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Enhancing the nutritional value of sweet bell pepper through moderate NaCl salinity

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ABSTRACT

Salinity presents a significant obstacle to crop productivity, particularly in dry and semi-arid regions. Sweet bell pepper (*Capsicum annuum* L.), a widely grown and consumed horticultural crop, is especially vulnerable to salinity. Consequently, it is vital to determine the salinity threshold that impacts bell pepper growth and quality, enabling sustainable production in salinized areas. This study aimed to evaluate the effects of varying sodium chloride concentrations (0, 50, and 75 mM) on bell pepper growth, nutritional value, and phytochemical composition, aiming to identify the adaptable threshold in salinized environments. The results suggested that the application of 75 mM NaCl not only had no adverse impact on fruit quality in terms of biomolecules and phytochemicals but also led to significant improvements. Specifically, under these conditions, there was a remarkable increase, in respect to control, in total protein (TPRO by 50 %), total carbohydrates (TCARB by 18 %), lycopene (LIC by 68 %), total Carotenoids (TCAR by 13 %), and total phenols (TPHE by 18 %) in terms of antioxidants.In contrast, the content of ascorbic acid and antioxidant activities remained consistent. Moderate salt stress exhibited the most positive influence on sweet bell pepper quality, leading to higher concentrations of essential nutrients and nutraceutical compounds, including minerals, phenolic acids, and flavonoids.

1. Introduction

Salinity, a critical environmental factor, significantly restricts crop productivity, especially in arid and semi-arid regions [1,2]. Climate change and extreme weather events are exacerbating the variability of temperature and precipitation, leading to water scarcity, particularly in Mediterranean areas where there is an increasing need for irrigation [3]; Pannell et al., 2006; [4,5]. Soil salinization poses a major problem in irrigated agriculture due to water shortages, poor water quality, and inadequate soil drainage, resulting in reduced crop growth, productivity, and negative economic, environmental, and social impacts [3]; Pannell et al., 2006). Excessive salts in the soil solution hinder water and nutrient uptake by plants, causing osmotic stress, ion toxicity, nutrient imbalances, and water deficit, ultimately leading to chlorosis, early leaf senescence, and significant crop yield reductions (Souri et al., 2019; [6].

According to the Food and Agriculture Organization (FAO), approximately 424 million hectares of topsoil (0–30 cm) and 833 million hectares of subsoil (30–100 cm) are affected by salinity globally [7]. Currently, salinity affects over 74 % of agricultural lands [8,9]). Horticultural crops, including sweet bell pepper (*Capsicum annuum* L.), are generally moderately sensitive, sensitive, or highly

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Table 1
Chemical and biochemical properties of soil located in Motta San Giovanni, Soil texture (percentage of sand, silt and clay); pH_{H2O} in water and pH_{KCl} in potassium chloride; EC = electric conductivity (μS/cm); TP = total water soluble phenols (μg TAE g^{-1} ds): OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon nitrogen ratio; OM = organic matter (%); MBC = Microbial biomass Carbon (μg C g^{-1} soil); CEC= Cation Exchange Capacity (cmol(+) Kg^{-1}).

	CTR
Sandy[a]	64.94 ± 10
Clay	11.85 ± 4
Silt	23.21 ± 3
Texture	Sandy-loam
pH $_{(H2O)}$	8.43 ± 0.1
pH $_{(KCl)}$	6.94 ± 0.1
EC	302 ± 10
TP	282 ± 12
MBC	835 ± 12
CEC	27.8 ± 1
OC	1.98 ± 0.5
TN	0.20 ± 0.02
C/N	9.9 ± 1.5
OM	3.42 ± 0.6

[a] Soil texture was detected using the hydrometer method (Bouyoucos, 1962); Electric conductivity (EC) was determined in distilled water by Hanna instrument conductivity meter; pH was measured in distilled water (soil/solution ratio 1:2.5) with a glass electrode; organic carbon and nitrogen were assessed with TOC analyser; cation exchange capacity was detected by using Barium Chloride Method as reported by (Hendershot and Duquette, 1986); Total water-soluble phenols (monomeric and polyphenols) were determined by using the Folin-Ciocalteau reagent (Box, 1983); microbial biomass C was detected by using the method of Vance et al. (1987).

susceptible to salinity [10,11]. With the world population projected to reach 9 billion by 2050 and the expected decrease in cultivable land due to increasing salinity by approximately 34 % (Boretti et al., 2019), it is crucial to identify vegetable species that naturally adapt to saline soils while producing high-quality crops to meet the nutritional needs of the growing population, including vegetarian and vegan individuals.

Sweet bell pepper is among the most important cultivated horticultural species globally, with production increasing from 17 to 36 million tons over the past 20 years and a corresponding expansion in cultivated areas by approximately 35 % [12,13]. Previous studies, such as Shaid et al. (2018) and [14]; have identified pepper plants as sensitive to salinity, with a tolerance limit of up to 1.5–2 dS m^{-1}. Each additional electrical conductivity (EC) unit beyond this threshold led to a 14 % decrease in pepper biomass production, and prolonged exposure to high salinity levels resulted in plant death [15]. Salinity affects bell pepper growth by disrupting water and nutrient uptake. Excessive salts in the soil can create an osmotic imbalance, leading to water stress and reduced cell expansion. This can result in stunted growth, decreased leaf area, and overall plant size. The accumulation of salts in the plant tissue can also disrupt nutrient uptake, leading to nutrient deficiencies and imbalances. Furthermore, salinity can directly impact bell pepper fruit quality (Oliveira et al., 2016) [16]. evidenced, in pepper plants, that dry weight and marketable yield diminished by 46 and 25 %, respectively when irrigated with water at 4.4 dS m^{-1}. From a physiological point of view, they evidenced that root was the first organ affected after the exposure to Na+ and Cl− concentration, these ions cause not only an osmotic and ionic stress but also, they move rapidly to photosynthetic organs that lead to reduce biomass and crop production.

[17] evidenced that low and moderate salt stress affected four *Capsicum annuum* L. genotype growth and treatment with 40 mM NaCl caused the highest increase in individual phenols, followed by treatment with 20 mM NaCl. The authors confirmed that the reaction and metabolic content to salt stress within the genus Capsicum is genotype-, fruit part-, and salinity level-dependent.

High salt levels can affect the accumulation of essential nutrients in the fruits, leading to nutritional imbalances and reduced nutritional value. Salinity stress can also affect the flavour, colour, and texture of bell peppers, making them less desirable for consumers. In addition to these direct effects, salinity-induced stress can trigger physiological responses in bell pepper plants. It can lead to the production of reactive oxygen species (ROS) [18], causing oxidative stress and damage to cell membranes, proteins, and DNA. This

Table 2
Climatic conditions during the experimental time in Motta San Giovanni.
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	May	June	July	August
Medium Temperature (°C)	17.7	22.3	25.3	25.5
Minimum temperature (°C)	13.7	17.9	20.8	21.4
Maximum temperature (°C)	21.6	26.4	29.6	29.7
Precipitation (mm)	30	21	8	17
Moisture (%)	67%	61%	57%	59%
Rainy days per month (g.)	4	2	1	2
Hours of sunshine per day	11.7	12.7	12.7	11.9

oxidative damage can further contribute to reduced growth and quality of bell peppers [19–21]. Overall, the negative effects of salinity on bell pepper quality and growth highlight the importance of managing and mitigating salinity stress in agricultural practices. This can be achieved through various strategies such as proper irrigation management, soil amendments, and the use of salt-tolerant varieties. The hypothesis driving this study is that plants can adapt to salinity induced by NaCl by making necessary biochemical adjustments to survive under stress. Therefore, an efficient antioxidant system consisting of antioxidant compounds and enzymes could potentially mitigate oxidative stress induced by reactive oxygen species (ROS). This approach not only aims to increase salinity resistance in salt-sensitive species but also to enhance valuable bio-compounds that can provide nutritional benefits, adding value to crops. Currently, there are limited studies that have assessed the quality and growth of bell peppers in open fields with increasing salinity levels. Therefore, the specific objective of this study was to examine the effects of 0, 50, and 75 mM NaCl on bell pepper growth, nutritive values, and phytochemicals in order to determine the threshold salinity level for possible adaptability. Understanding the responses of bell peppers to varying NaCl concentrations is crucial for ensuring the production of this widely cultivated crop, particularly in areas where salinity poses a growing constraint.

2. Materials and methods

2.1. Soil analysis, growth conditions and stress treatments

The experiment has been carried out in open field in Motta San Giovanni, in the Agricultural Farm Orfei Loc. Liso, Italy (x: 561023,1; y: 4204908,9; WGS 84 UTM Zone 33 N). The soil belongs to sandy-loam (11.85 % clay, 23.21 % silt and 64.94 % sand) textural class according to the soil classification system of USDA (1999). The soils were slightly alkaline and contained 3.09 % organic matter and 0.17 % nitrogen (Table 1).

Prior to transplanting the 25-day-old pepper seedlings, which had been germinated in a climatic cell, the soil was divided into 1 m^2 parcels. The soil used in this study initially had a non-saline composition with a constitutive electrical conductivity (EC) of 300 μS/cm. To induce salinity conditions, the parcels were treated with a sodium chloride (NaCl) solution. Despite, different salts contribute to the soil salinity in many cases [22], NaCl was chosen as it is the most common salt in agricultural lands and negatively affects plant growth and productivity. The NaCl solution was prepared using deionized water and different concentrations of NaCl, specifically 50 mM and 75 mM. The control group consisted of untreated plots. The selection of 50 mM and 75 mM NaCl concentrations was based on previous results obtained from growing bell peppers in 30 cm diameter pots within a climatic chamber (data not shown). The results indicated that at 100 mM NaCl, the plants exhibited growth difficulties with a significant reduction of 55 % in growth and performance. For irrigation purposes, deionized water (control) or a solution of 50 mM or 75 mM NaCl was used. The salinized water was applied to the parcels until the desired pre-established salinity conditions were achieved. The attainment of 50 mM or 75 mM NaCl concentration was measured using a conductometer. Soil salinity levels were determined by measuring the electrical conductivity of the soil. The soil was dried, and a mixture of 5 mL of deionized water per 1 g of soil was prepared. The suspension was then shaken, and the probe of a handheld conductivity meter (Hanna Instrument) was immersed in the mixture to measure the electrical conductivity (EC). It should be noted that an EC value of 1 dS/m corresponds to a pure NaCl concentration of 10 mM. Once the soils reached the desired salinity concentrations of 50 and 75 mM NaCl, the soil moisture level was maintained at approximately 70 % of field capacity throughout the entire experiment by supplying fresh water. This was done to prevent excessive NaCl accumulation and salt leaching. The EC of the soils was monitored every 2 days using a portable conductometer to ensure that the soil salinity remained constant at 50 and 75 mM. Each independent experiment was conducted for three consecutive years in triplicate in the field, with 10 plants per square meter cultivated in each parcel. The life cycle of each pepper plant was completed in soil conditioned with NaCl, and the plants were compared to those grown in unsalinized soils (control). The experiment followed a randomized complete block design. The reported data represents the mean of three independent experiments conducted in triplicate ($n = 9$) in parallel. Throughout the entire three-month experiment, which included transplanting in May and data collection at the beginning of August, soil salinity was monitored weekly by collecting soil samples up to a depth of 50 cm, corresponding to the explored root zone. This was done to maintain a constant concentration of 50 and 75 mM NaCl throughout the experiment. Additionally, the moisture level was maintained at approximately 70 % of field capacity by supplying fresh water, as described by Kirkham et al. (2014). The specific climatic conditions during the experimental periods are detailed in Table 2. The primary goal of this study was to undertake an experiment focused solely on investigating the effects of sodium chloride (NaCl) on plants, deliberately opting not to apply soil fertilization. This deliberate choice was made to segregate and closely examine the influence of NaCl in isolation on plant growth, devoid of any potential interference stemming from supplementary nutrients found in fertilizers. Such an experimental framework proves valuable when the objective is to precisely comprehend the unmediated impact of a particular factor—in this instance, salt (NaCl)—on the growth and development of plants.

2.2. Plant analysis

The experiment concluded upon reaching bell pepper maturity, which occurred three months after the seedlings were transplanted (July). Several growth parameters were measured, including plant height (PH) which was measured from the soil level to the highest point of the plant, and leaf number (LF) which represented the count of leaves from the basal leaves to the last open leaf. Additionally, fruit size was measured in terms of diameter (in cm), and fruit number was recorded.

Moreover, the evaluation extended to the quality of mature pepper fruits (selected randomly and representing each treatment), encompassing an analysis of antioxidants, primary metabolites, and secondary metabolites. These comprehensive evaluations were undertaken to gauge the existence and concentrations of these constituents, which serve as pivotal benchmarks for assessing both fruit quality and nutritional significance.

2.3. Determination of antioxidant activities and compounds in pepper fruits

Antioxidant compounds and antioxidant activities were detected in the mature bell pepper fruit [23,24].

Fruits were blended and homogenized with 15 mL of EthOH: H2O (80:20), centrifuged at 3000 rpm for 15 min, and filtered through a 0.45 μm filter (Millipore Corporation, Bedford, TX, USA). The extracts were frozen at −80 °C until analysis.

Total carbohydrates (TCARB) and total protein (TPRO) were determined as reported in Ref. [25] and expressed as mg glucose g-1 DW and mg*g^{-1} DW), respectively.

Total phenols (TPHE), were detected using the Folin–Ciocalteu assay [25]. Absorbance was read at 760 nm, and results reported as μg GAE * g^{-1} DW.

Total flavonoids (TFLA) were assessed as reported into [25]. Absorbance was measured at 430 nm and the data expressed as g quercetin g^{-1} DW.

Vitamin C (VIT C) was assayed following Muscolo et al., method [25] using an ascorbic acid calibration curve. The results were reported as mg alpha-tocopherol 100 g^{-1} DW.

Vitamin E (VIT E) was analyzed as reported in Ref. [26] measuring the absorbance at 695 nm. Data were quantified on the molar absorption coefficient of the phosphor-molybdenum complex, and expressed as mg alpha-tocopherol 100 g^{-1} dry weight (DW).

Vitamin A (VIT A) was assessed following [27] method, reading the absorbance at 436 nm, and expressing the results as μg retinol 100 g^{-1} dry weight (DW).

Total Carotenoids (TCAR) were extracted by pounding the fruits 50 mg in 25 mL cold acetone [28]. The sample absorbances were read at 537, 647, and 663 nm. The amount of carotenoids was expressed as mg g^{-1} dry weight (DW).

Lycopene (LIC) was detected as reported by Ref. [29] and expressed as mg 100 g^{-1}.

The antioxidant activity against DPPH radical (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was detected following the method reported in Ref. [25]. Changes in ABS have been read at 517 nm.

Total antioxidant capacity (TAC) was assessed following [25] method, measuring the absorbance at 695 nm and expressing the antioxidant activity as μg of α-tocopherol g^{-1} DW.

The ABTS assay (TE antioxidant capacity assay TEAC) was done according to Ref. [30] with a few modifications. Solutions of 7 mM of ABTS (final concentration) and 2.45 mM ammonium persulfate (final concentration) in phosphate buffered saline (PBS) were mixed and left in the dark at room temperature for 12–16 h. Before use, the absorbance of $ABTS^{+}$ solution was fixed at 0.70 ± 0.02 at 734 nm, and diluted if necessary with PBS as reported in Ref. [25].

2.4. Mineral detection

Pepper samples were used to extract cations, including Na, K, Ca, and Mg, which were subsequently analyzed using ion chromatography (DIONEX ICS-1100, Thermo Fisher Scientific Waltham, MA, USA). For this analysis, 1 g of dry material was ashed in a porcelain capsule at a temperature of 550 °C for 6 h. The resulting ash was then subjected to acidification using a 1 M HCl solution (10 mL) at 100 °C for 30 min. The acidified solution was filtered using Whatman 1 filter paper, and the filtered solution was measured using an ion chromatograph with a 20 mM methane-sulfonic acid eluent. The concentration of Fe was determined separately using atomic absorption spectrophotometry (model 2380, PerkinElmer Co., Waltham, MA, USA). The amount of each cation was calculated based on its own standard curve. Furthermore, phosphorus (P) was measured using ion chromatography (DIONEX) and the results were compared with a multi-ion cation standard curve (Multi Ion Cation IC standard solution, Specpure®, Dionex) according to the method described by Ref. [31].

All solvents and reagents used in the analysis were obtained from Panreac (Barcelona, Spain).

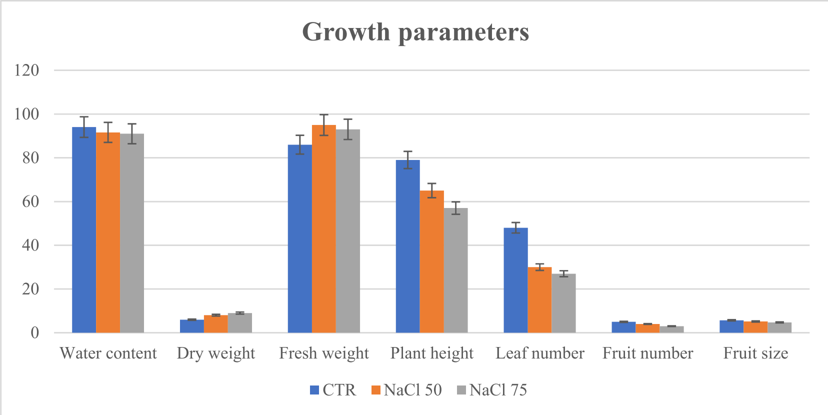

Fig. 1. Pepper growth parameters: water content (%) biomass as dry weight (%) and fresh weight (g/plant), leaf number (n), plant height (cm), fruit number (n) and fruit size (diameter, cm), detected at the end of the growth period (3 months) in soil without salinity (CTR, control), NaCl 50 (50 mM NaCl) and NaCl 75 (75 mM NaCl). Data are the mean ± standard error of three replicates of three independent experiments (n = 36).

Table 3
Correlation matrix (Pearson r) of pepper growth parameters: water content (WC, %) biomass as dry weight (DW, g/plant) and fresh weight (FW, g/plant), leaf number (n), plant height (cm), fruit number (n) and fruit size (diameter, cm), detected at the end of the growth period (3 months) in 0, 50 and 75 mM NaCl. Values in bold denote significant correlations with significance level 0.05. The green color denotes high correlation, the red color indicates no/or inverse correlation.

Variables	WC	DW	FW	PH	Leaf number	Fruit number	Fruit size
WC	1	.992	.919	.985	**0.998**	0.946	0.966
DW	.992	1	.862	**.999**	-0.983	-0.979	-0.991
FW	.919	.862	1	.836	-0.941	-0.741	-0.786
PH	.985	**.999**	.836	1	0.973	0.988	0.996
Leaf number	**.998**	.983	.941	.973	1	0.924	0.949
Fruit number	.946	.979	.741	.988	0.924	1	**0.998**
Fruit size	.966	.991	.786	.996	0.949	**0.998**	1

2.5. Statistical analysis

A statistical analysis was conducted to analyse the data sets, and analysis of variance (ANOVA) was performed. One-way ANOVA with Tukey's Honestly Significant Difference test was used to assess the effects of salinity on each of the measured parameters. The statistical software SYSTAT 13.2, Inc. Richmond, CA USA, a powerful statistical analysis and graphics software for Windows 7, was utilized for all the statistical analyses. Effects were considered significant at a p-value of ≤ 0.05. For single phenolic acids and single flavonoids (data presented in Tables 5 and 6), effects were considered significant at a p-value of ≤ 0.01, while for the other parameters, effects were significant at a p-value of ≤ 0.05. Principal Component Analysis (PCA) was employed to analyse the datasets. PCA was also applied to process the results obtained from the Ultra-Fast Gas Chromatography (UFGC), selecting features that exhibited the highest discriminatory power between samples. For visualization purposes, the AlphaSoft 2020 version 7.2.5 (Alpha MOS, Toulouse, France), the native UFGC program, was used.

3. Results and discussion

The experimental data unveiled the varied effects of NaCl salinity on the growth parameters of pepper plants, with the extent of impact dependent on both the salt concentration and the specific parameter under analysis. Notably, the water content as well as the

Table 4
Correlation matrix (Pearson r) of sodium, potassium, calcium and magnesium in bell pepper fruits. Values in bold denote significant correlations with significance level 0.05. The green color denotes high correlation, the red color indicates no/or inverse correlation.

Variables	Sodium	Potassium	Magnesium	Calcium
Sodium	**1**	-0.185	**0.999**	**1.000**
Potassium	-0.185	**1**	-0.222	-0,165
Magnesium	**0.999**	-0.222	**1**	**0.998**
Calcium	**1.000**	-0.165	**0.998**	**1**

Table 5
Total proteins (TPRO, mg g^{-1} DW); total carotenoids (TCAR, μg 100 g^{-1} DW); lycopene (LIC, mg 100 g-1 DW); total carbohydrates (TCARB, mg glucose g^{-1} DW); total phenols (TPHE, μg GAE * g^{-1} DW); total flavonoids (TFLA, μg quercetin g^{-1} DW); vitamin A (VIT A, μg retinol 100 g^{-1} DW); vitamin C (VIT C, mg ascorbic acid g^{-1} DW.); vitamin E (VIT E, mg alpha-tocopherol 100 g^{-1} DW.); total antioxidant capacity (TAC, mg α-tocopherol *100 g^{-1} d.w.); 2,2-diphenyl-1-picryl-hydrazyl (DPPH, % inhibition); 1,1-diphenyl-2-picrylhydrazyl (DPPH radical, μM Trolox g^{-1} dawn.) 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid,(ABTS, mg Trolox 100 $^{-1}$ g dw) *Different letters in the same row indicate significant difference at $p \leq 0.05$, ANOVA with Tukey post hoc test.

ID	CTR	NaCl 50 mM	NaCl 75 mM
TPRO	1.0^{c*}	1.3^{b}	1.5^{a}
TCAR	270^{b}	310^{a}	300^{a}
LIC	14^{c}	19^{b}	23^{a}
TCARB	2.2^{b}	2.4^{ab}	2.6^{a}
TPHE	21095^{c}	24015^{b}	24198^{a}
TFLA	9295^{a}	5999^{c}	61450^{b}
VITA	29.2^{c}	57.7^{a}	43.3^{b}
VITC	1.16^{a}	1.0^{a}	1.0^{a}
VIT E	0.32^{c}	0.5^{a}	0.6^{a}
TAC	1.8^{b}	1.9^{b}	2.1^{a}
DPPH %	32.7^{b}	34.6^{a}	33.3^{ab}
DPPH	4.1^{b}	4.6^{a}	4.2^{b}
ABTS	49^{a}	45^{b}	46^{b}

dry and fresh weight of the plants, did not show significant changes in response to the salinity treatments of 50 mM and 75 mM NaCl. In contrast, plant height exhibited a reduction, which further increased with higher NaCl concentrations. The leaf number also significantly decreased under salinity compared to the control, indicating a negative impact on leaf development. However, the fruit number and size were not significantly affected by NaCl when compared to the control (Fig. 1), suggesting that the plants prioritized their energy allocation towards fruit production rather than leaf growth. The lack of changes in biomass and water content could be attributed to the presence of certain biomolecules that act as osmolytes, protecting the plants from dehydration under salinity stress. Moreover, the increase in potassium, magnesium, and calcium, which have physiological regulatory roles, contributed to biomass production, as reported in other crops by Ref. [32] and Thomas et al. (2017). The Pearson matrix data revealed a positive correlation between water content and all the growth parameters, except for dry and fresh weight, with the strongest correlation observed with the leaf number (Table 3). Although tissue water content is an under-explored plant functional trait, it plays a fundamental and regulatory role in plant metabolic reactions, directly influencing photosynthetic rates, productivity, and the carbon cycle [33]. Furthermore, crop growth is known to be influenced by the competition among certain ions, which can lead to mineral imbalances or deficiencies, thereby affecting important metabolic pathways associated with crop growth.

NaCl had a significant impact on ion uptake in pepper plants, resulting in higher levels of sodium in pepper fruits treated with 75 mM NaCl, along with unexpected increases in calcium and magnesium concentrations at the highest salt concentration (Fig. 2). In contrast, when treated with 50 mM NaCl, there was a lower accumulation of sodium and a higher potassium content compared to the control, while the concentrations of magnesium and calcium remained unchanged (Fig. 2). The Pearson matrix data revealed a strong positive correlation between sodium, magnesium, and calcium, as well as a negative inverse correlation between sodium and potassium (Table 4). These findings support the hypothesis that sodium stimulates cation exchanges at the soil level, causing the release of adsorbed cations on the colloid surface, with higher sodium concentrations leading to greater cation mobility, as previously demonstrated by Ref. [15]. In soils the effect of increased sodium adsorption is particularly noticeable. With the rising concentration of sodium ions in the soil solution, these ions compete with other cations like calcium (Ca) for binding sites on the soil colloids. As a result, sodium ions can displace calcium ions from the soil colloids, leading to the release of calcium into the soil solution [34]. The changes in ion availability and cation exchange that occur due to increased sodium adsorption and calcium release can impact plant nutrition with an increased magnesium and calcium uptake by plant roots, calcium is particularly important for cell wall structure integrity in plants (Endo.

Table 6
Correlation matrix (Pearson r) between total proteins (TPRO, mg g-1 DW); total carotenoids (TCAR, µg 100 g-1 DW); lycopene (LIC, mg 100 g-1 DW); total carbohydrates (TCARB, mg glucose g-1 DW); total phenols (TPHE, µg GAE * g-1 DW); total flavonoids (TFLA, µg quercetin g-1 DW); vitamin A (VIT A, µg retinol 100 g-1 DW); vitamin C (VIT C, mg ascorbic acid g-1 DW.); vitamin E (VIT E, mg alpha-tocopherol 100 g-1 DW.); total anti-oxidant capacity (TAC, mg α-tocopherol *100 g-1 d.w.); 2,2-diphenyl-1-picrylhydrazyl (DPPH, % inhibition); 1,1-diphenyl-2-picrylhydrazyl (DPPH radical, µM Trolox g-1 DW); 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid, (ABTS µM Trolox g-1 DW). The number in bold denote significant and positive correlation.

Variables	TPRO	TCAR	LIC	TCARB	TPHE	TFLA	VITA	VITC	VIT E	TAC	DPPH	ABTS
TPRO	**1**	0.795	**0.999**	**0.993**	**0.937**	0.770	0.592	−0.888	**0.970**	**0.945**	0.416	−0.795
TCAR	0.795	**1**	0.763	0.721	**0.957**	0.226	0.959	−0.985	**0.919**	0.553	**0.882**	−1
LIC	**0.999**	0.763	**1**	**0.998**	**0.918**	0.802	0.550	−0.863	**0.956**	**0.960**	0.369	−0.763
TCARB	**0.993**	0.721	**0.998**	**1**	**0.891**	**0.838**	0.495	−0.829	**0.936**	**0.976**	0.309	−0.721
TPHE	**0.937**	**0.957**	**0.918**	**0.891**	**1**	0.500	**0.836**	−0.993	**0.994**	0.772	0.707	−0.957
TFLA	0.770	0.226	0.802	**0.838**	0,.500	**1**	−0.058	−0.391	0.592	**0.937**	−0.260	−0.226
VITA	0.592	**0.959**	0.550	0.495	**0.836**	−0.058	1	−0.896	0.770	0.295	**0.979**	−0.959
VITC	−0.888	−0.985	−0.863	−0.829	−0.993	−0.391	−0.896	1	−0.973	−0.689	−0.788	**0.985**
VIT E	**0.970**	**0.919**	**0.956**	**0.936**	**0.994**	0.592	0.770	−0.973	1	**0.837**	0.624	−0.919
TAC	**0.945**	0.553	**0.960**	**0.976**	0.772	**0.937**	0.295	−0.689	**0.837**	1	0.095	−0.553
DPPH	0.416	**0.882**	0.369	0.309	0,.707	−0.260	**0.979**	−0.788	0.624	**0.095**	1	−0.882
ABTS	−0.795	−1.000	−0.763	−0.721	−0.957	−0.226	−0.959	0.985	−0.919	−0.553	−0.882	**1**

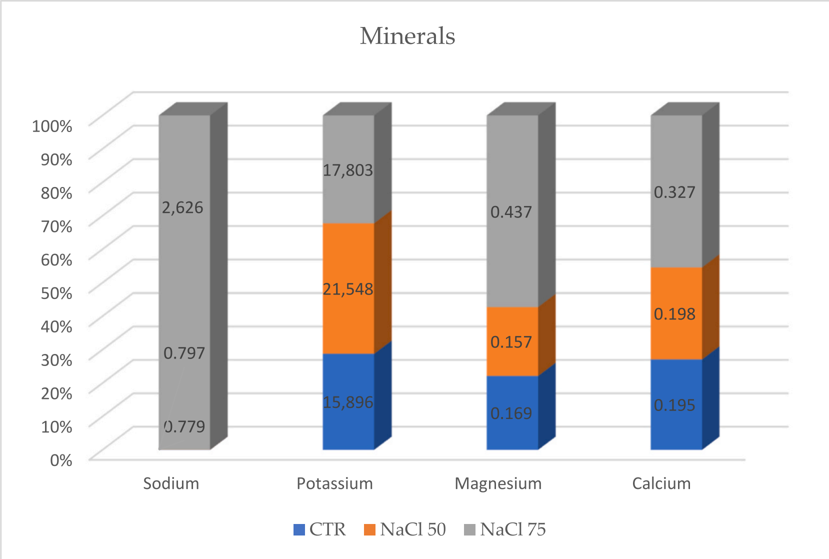

Fig. 2. Sodium, potassium, calcium, magnesium in bell pepper fruits cultivated with 0, 50 and 75 mM NaCl. Data are the mean of three replicates of three independent experiments.

Table 7
Single phenolic acids of pepper cultivated in soils without salinity (CTR) and with 50 mM NaCl and 75 mM NaCl.

	CTR	NaCl 50 mM	NaCl 75 mM
	mg/g SS	mg/g SS	mg/g SS
Phenolic acids			
Gallic	35.2^{a*}	11.3^{c}	25.1^{b}
Protocatechuic	0.15^{b}	1.75^{a}	ND$^{\&}$
Syringic	ND	1.90	ND
***p*-coumaric**	0.15^{b}	0.76^{a}	0.14^{b}
***m*-coumaric**	0.06^{b}	0.38^{a}	ND
***o*-coumaric**	ND	ND	0.09
Trans-cinnamic	0.06^{b}	0.15^{a}	0.09^{b}
3-hydroxycinnamic	0.09	ND	ND
Trans-4-hydroxycinnamic	6.1^{b}	8.76^{a}	3.89^{c}
Synaptic	3.0^{a}	2.28^{b}	0.68^{c}
2,5 dihydroxy-benzoic	ND	0.04^{a}	0.017^{b}
Caffeic	0.21^{a}	0.15^{b}	0.068^{c}
Chlorogenic	ND	0.08	0.034
Ferulic	0.06^{b}	0.08^{a}	0.085^{a}
Ellagic	0.03^{c}	0.38^{a}	0.119^{b}
Vanillic	ND	2.29^{a}	1.79^{b}

*Different letters in the same row indicate significant differences among the treatments at $p \leq 0.01$.
§ND = below the detection limit.

It is worth noting that [35] found that sodium is generally not accumulated in the above-ground organs of pepper, suggesting that the decrease in biomass production (plant height and leaf number) observed in our study could be attributed to osmotic stress or ion toxicity at the root level. The salinity caused by NaCl, as demonstrated by Baby and Jin (2011) and Asharaf and Harris (2013), can induce various secondary effects in plant metabolism, including the overproduction of reactive oxygen species (ROS).

In response to salinity stress, plants activate robust antioxidant systems, encompassing both enzymatic and non-enzymatic components. Our data revealed an increase in total carbohydrates and total proteins as salinity stress intensified (Table 5), suggesting that these enhancements in primary metabolites were driven by their role as osmolytes [36]. Total carotenoids, including lycopene, along with phenolic compounds, hold significant value in promoting health benefits and are essential components of human and animal diets. These metabolites are influenced by genetic, environmental, and agronomic factors [37]. The augmentation of plant secondary metabolites, which are synthesized for defence and protection purposes [38], leads to the production of high-quality fruits that contribute to human health. Our data demonstrated a substantial increase in lycopene, vitamin A, vitamin E, and total phenols in response to salinity, indicating an improvement in fruit quality. However, flavonoids and vitamin C exhibited a decrease. The Pearson matrix data revealed positive correlations among the biomolecules and antioxidant compounds that increased in the presence of salinity, as they possess well-established antioxidant and osmoprotective roles. Furthermore, these compounds exhibited positive

Table 8
Single flavonoids detected in sweet bell pepper cultivated in soils without salinity (CTR) and with 50 mM NaCl and 75 mM NaCl. *Different letters in the same row.

ID	CTR	NaCl 50 mM	NaCl 75 mM
	mg/g SS	mg/g SS	mg/g SS
Flavonoids	ND§	ND	
Procyanidin 2	0.212c*	0.838a	0.254b
Pelargonidine	1.364	ND	ND
Cyanidine 3 O-glucoside	0.091c	1.524a	0.339b
Catechin	ND	0.114b	0.847a
Epicatechin	0.061c	0.495a	0.337b
Delphinidin	0.152b	0.343a	0.102c
Myricetin	ND	ND	0.932
Luteolin	0.153b	0.190a	0.085c
Punicalagin	0.061a	ND	0.017b
Naringin	ND	0.229a	0.153b
Apigenin-7-neohesperosside	0.364b	0.381a	0.220c
Quercetin	2.121a	0.267b	0.068c
Quercitin-3 beta-D glucoside	14.152a	0.076b	ND
Kaempferol	0.121b	1.524a	0.102c
Procyanidin 1	2.727b	3.048a	1.525c
Vicenin 2	3.333a	2.286b	1.102c
Erythrocin	ND	0.762a	0.237b
Rutin	ND	0.533b	0.847a
Apigenin	0.152c	0.762b	1.017a

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Apigenin	0.152c	0.762b	1.017a

correlations with total antioxidant capacity (TAC) (Table 6).

Among the single phenolic acids analyzed, 50 mM NaCl-treated peppers exhibited higher abundance compared to both the control and 75 mM NaCl-treated peppers. The most abundant single acids, in descending order, were *trans*-4-hydroxycinnamic, synaptic, vanillic, syringic, protocatechuic, and ellagic (Table 7). These phenolic acids are known to possess significant health-promoting properties. For instance, ellagic acid has been shown to reduce cholesterol accumulation (Teklic et al., 2020), while vanillic acid has demonstrated efficacy in reducing heart attack size and improving ventricular function in isolated rat hearts subjected to ischemia/reperfusion [39]. Ferulic acid has been identified as an enhancer of insulin sensitivity and has shown benefits in reducing high blood pressure and preventing vascular remodeling in a high carbohydrate, high-fat diet model [40]. Interestingly, at the highest salinity concentration, there was a lower presence of single phenolic acids, although five of them (chlorogenic, ferulic, 2-5dihydroxy benzoic, ellagic, and vanillic) with notable anti-inflammatory, antioxidant, and anticarcinogenic properties were still present in greater amounts compared to the control (Table 8).

Regarding the single flavonoids, a significantly greater quantity was observed in peppers treated with 50 mM NaCl, followed by those treated with 75 mM NaCl, compared to the control. Erythrocin, rutin, catechin, and naringin were exclusively detected in the salt-treated fruits. Notably, kaempferol and procyanidin, known for their significant effects in reducing cardiovascular disease, exhibited the highest abundance, being ten times more prevalent in the peppers treated with 50 mM NaCl. These specific flavonoids have been found to exert beneficial effects primarily through their ability to reduce the oxidation of low-density lipoproteins, thereby improving lipid profiles and inducing vasodilation, which regulates apoptotic processes in the endothelium (Table 6). Numerous studies [41–44] have provided evidence that these positive effects, including their anti-inflammatory role, can be attributed to the potent antioxidant properties exhibited by these flavonoids.

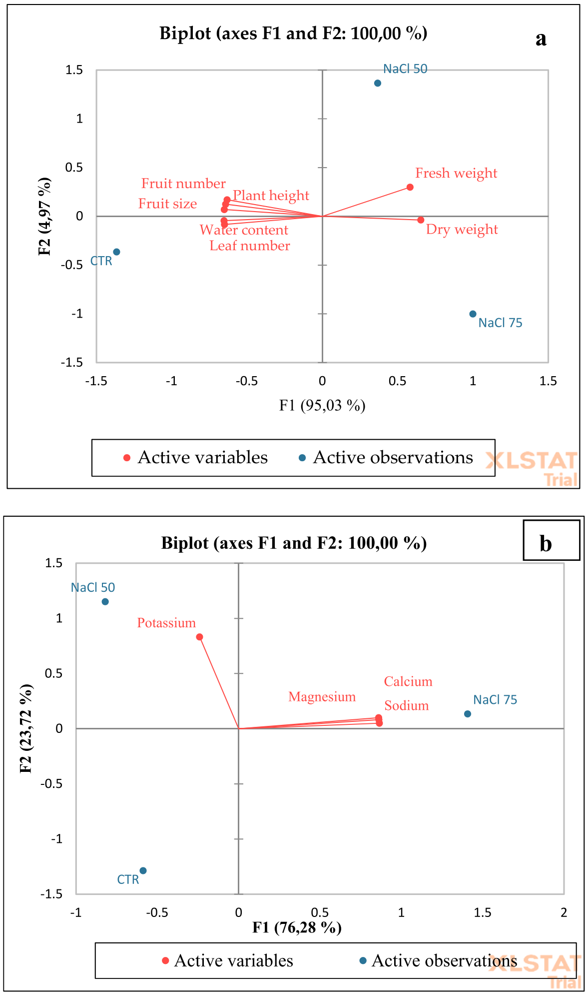

Fig. 3. PCA (principal component analysis) diagram of growth parameters (a) and minerals (b) of pepper cultivated in soils without salinity (CTR) and with 50 mM NaCl and 75 mM NaCl.

A principal component analysis (PCA) was conducted to visually explore the relationship between the treatments and sensory descriptive attributes, represented by the growth parameters. The first principal component (PC1), accounting for 95.03 % of the variance, and the second principal component (PC2), accounting for 4.97 % of the variance, were plotted in Fig. 3A. The data revealed that fresh weight (FW) was primarily influenced by the 50 mM NaCl treatment, while dry weight was more affected by the 75 mM NaCl treatment. Water content and leaf number were positively correlated with the absence of salinity (CTR), as shown in Fig. 3A.

Regarding cation content, PC1 explained 76.28 % of the variance, while PC2 explained 23.72 %, collectively accounting for 100 % of the total variance (Fig. 3B). The data indicated a correlation between the 50 mM NaCl treatment and potassium levels, whereas calcium, magnesium, and sodium were more strongly associated with the 75 mM NaCl treatment.

In terms of bioactive compounds (Fig. 4), the data revealed different correlations between salinity and important biocompounds, depending on the NaCl concentration. The 50 mM NaCl treatment showed positive correlations with vitamin A, total phenols, total carotenoids, and DPPH (a measure of antioxidant activity). On the other hand, the 75 mM NaCl treatment showed positive correlations with primary metabolites, total flavonoids, lycopene, and total antioxidant capacity. The absence of salinity (CTR) was only correlated with vitamin C and ABTS (another measure of antioxidant activity).

In terms of single flavonoids (Fig. 5a), punicalagin was found to have no correlation with the treatments, while all other single flavonoids were mainly correlated with the absence of salinity (CTR), 50 mM NaCl, and to a lesser extent with 75 mM NaCl. Flavonoids that showed stronger correlations with the highest salinity concentration (75 mM NaCl) were apigenin, rutin, catechin, and myricetin.

Fig. 5b provided additional insights, where the top-right quadrant represented the association of NaCl 50 mM with important single phenolic acids. The top-left quadrant corresponded to the absence of salinity (CTR) and showed correlations with caffeic acid, hydroxycinnamic acid, synaptic acid, and gallic acid. On the other hand, in the negative quadrant on the left, *o*-coumaric acid was associated with 75 mM NaCl. Some phenolic acids in the negative quadrant on the right were not influenced by any treatment.

In summary, our findings have contributed a novel perspective to the existing body of knowledge, shedding light on our

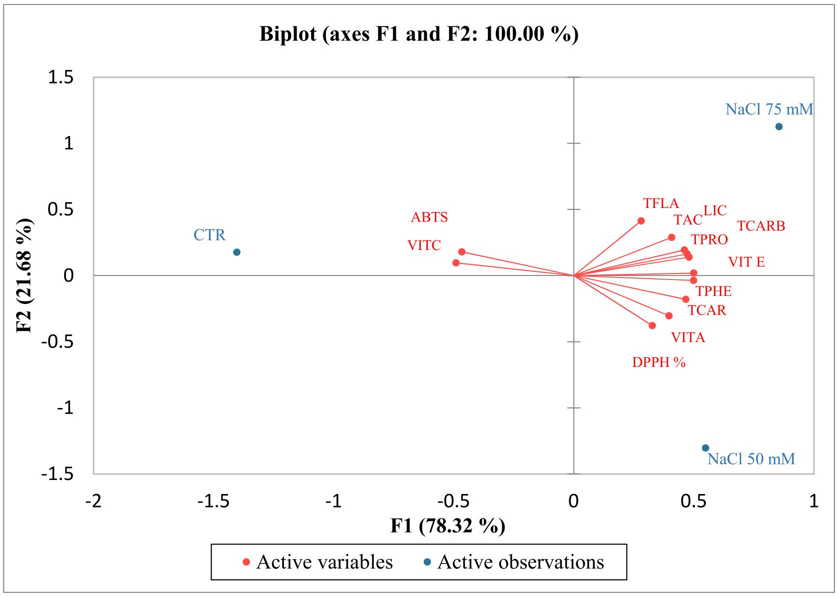

Fig. 4. PCA (principal component analysis) diagram of biomolecules, antioxidant compounds and antioxidant activities of pepper cultivated in soils without salinity (CTR) and with 50 mM NaCl and 75 mM NaCl.

observations that NaCl salinity exerts a positive influence on pepper metabolism. This stimulation is evident in the increased synthesis of bioactive compounds and heightened antioxidative activities, particularly notable at a concentration of 50 mM NaCl. This study's uniqueness lies in its departure from prior research conducted on inert substrates like perlite and coconut fiber, or involving lower NaCl concentrations (15, 20, 30, and 40 mM). These earlier studies have consistently demonstrated a pronounced detrimental impact of salinity on factors such as marketability, total and individual organic acid content, as well as sugar content [17,45–57].

4. Conclusions

To summarize, while plant height and leaf number experienced adverse effects, the salinity had no impact on the quantity and dimensions of fruits in pepper plants. This intriguingly indicates a resource allocation shift towards bolstering fruit production. Of particular interest, exposure to NaCl triggered the synthesis of diverse secondary metabolites recognized for their favorable influence on various target organs, presenting a multitude of benefits in combating human ailments, including specific types of cancer and cardiovascular disorders. These findings underscore the capacity of salinity to activate secondary metabolic pathways, culminating in the generation of fruits enriched with potent bioactive compounds. This proves especially advantageous in regions grappling with water or soil salinization constraints, where the cultivation of salt-sensitive crops is limited. Consequently, this study emerges as a valuable contribution toward elevating pepper productivity and enhancing the economic viability for farmers in marginal areas.

Additional information

No additional information is available for this paper.

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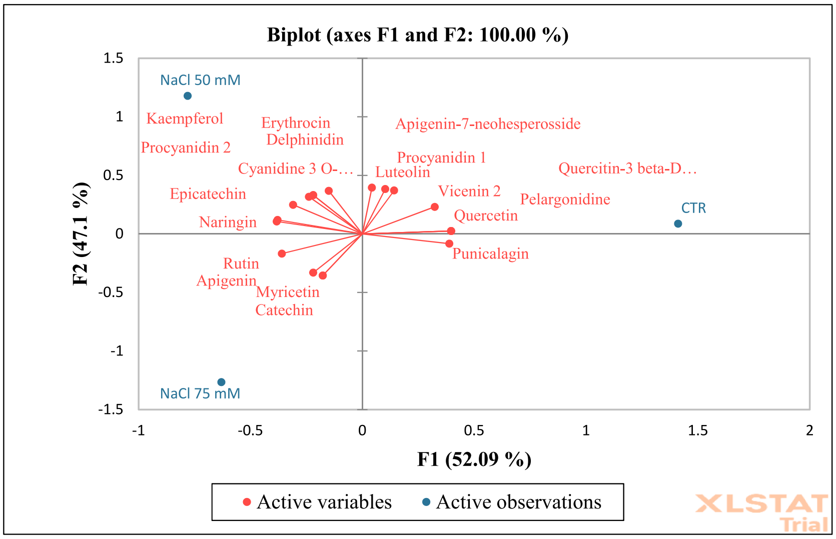
CRediT authorship contribution statement

F. Marra: Formal analysis, Investigation, Methodology. **A. Maffia:** Methodology, Software, Writing – original draft. **F. Canino:** Validation. **B. Petrovicova:** Formal analysis, Software, Validation. **C. Mallamaci:** Investigation, Validation. **Mt Russo:** Writing – original draft. **Muhammad Iftikhar Hussain:** Writing – original draft. **A. Muscolo:** Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.



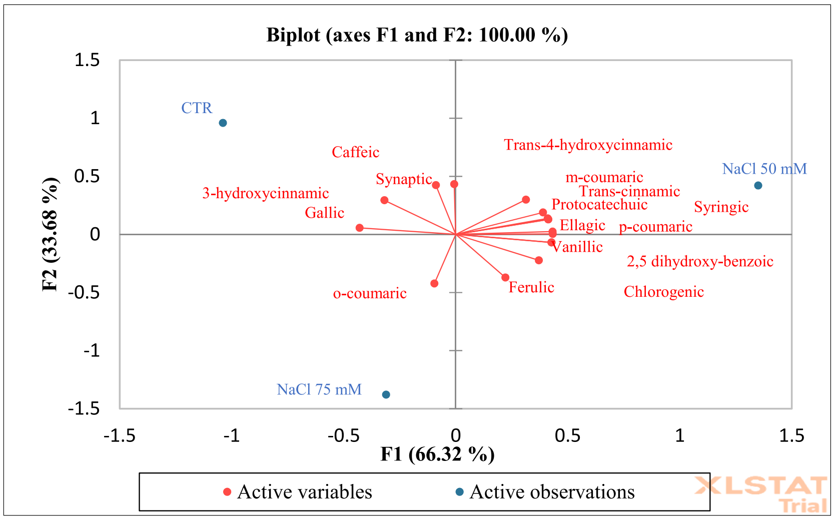


Fig. 5. PCA (principal component analysis) diagram of single phenolic acids (a) and single flavonoids (b) of pepper cultivated in soils without salinity (CTR) and with 50 mM NaCl and 75 mM NaCl.

Declaration of competing interest

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

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