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Production of nutraceutical compounds from *Cannabis indica* by different integrative treatments in nutritive solution

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ABSTRACT

Cannabis indica is a multipurpose species rich in phytochemical compounds with nutraceutical and medicinal properties. Mineral nutrition has been shown to be an important factor for the synthesis of these molecules. However, the influence of ion balances with signaling and stimulating molecules applied through the nutrient solution on the synthesis of metabolites has not been studied. Therefore, the purpose of this work was to delimit the impact of ion balances complemented with acetylsalicylic acid (ASA) and phenylalanine (Phe) on the development of the plant and on the production of nutraceutical compounds. Nine treatments were evaluated: 12 $mmol_c$ L^{-1}; 15 $mmol_c$ L^{-1}; 15 $mmol_c$ L^{-1} + 20 mg L^{-1} ASA; 15 $mmol_c$ L^{-1} + 500 mg L^{-1} Phe; 15 $mmol_c$ L^{-1} + 20 mg L^{-1} ASA + 500 mg L^{-1} Phe; 19 and 20 $mmol_c$ L^{-1}; 19 and 20 $mmol_c$ L^{-1} + 20 mg L^{-1} ASA; 19 and 20 $mmol_c$ L^{-1} + 500 mg L^{-1} Phe; 19 and 20 $mmol_c$ L^{-1} + 20 mg L^{-1} ASA + 500 mg L^{-1} Phe in a randomized block design under controlled conditions. The commercial solution (12 $mmol_c$ L^{-1}) performed better in terms of quercetin production (1878 mg 100 g^{-1}) in inflorescences, accompanied by an antagonism for Ca. The Hoagland solution (19 $mmol_c$ L^{-1}) obtained better vegetative development with higher heights (9.9 - 10.8 cm) and SPAD indices (50.1 - 52.8). The combination of Hoagland and Steiner generated high levels of foliar potassium (7.74 to 8.86 %) due to cationic antagonisms, but produced lower concentration of quercetin. Supplementation of the nutrient solution promoted higher leaf biomass in treatments 3 and 8 with 8.3 g of dry matter, and improved calcium absorption in treatments 3 and 5.

Keywords: Hemp, phenylalanine, ionic balances, acetylsalicylic acid, flavonoids.

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INTRODUCTION

Cannabis (*Cannabis indica*) has been utilized by Central Asian countries from the Neolithic period to the present (Booth and Bohlmann, 2019). Its importance lies in being a multipurpose species for various sectors, including industry, environment, livestock, and pharmaceuticals. The latter is particularly relevant due to its potential for stimulating phytochemical compounds with nutraceutical and medicinal properties beneficial to human health (Andre et al., 2016). These molecules result from secondary metabolism through the following biochemical pathways: shikimic acid, polyketide, and plastidial (Gordo, 2018). Key enzymes involved in these processes include phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), chalcone synthase (CHS), and polyketide synthase (PKS) (Bautista et al., 2021).

On one hand, previous research has correlated the impact of mineral nutrition with the activation of the aforementioned pathways. Nitrogen is essential for the

synthesis of amino acids and proteins, directly influencing photosynthesis and growth (Song et al., 2023). Saloner and Bernstein (2021) demonstrated that increasing nitrogen doses from 30 to 320 mg L^{-1} in the nutrient solution resulted in a 60–70% decrease in cannabinoid concentration compared to the control. A similar effect was observed when the NH_4^+/NO_3^- ratio exceeded 30%, as the plant is highly sensitive to ammonium toxicity (Saloner and Bernstein, 2022b). The optimal average value is 160 mg L^{-1} N with an NH_4^+/NO_3^- ratio of 0 to 30%.

Phosphorus is an essential element for the formation of nucleic acids, nucleoproteins, phospholipids, and energy storage molecules, as well as for stimulating metabolomic pathways (Kochhar and Gujral, 2020). Shiponi and Bernstein (2021) stated that phosphorus application linearly modifies cannabinoid production and determined that a concentration of 30 to 90 mg L^{-1} of P in solution is required for optimal plant development without affecting cannabinoid synthesis. Potassium is an essential macronutrient that plays a crucial role in various physiological processes, acting as an activator of catalytic enzymes during photosynthesis and respiration (Mengel and Kirkby, 2000). Saloner and Bernstein (2022a) outlined the effects of potassium on the production of medicinal compounds, noting that concentrations below 15 mg L^{-1} indicate visual deficiency symptoms, while concentrations above 175 mg L^{-1} resulted in high inflorescence yields but significantly decreased the production of cannabinoids and terpenes. Magnesium is an essential element whose primary function is to be a structural component of chlorophyll, as well as to stabilize ribosome structures and activate enzymes such as RuBP carboxylase and PEP carboxylase (Kochhar and Gujral, 2020). Optimal development requires a magnesium supply of 35–70 mg L^{-1} in solution; lower concentrations lead to visual deficiency symptoms, physiological disorders, and a 28% reduction in the total biomass of *Cannabis sativa* (Morad and Bernstein, 2023).

On the other hand, Phe has been studied for its high potential in agriculture as a stimulant that enhances plant tolerance and resistance. Phe is an essential aromatic amino acid with neutral and nonpolar properties (Das et al., 2018). It serves as a precursor to the enzyme phenylalanine ammonia-lyase (PAL) in the phenylpropanoid pathway and the shikimic acid pathway (Aghdam et al., 2019). Exogenous applications of Phe have been shown to increase the concentration of metabolites such as phenols, flavonoids, anthocyanins, and lignin in harvested crops (Das et al., 2018).

Another molecule with hormonal effects capable of influencing the plant metabolomic system is salicylic acid (SA). Within this group, there is an analog known as ASA, which performs the same function as salicylic acid despite being of synthetic origin and easily accessible in Mexico. SA is a hormone associated with plant defense and, therefore, promotes immunity against pathogens (Ding and Ding, 2020). According to Tucuch-Haas et al. (2021), exogenous application of ASA to foliage has been documented to regulate stomatal opening and closing, promote systemic acquired resistance (SAR), induce cell apoptosis, and stimulate metabolite production through the expression of regulatory genes in the aforementioned metabolic pathways.

However, little research has been conducted on the impact of ionic balance and the described molecules on the secondary metabolism of *C. indica*. For this reason, the present study aimed to determine the impact of ionic balance, supplemented with Phe and ASA in the nutrient solution, on the development of *C. indica* (hemp) and the production of phenols and total flavonoids.

MATERIALS AND METHODS

Plant material

The hemp seeds (*C. indica*) used in this research were of the regular (dioecious) type, intended for medicinal purposes. They were donated by producers from the community of La Cercada, Dolores Hidalgo, Guanajuato, located at 21º 11' 10.87'' N latitude and 100º 53' 40.79'' W longitude.

Seedling management

The seeds were soaked the night before sowing. The most vigorous seeds were selected and sown in polystyrene trays with 72 cavities. The substrate used was a 50:50 mixture of peat and perlite. Watering was done manually according to the substrate's needs. The seedlings were placed under artificial lighting at an intensity of 150 µmol s^{-1} m^{-2} using 100 W Citizen COB LED lamps emitting white light, positioned 30 cm above the plants. The photoperiod consisted of 16 hours of light and 8 hours of darkness throughout this stage. The seedling phase lasted for 30 days.

Treatment design

Nine treatments were evaluated (Table 1). Some treatments were supplemented with 20 mg L^{-1} acetylsalicylic acid (ASA) (treatments 3 and 7), 500 mg L^{-1} phenylalanine (Phe) (treatments 4 and 8), and a combination of 20 mg L^{-1} ASA + 500 mg L^{-1} Phe (treatments 5 and 9), which were provided in the nutrient solution during the flowering stage.

As a source of micronutrients, all treatments included Fe^{2+} (2 mg L^{-1}), Mn^{2+} (1 mg L^{-1}), Zn^{2+} (0.1 mg L^{-1}), Cu^{2+}

Table 1. Design of treatments applied via nutrient solution under different total ionic balances and biostimulants in hemp (*Cannabis indica*).

Treatment[†]	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	RIT[††]	Stage [¶]	Biostimulant [¶¶]
				($mmol_c\ L^{-1}$)						
1	7.5	1.0	3.5	1.5	3.0	5.0	2.5	12.0	V	A
	9.0	1.5	1.5	-	5.0	4.0	3.0	12.0	F	A
2	10.0	1.0	4.0	0.5	4.5	7.0	3.0	15.0	V, F	A, A
3	10.0	1.0	4.0	0.5	4.5	7.0	3.0	15.0	V, F	A, P
4	10.0	1.0	4.0	0.5	4.5	7.0	3.0	15.0	V, F	A, P
5	10.0	1.0	4.0	0.5	4.5	7.0	3.0	15.0	V, F	A, P
6	14.0	1.0	4.0	1.0	6.0	8.0	4.0	19.0	V	A
	12.0	1.0	7.0	-	7.0	9.0	4.0	20.0	F	A
7	14.0	1.0	4.0	1.0	6.0	8.0	4.0	19.0	V	A
	12.0	1.0	7.0	-	7.0	9.0	4.0	20.0	F	P
8	14.0	1.0	4.0	1.0	6.0	8.0	4.0	19.0	V	A
	12.0	1.0	7.0	-	7.0	9.0	4.0	20.0	F	P
9	14.0	1.0	4.0	1.0	6.0	8.0	4.0	19.0	V	A
	12.0	1.0	7.0	-	7.0	9.0	4.0	20.0	F	P

[†]1: Trade balance; 2, 3, 4, and 5: Modified equilibrium (Saloner and Bernstein, 2022b); 6-V, 7-V, 8-V, and 9-V: Equilibrium (Hoagland and Arnon, 1938); 6-F, 7-F, 8-F, and 9-F: Equilibrium (Steiner, 1980). [††]Total ionic balance of anions and cations in the nutrient solution. [¶]V: Vegetative phase, F: Flowering phase. [¶¶]A: Without biostimulant, P: With biostimulant.

($0.1\ mg\ L^{-1}$), B^{2-} ($0.5\ mg\ L^{-1}$), and EDTA-chelated Mo^{2-} ($0.05\ mg\ L^{-1}$). A completely randomized block design (CRBD) was used, with three replications and a planting density of 9 plants/m². The nutrient solution was applied immediately after transplanting. Each experimental unit consisted of 4 L pots filled with the same substrate mixture used for sowing, following a "Sea of Green" (SOG) cultivation system.

During the vegetative phase (weeks 1-4), the photoperiod was set to 16 hours of light and 8 hours of darkness. During the flowering phase (weeks 5-12), the photoperiod was adjusted to 12 hours of light and 12 hours of darkness. Harvesting took place between 120 and 130 days after transplanting (DAT).

Sample pretreatment

All fresh and senescent foliar tissues were washed with distilled water to remove impurities. The foliar samples and inflorescences were dried in an oven at 50 ± 5 ºC until reaching a constant weight. The dried samples were ground using a 20-mesh sieve and homogenized for subsequent measurements, including total flavonoids, total phenols, and nutrient content.

Evaluation of physiological variables

Plant height

Height was measured using a tape measure from the base to the apex of the main stem at 30 days after transplanting (DAT) in each experimental unit before apical pruning.

SPAD indices

SPAD indices were determined using a SPAD 502 Minolta LTD in physiologically mature leaves at 30 and 60 DAT. Measurements were taken with five repetitions per experimental unit, and the obtained values were averaged.

Chlorophyll a and b

Chlorophyll determination was conducted following the methodology of Witham et al. (1971). Absorbance was measured at 645 nm and 663 nm for chlorophyll b and a, respectively, using a UV-visible spectrophotometer (10UV, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The obtained values were applied to the following equations:

- Chlorophyll a ($\mu g\ mL^{-1}$) = 12.21 × Abs 663 - 2.81 × Abs 645
- Chlorophyll b ($\mu g\ mL^{-1}$) = 20.13 × Abs 645 - 5.03 × Abs 663.

Biomass

Foliar tissue and inflorescences were harvested at 130 DAT. The samples were stored in paper bags at room temperature for two weeks. The dry plant parts were weighed to a constant weight using a precision balance.

Evaluation of nutraceutical compounds

Total flavonoids

The total flavonoid content in inflorescences was determined using the UV-VIS spectrophotometric method proposed by Bakar et al. (2009). A calibration curve was prepared using ten points (0 to 450 µg mL^{-1}) of quercetin (95%, Sigma-Aldrich).

Total phenols

The total phenol content in leaves was determined using the Folin–Ciocalteu spectrophotometric method, following the procedure described by Koch et al. (2015). From a stock solution of 1128 µg mL^{-1}, a series of dilutions were prepared: 112, 225, 338, 451, 563, 676, 789, 902, and 1015 µg mL^{-1} of gallic acid (98%, Sigma-Aldrich).

Nutrient content evaluation

The macronutrient content (N, P, K, Ca, and Mg) was determined using a wet digestion method with an H_2SO_4:$HClO_4$ (4:1, v/v) mixture. Digestion was carried out with 0.25 g of foliar sample, adding 4 mL of the acid mixture and 2 mL of H_2O_2. The digestate was diluted to 50 mL with distilled water and stored at 4°C until analysis. Nitrogen (N) was quantified using the micro Kjeldahl method (Fleck and Munro, 1965). Phosphorus (P) was determined by forming a vanadomolybdate complex and measuring absorbance using UV-VIS spectrophotometry at 420 nm. Potassium (K) was analyzed via flame photometry using a 410 flame photometer (Sherwood, Cambridge, UK), while calcium (Ca) and magnesium (Mg) were measured using atomic absorption spectrophotometry (GBC-SavantAA, Victoria, Keysborough, Australia).

Statistical analysis

The results for plant height, SPAD readings, chlorophyll a and b, foliar biomass and inflorescences, total flavonoids, total phenols, and nutrient concentration in foliar tissue were statistically analyzed using SAS Student (Statistical Analysis System). The analysis included orthogonal contrasts, multiple mean comparisons, and mixed models (Montesinos et al., 2022).

RESULTS

Evaluation of physiological variables

The results (Table 2) show that the evaluated ionic relationships, both alone and in combination with ASA and Phe, did not induce significant effects on SPAD indices during flowering, chlorophyll a and b levels, or inflorescence biomass.

Table 2. Average values of height, indices, photosynthetic pigments, and hemp (*Cannabis indica*) biomass under different nutrient solutions.

Treatment	Height (cm)	SPAD		Clorophyll		Biomass	
				a	b	foliage	flowers
		30 dat	60 dat	(mg g^{-1})		(g)	
1	9.35 ab	51.23 a	63.00 a	1.93 a	0.52 a	5.22 b	1.70 a
2	6.70 bc	46.53 ab	65.40 a	1.37 a	0.39 a	6.88 ab	3.41 a
3	8.30 abc	48.90 a	65.97 a	1.52 a	0.41 a	8.30 a	2.30 a
4	9.10 ab	46.70 ab	66.50 a	1.60 a	0.40 a	6.86 ab	3.49 a
5	6.10 c	47.83 ab	66.80 a	1.47 a	0.37 a	6.78 ab	2.35 a
6	9.90 a	52.80 a	66.57 a	1.21 a	0.31 a	6.73 ab	2.11 a
7	10.80 a	55.17 a	67.70 a	1.70 a	0.43 a	6.69 ab	1.78 a
8	10.35 a	50.13 a	66.37 a	1.56 a	0.39 a	8.29 a	1.21 a
9	9.20 ab	38.20 b	67.10 a	1.11 a	0.26 a	5.52 ab	2.28 a
p-value	0.0400	0.0423	0.8407	0.3199	0.1452	0.0400	0.6314
DMS	2.70	10.65	6.27	1.09	0.27	2.93	2.84

Different letters within rows indicate statistically significant differences (LSD, P ≤ 0.05).

During the vegetative stage, Treatment 7, corresponding to 19 mmolc L^{-1}, resulted in the greatest height and SPAD index, measuring 10.8 cm and 55.17, respectively, compared to solutions with total ionic balances of 12 and 15 mmolc L^{-1}. Treatments 3 and 8 (Bernstein + 20 mg L^{-1} ASA and Hoagland + Steiner + 500 mg L^{-1} Phe) produced the highest foliar biomass, both yielding 8.30 g of dry matter. In contrast, Treatment 1 promoted the lowest foliar biomass accumulation, with 5.22 g.

Table 3. Average concentration of secondary metabolites and macronutrients in the foliar tissue of hemp (*Cannabis indica*) under different ionic balances and biostimulation in a nutrient solution.

Treatment	Total Flavonoids†	Total Phenols††	N	P	K	Ca	Mg
	(mg 100 g^{-1})				(%)		
1	1878.00 a	438.53 a	2.61 a	0.26 a	5.03 d	0.24 ab	0.10 a
2	1340.22 bc	394.41 a	2.89 a	0.22 a	7.15 bc	0.23 abc	0.11 a
3	1339.48 bc	360.29 a	2.60 a	0.22 a	7.62 b	0.25 a	0.11 a
4	1483.19 ab	436.76 a	2.59 a	0.20 a	7.26 bc	0.22 bcd	0.10 a
5	1687.63 ab	382.65 a	2.71 a	0.23 a	6.22 c	0.25 a	0.09 a
6	1374.30 bc	316.76 a	2.82 a	0.22 a	8.19 ab	0.23 abcd	0.10 a
7	1229.75 bc	393.82 a	3.12 a	0.22 a	7.74 ab	0.21 cd	0.09 a
8	999.48 c	326.76 a	2.79 a	0.21 a	8.86 a	0.21 d	0.10 a
9	1308.05 bc	397.94 a	3.07 a	0.23 a	7.81 ab	0.24 ab	0.09 a
p-value	0.0499	0.4034	0.2674	0.7457	0.0020	0.0118	0.4409
DMS	457.68	114.05	0.4706	0.1223	1.101	0.0218	0.0177

Different letters within rows indicate statistically significant differences (LSD, $P \leq 0.05$).
† Total flavonoids quantified as total quercetin in inflorescences ($n = 3$).
†† Total phenols quantified as total gallic acid in mature leaves ($n = 3$).

Evaluation of nutraceutical compounds

According to Table 3, the evaluated treatments did not have a significant effect on the total phenol content in leaves. Treatment 1 (12 mmolc L^{-1}) exhibited the highest yield of nutraceutical metabolites, expressed as total flavonoids (1,878 mg per 100 g of quercetin) in flowers, and showed the highest trend in total phenol content (439 mg per 100 g of gallic acid) in leaves.

Evaluation of nutrient content

The average macronutrient concentrations (Table 3) showed no significant differences among treatments concerning RIT or the application of ASA and Phe for N, P, and Mg in foliar tissue. The highest potassium absorption was observed in Treatment 8 (20 mmolc L^{-1} + 500 ppm Phe), with an average concentration of 8.86%, while the lowest was recorded in Treatment 1 (12 mmolc L^{-1}), with 5.03% in foliar tissue. Treatments 3 and 5 resulted in the highest calcium absorption in foliar tissue, at 0.25%, whereas Treatments 7 and 8 exhibited the lowest values at 0.21%.

DISCUSSION

Interactions between nutrient solutions and physiological parameters

The behavior of plant height and SPAD variables can be attributed to the proportions of NO_3^- and NH_4^+ present in the nutrient solutions. Saloner and Bernstein (2022b) obtained similar effects by modifying the NH_4^+/NO_3^- ratio, determining that when this ratio exceeded 10%, total plant height and inflorescence length decreased due to NH_4^+ phytotoxicity in *C. sativa*. In Treatment 7, the NH_4^+/NO_3^- ratio was 7%, compared to 5% in Treatment 5 and 20% in Treatment 1. This effect can be explained by the role of nitrogen and its ionic form in determining plant size, as nitrogen is an essential component of proteins, porphyrins, and the enzyme Rubisco (Bevan et al., 2021). Although plants can absorb the reduced form of nitrogen to save energy during cellular denitrification and more efficiently form amino groups, they are also susceptible to ammonia toxicity within vacuoles (Yep and Zheng, 2020).

In Solutions 3 and 8, the presence of ASA and Phe enhanced foliar biomass accumulation. ASA is processed and converted into salicylic acid within the cell (Peng et al., 2017), and the evaluated dose (20 mg L^{-1}) improved CO_2 fixation during photosynthesis, resulting in a greater amount of structural carbon in the foliage of *C. indica*, as reported by Tucuch-Haas et al. (2020) for maize treated with 0.2 mg L^{-1} ASA. The positive effect of Phe application (500 mg L^{-1}) via the root system in *C. indica* can be attributed to the plant's ability to use it as a temporary nitrogen source. This behavior aligns with a study on *Populus* × *canescens*, in which partial or total substitution of inorganic nitrogen with Phe stimulated greater aerial and root biomass production (Jiao et al., 2018). However, it has been studied that other amino acids have the same property as Phe as a provisional source (Näsholm et al. 2009).

Interactions between nutrient solutions and nutraceutical compounds

During this research, the behavior of cannabinoids was not addressed because, at the time of laboratory determinations, the reference standards had import

restrictions in Mexico. When analyzing the trend of flavonoid data across treatments, it was observed that increasing the total ion concentration in the solution significantly reduced the quercetin concentration in *C. indica* flowers. This response may be influenced by the nutritional dynamics present in the plant's nutrient medium. Saloner and Bernstein (2020) proposed optimal values using response surface methodology for a specialized nutrient solution for *C. sativa*, with concentrations ranging from 11 mmolc L^{-1} of NO_3, 1 to 3 mmolc L^{-1} of H_2PO_4 (Shiponi and Bernstein, 2021), 4.5 mmolc L^{-1} of K (Saloner and Bernstein, 2022a), and 3 to 6 mmolc L^{-1} of Mg (Morad and Bernstein, 2023). These values successfully optimized primary growth while maintaining the synthesis of terpenes and therapeutic cannabinoids. However, in *C. indica*, the production of inflorescences with the highest nutraceutical quality was not observed. This contrasts with Treatment 1, based on the principle of electroneutrality, which resulted in a higher flavonoid concentration in the flowers compared to Treatment 2, which was designed using response surface methodology and yielded lower flavonoid concentrations in this hemp variety.

The group of treatments (2, 3, 4, and 5), according to orthogonal contrast analysis (data not shown), exhibited a synergy with ASA and Phe, possibly due to the overexpression of enzymatic genes activating the plastidial (MEP) and shikimic acid pathways. This led to abnormal enzymatic activity, as noted by Pott et al. (2019), who stated that the internal accumulation of Phe and ASA stimulates precursors of lignin, terpenes, and flavonoids, as well as precursors for phenylpropanoid synthesis. This phenomenon is related to the disruption of the natural balance between primary and secondary metabolism, shifting the demand and transport of sugars toward secondary metabolism to enhance the plant's defense mechanisms (Sun et al., 2019). Naturally, 30% of the fixed carbon is allocated to phenylpropanoid production (Pott et al., 2019).

The Hoagland (1938) and Steiner (1980) solutions were developed to enhance productivity and yield in high-value commercial vegetables. However, the results indicated that *C. indica* does not conform to the principles established by these solutions, as the modified treatments (6, 7, 8, and 9) did not promote the production of nutraceutical metabolites. On the contrary, the addition of ASA and Phe inhibited flavonoid synthesis, possibly because their potassium levels were higher than those in the other solution groups. Saloner and Bernstein (2022a) reported that elevated potassium levels could hinder the biosynthesis of certain secondary metabolites by altering enzymatic activity, potentially leading to excessive vegetative growth at the expense of defensive compound production. The data from this study align with that statement; however, there is no evidence to support the presence of antagonism between ASA and Phe in flavonoid production in *C. indica*.

Interactions between nutrient solutions and nutritional content

The explanation for these results may lie in the selectivity exerted by root membranes. Despite working with widely differing concentrations of nitrogen and magnesium in solution, there was no reciprocal absorption of these nutrients. Rengel et al. (2022) state that the primary site of selectivity in the absorption of cations and anions is the plasma membrane of the cells, where the lipid bilayer prevents the indiscriminate flow of ions and polar molecules from the apoplast to the cytoplasm (influx) and from the cytoplasm to the apoplast (efflux).

The results exceeded those reported by Cockson et al. (2019) for the 'T1' variety of *C. sativa* using Hoagland and Arnon (1938) as a nutrient medium throughout the production cycle. In their study, the average foliar potassium value was 2.85%, and as the concentration decreased to 0.41%, biomass was reduced by 27% compared to the control. Yep and Zheng (2020) tested lower potassium doses (1.9 and 2.9 mmolc L^{-1}) than those used in the present study on *C. sativa* cv. Mandarin and found an increase in the number of marketable inflorescences by 16% and 22%, associated with foliar potassium concentrations of 1.95% and 2.69%, respectively.

Furthermore, orthogonal contrast analysis (data not shown) indicated that potassium absorption was more significantly influenced by the RIT than by the effects of ASA and Phe. Therefore, foliar potassium dynamics are directly proportional to the ion's available concentrations in the nutrient solution. When comparing flavonoid content with foliar potassium levels, it was observed that as potassium concentration in the foliage increased, total flavonoid production in the inflorescences decreased. This confirms the premise of Saloner and Bernstein (2022a) that high potassium concentrations inhibit the production of certain secondary metabolites, leading to greater vegetative growth.

The treatment group analysis (data not shown) indicated that calcium absorption is more closely linked to signaling molecules than to the RIT. The impact of ASA and Phe on calcium assimilation lies in their ability to directly influence calcium metabolism by regulating specific ion transporters in cell membranes and enhancing cell permeability (Khan et al., 2015). This facilitates greater calcium ion uptake by the roots. The absorbed calcium followed an inverse relationship with the RIT, similar to the behavior of quercetin in inflorescences. However, when comparing the trends of potassium and calcium, an antagonistic effect can be inferred.

In agreement with these results, Yep and Zheng (2020)

found that an increase in potassium ions in solution reduced the concentration of divalent cations (calcium and magnesium) in the leaf tissue of *C. sativa*. This antagonism arises from competition among ions with similar properties (group, valency, and ionic radius) for transport sites (carrier proteins), as transporters are rarely specific and depend on ion concentrations in solution (Rengel et al., 2022). This highlights that potassium may have inhibited or blocked enzymatic activities in the secondary metabolic system in solutions combined with Hoagland and Steiner. In contrast, calcium exerted a synergistic effect on the metabolomic system of *C. indica* with Bernstein and commercial solutions.

This research supports the claims of Yep and Zheng (2020) and Bevan et al. (2021) regarding the taboos surrounding the empirical management of this crop's nutrition by commercial companies and technical advisors. It is common practice to apply excessive doses of phosphorus and potassium starting from the pre-flowering stage. However, evidence shows that this approach is counterproductive, as it promotes the allocation of sugars to primary pathways rather than secondary ones, resulting in raw materials of very low nutraceutical and medicinal quality. Consequently, Treatment 1 (12 mmolc L^{-1}) is recommended as the best-performing option for both stages of medicinal compound production, not only for its productivity but also for its contribution to sustainable cultivation.

CONCLUSIONS

The results showed that during the vegetative stage, Treatments 6, 7, and 8, based on Hoagland's solution, achieved better development, with plant heights of 9.9, 10.8, and 10.3 cm and SPAD indices of 52.8, 55.2, and 50.1, respectively. During the reproductive stage, the group of treatments (6, 7, 8, and 9) using the Steiner solution base generated high foliar potassium levels (7.74-8.86%) but resulted in lower metabolite production compared to other groups. Treatment 1 exhibited the highest quercetin concentration in inflorescences (1878 mg 100 g^{-1}) and the lowest potassium level (5%) in foliar tissue. The addition of ASA and Phe in the solution promoted greater foliar biomass in Treatments 3 and 8, with 8.3 g of dry matter, and improved calcium absorption in Treatments 3 and 5.

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