h, and *c* the number of cracked fruits after 6 h. The fruit cracking index was determined over a one-year period (2025). The ripening date of fruits from the examined sweet cherry cultivar/rootstock combinations was recorded at the beginning of commercial harvest for fresh consumption and expressed as the number of days from 1st January. In addition, the correlation between ripening date and fruit cracking index was analysed in order to examine the relationship between these two important parameters. The obtained data for fruit cracking of fruit are presented as mean values for three replicates ± standard error and were statistically processed using Fisher's model of analysis of variance (ANOVA) for a two-factorial experiment, applying the F-test [Fisher 1953] for P ≤ 0.05 (using the statistical package IBM SPSS Statistics 20 (SPSS Inc, Chicago, IL, USA)). In the two-factorial analysis of variance, the factors used were the cultivar and the rootstock. When the F-test was significant, differences between arithmetic means were tested using Duncan's multiple range test at a significance level of P ≤ 0.05. The results are presented in graphs. Correlation analysis between fruit cracking and time of maturating was performed using IBM SPSS Statistics 20 (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Ripening time in sweet cherry is a genetically controlled trait primarily determined by the cultivar, while rootstocks may modify the onset of maturity through their effects on tree vigor, assimilate distribution, and fruit development dynamics. Figure 1 shows the number of days from the January 1st to harvest of the studied combinations cultivar/rootstock (red color), sweet cherry cultivars (green color) and rootstocks (yelow color) in the agroecological conditions of Trebinje. Under the conditions of Trebinje, the combinations cultivar/rootstock ripened from the third decade of May to the first decade of June, covering a period of 13 days. In 2025, the earliest onset of fruit ripening was recorded for the cultivar Marysa grafted onto the vegetative rootstock Gisela 6 (May 24), while on the same rootstock the latest ripening was observed for Sweet Stephany (June 6). On average across both rootstocks, fruits of the cultivar Marysa ripened the earliest (May 25), whereas the latest ripening time was recorded for Sweet Stephany (June 6). The mean fruit ripening date for both studied rootstocks was June 1. According to descriptions by the Alma Mater Studiorum – University of Bologna breeding program, Sweet Gabriel and Sweet Valina exhibit intermediate and mid-late ripening windows respectively when compared to the standard cultivar Burlat, while Sweet Stephany is categorized as a late-season cultivar, with ripening occurring approximately 25–27 days after Burlat. Information for Marysa classifies it generally as mid-season based on orchard production data. Previous studies have shown that both cultivar genetics and rootstock choice significantly influence sweet cherry phenology, including the timing of ripening [Duralija et al. 2007, Usenik et al. 2010, Boskov et al. 2022, Filyova 2024].

Figure 1. Number of days from the January 1st to harvest (2025)

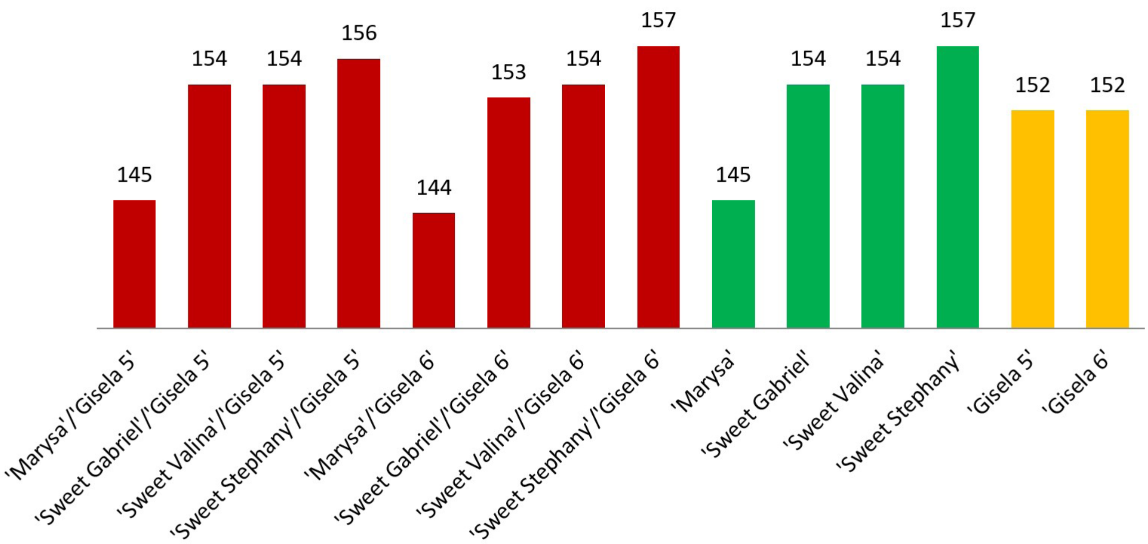
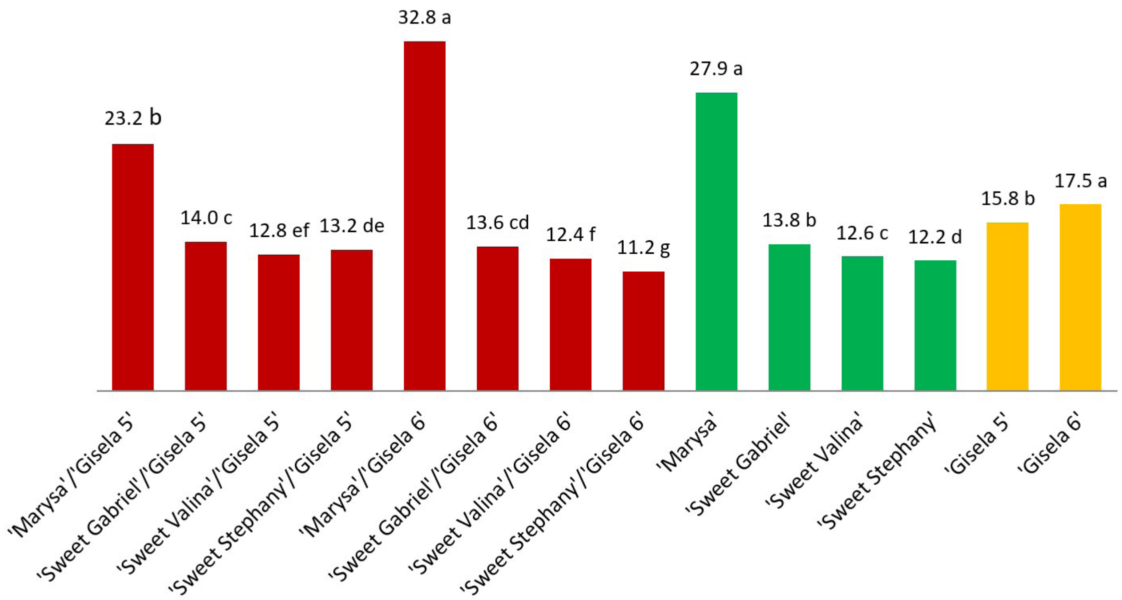


Figure 2 shows data related to the susceptibility of sweet cherry cultivars and rootstocks to fruit cracking. The lowest fruit cracking index was recorded for the cultivar Sweet Stephany grafted onto the rootstock Gisela 6 (11.2), while the highest value was observed for the cultivar Marysa grafted onto the same rootstock (Gisela 6) (32.8), which was statistically significantly higher compared to the other combinations. The examined cultivars, rootstocks and cultivar/rootstock combinations differed significantly with respect to the fruit cracking index (Figure 2). When comparing cultivars, fruits of Marysa showed the highest susceptibility to cracking, which was statistically significant in relation to the other cultivars. The trend of the fruit cracking index among cultivars was as follows: Marysa (27.9) > Sweet Gabriel (13.8) > Sweet Valina (12.6) > Sweet Stephany (12.2). Conversely, fruits sampled from trees grafted onto the vegetative rootstock Gisela 6 showed a statistically significantly higher susceptibility to fruit cracking (17.5) compared to fruits sampled from trees grafted onto the rootstock Gisela 5 (15.8). According to the values of the fruit skin cracking index, all cultivars can be classified into four groups: slightly susceptible (cracking index <10.0), moderately susceptible (cracking index 10.1–30.0), susceptible (cracking index 30.1–50.0), and highly susceptible (cracking index >50) [Milatović and Đurović 2010]. The studied cultivar/rootstock combinations could be divided into two groups: moderately susceptible (Sweet Gabriel/Gisela 5; Sweet Valina/Gisela 5; Marysa/Gisela 5; Sweet Stephany/Gisela 5; Sweet Gabriel/Gisela 6; Sweet Valina/Gisela 6; Sweet Stephany/Gisela 6) and susceptible (Marysa/Gisela 6). The results indicate that cracking susceptibility is strongly dependent on cultivar characteristics and may be modified by rootstock choice, emphasizing the importance of selecting appropriate combinations for specific agro-ecological conditions. Although the susceptibility of sweet cherry cultivars to fruit cracking has been studied extensively in a wide range of genotypes using standardized cracking indices, no scientific publications to date have reported cracking susceptibility measurements specifically for the cultivars Sweet Gabriel, Sweet Valina, Sweet Stephany, or Marysa. This highlights a gap in the literature regarding cracking behavior in newly introduced cherry cultivars from the Bologna breeding program. A correlation was established between the fruit cracking index and the ripening time. This correlation was negative and highly significant (–0.91**). Specifically, cultivars with earlier ripening dates exhibited higher fruit cracking index values, indicating they were significantly more susceptible to cracking. From an economic perspective, this information is particularly important when selecting sweet cherry cultivars.

Figure 2. The fruit cracking index value depending on cultivar/rootstock combination



Mean values following the same letter within a column for each cultivar are not significantly different according to the Duncan multiple range test (P ≤ 0.05)

CONCLUSION

The results revealed significant differences in fruit cracking susceptibility among the examined cultivar/rootstock combinations, confirming the strong influence of both genetic and agronomic factors. Higher susceptibility observed in certain combinations, particularly on Gisela 6, may be associated with differences in fruit skin structure, water uptake dynamics, and osmotic pressure within the fruit during ripening. The strong negative correlation between harvest date and cracking index indicates that earlier-ripening cultivars are more exposed to environmental stress, especially rainfall, which can intensify cracking.

However, it should be emphasized that the results obtained in this study are based on a one-year investigation (2025), which represents a limitation when drawing broader conclusions. Therefore, in order to obtain more reliable and comprehensive results, it is necessary to conduct multi-year research under similar agroecological conditions. Only on the basis of such studies can more precise recommendations be made regarding the selection of sweet cherry cultivars and rootstocks for cultivation in environments similar to those of Trebinje. Generally, these are preliminary one-year studies and that in order to provide clear guidance to growers and nursery producers, they need to be repeated.

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DECLARATION OF THE USE OF GENERATIVE AI IN THE PROCESS OF PREPARING THE PAPER

No generative AI tools were used in the preparation, writing, editing, analysis, or revision of this manuscript. All ideas, research, analysis, and written content were produced solely by the author.

AUTHORS' CONTRIBUTIONS

M.R. – working on a conception and assumptions, developing the methods, conducting research, supervision, writing-review and editing; M.L. – working on a conception and assumptions, conducting research; S.J.Š. – developing the methods, supervision, writing-review and editing; J.A. – conducting research; A.D. – conducting research; J.H.S. – supervision, writing-review and editing; N.B. – supervision, writing-review and editing.

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