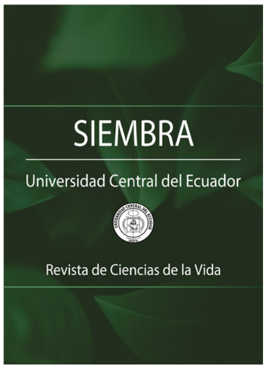
# *Evaluation of the Steiner nutrient solution applied to the cultivation of medical hemp flower (Cannabis sativa L.) var. Cherry Oregon, under greenhouse*




## *Evaluación de la solución nutritiva Steiner aplicada al cultivo de cáñamo medicinal (Cannabis sativa L.) var. Cherry Oregon, bajo invernadero*

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## Abstract

Proper nutritional management is crucial for the development and production of cannabinoids in the cultivation of *Cannabis sativa* var. Cherry Oregon. The objective of this research was to evaluate the effect of five concentrations of Steiner nutrient solution on the phenology of medicinal hemp, and to determine the optimal dosage of the nutrient solution for cannabinoid content (cannabidiol [CBD] and tetrahydrocannabinol [THC]). Research was conducted at the Instituto Nacional de Investigaciones Agropecuarias [INIAP], Estación Experimental Santa Catalina [EESC], in Ecuador, under greenhouse conditions. Five concentrations of Steiner nutrient solution (50, 75, 100, 125 and 150%). Agronomic variables such as plant height, diameter at collar height, yield, and cannabinoid content in flowers were evaluated. Significant effects ($p < 0.05$) were used. Agronomic variables such as plant height, diameter at neck height, yield, and cannabinoid content in flowers were evaluated. Significant effects ($p < 0.05$) were observed in plant height and diameter at neck height. Tetrahydrocannabinol (THC) remained at concentrations lower than 1%. Concentrations at 75% and 100% of the nutrient solution showed higher levels of cannabidiol (CBD) with 15.3% and 15.15%, respectively. The nutrient solution at 125% and 100% concentrations promoted greater growth in plant height and diameter. The 125% concentration also produced the highest dry biomass yield with 810 g $m^{-2}$ or 8.1 t $ha^{-1}$, surpassing the other concentrations. It is concluded that the optimal concentration of Steiner solution was 125% for accumulation of dry biomass, and 75 and 100% for cannabinoid production in flowers (CBD), complying with current regulations (< 1% THC).

**Keywords**: cannabinoids, hemp, nutrition, Steiner solution.

## Resumen

El adecuado manejo nutricional es crucial para el desarrollo y producción de cannabinoides en el cultivo de *Cannabis sativa* var. Cherry Oregon. Esta investigación tuvo por objetivo evaluar el efecto de cinco concentraciones de solución nutritiva Steiner en la fenología del cáñamo medicinal, y determinar la dosis óptima de la solución nutritiva para el contenido de cannabinoides (cannabidiol [CBD] y tetrahidrocannabinol

[THC]). La investigación fue realizada en el Instituto Nacional de Investigaciones Agropecuarias [INIAP], Estación Experimental Santa Catalina [EESC], en Ecuador, en condiciones de invernadero. Se emplearon cinco concentraciones de solución nutritiva de Steiner (50, 75, 100, 125 y 150%). Se evaluaron variables agronómicas como altura de planta, diámetro a la altura del cuello, rendimiento y contenido de cannabinoides en las flores. Se observó efecto significativo ($p < 0,05$) en altura de planta y diámetro del cuello. El THC se mantuvo < 1%. Concentraciones al 75% y 100% de la solución nutritiva mostraron niveles superiores de CBD con 15,3 y 15,15%, respectivamente. La solución nutritiva al 125 y 100% promovieron un mayor crecimiento en altura y diámetro a la altura del cuello. La concentración al 125% también produjo mayor rendimiento de biomasa seca con 810 g $m^{-2}$ o 8,10 t $ha^{-1}$, superando al resto de concentraciones. Se concluye que la concentración óptima de solución Steiner fue de 125% para acumulación de biomasa seca, y del 75 y 100% para la producción de cannabinoides en flores (CBD), cumpliendo con las regulaciones vigentes (< 1% de THC).

**Palabras clave:** cannabinoides, cáñamo, nutrición, solución Steiner.

## 1. Introduction

Hemp (*Cannabis sativa* L.) has gained global recognition for its medicinal properties and recreational use. In line with this, in 2019, Ecuador approved its use for therapeutic purposes, guiding research toward its adaptability and productivity (Bravo Avalos et al., 2023).

The Cherry Oregon hemp variety is high in cannabidiol [CBD], with a content of 15%, while the tetrahydrocannabinol [THC] content is approximately 1%, complying with regulatory standards (Acuerdo Ministerial No. 109). However, the CBD content depends on cultural factors, soil, nutrient absorption, water, light, and the harvest point (Garza, 2020).

Hemp producers have used nutrient solutions—either applied to soil or substrates—prepared according to nutritional requirements, growth stage, and the nutrient content of the irrigation water and/or substrate (Moreno Baque et al., 2021). A systematic method has been developed through which a formula can be calculated in advance for the composition of a nutrient solution that meets certain requirements regarding the relative proportions of nutrient ions, total ionic concentration, and pH, without complications from precipitation. This is known as the "universal Steiner nutrient solution" (Steiner, 1961; 1984). This nutrient solution is characterized by its content of essential macronutrients, where the ratios between anions and cations vary. It is considered ideal for analyzing plant growth, as it allows for balanced nutrition and the determination of how this affects cannabinoid concentrations (Trejo-Téllez & Gómez-Merino, 2012).

In this context, the aim of this research was to evaluate the effect of five concentrations of the Steiner nutrient solution on the phenology of medicinal hemp and to determine the optimal nutrient solution dose for cannabinoid content (CBD and THC).

## 2. Materials and Methods

This research was conducted from February 2022 to February 2023 in the greenhouse of the Department of Soil and Water Management [DMSA, Spanish acronym], at the Santa Catalina Experimental Station [EESC, Spanish acronym] of the National Institute of Agricultural Research [INIAP, Spanish acronym]. The greenhouse is in the parish of Cutuglahua, Mejía canton, Pichincha province, at an altitude of 3,059 m a.s.l., latitude 0° 22' 4.02" S, and longitude 78° 33' 15.96" W. The greenhouse had average maximum and minimum temperatures of 34.6°C and 20°C, respectively, as well as 75.60% relative humidity.

One factor was studied: the Steiner nutrient solution, applied at five concentrations (50%, 75%, 100%, 125%, and 150%) (Table 1), with corresponding variations in the concentrations of micronutrients (Fe: 3 mg $L^{-1}$; Mn: 1.52 mg $L^{-1}$; Zn: 0.35 mg $L^{-1}$; Cu: 0.23 mg $L^{-1}$; B: 0.71 mg $L^{-1}$; and Mo: 0.10 mg $L^{-1}$) depending on the treatments. A Completely Randomized Design [CRD] was applied, with 17 observations per treatment, resulting in a total of 85 observations. The experimental unit consisted of one plant grown in a 22-liter plastic bag, with 17 plants used for each treatment (85 plants in total).

Seeds of medicinal hemp (*Cannabis sativa* L.), Cherry Oregon variety, provided by the company BARAD, were used. The seedlings were 0.05 m tall and had 15 days of germination. An inert substrate mix composed of peat (50%), rice husk (30%), and pumice (20%)—previously sterilized—was used. A 1 kg sample of this substrate was taken for physical-chemical analysis at the Soil, Plant, and Water Laboratory of the EESC.

Additionally, a chemical analysis of the irrigation water was carried out using a 1-liter sample (Table 2).

**Table 1.** Ionic composition and osmotic potential of treatments, in the optimization of Steiner nutrient solution at different concentrations in medical hemp flower (*Cannabis sativa* L.) var. Cherry Oregon, under greenhouse conditions.

| Treatment | Concentration (%) | Ionic species (meq $L^{-1}$) | | | | | | EC* (dS $m^{-1}$) | OS** (MP) |
|---|---|---|---|---|---|---|---|---|---|
| | | $NO_3^-$ | $H_2PO_4^-$ | $SO_4^{2-}$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | | |
| T1 | 50 | 6.00 | 0.50 | 3.50 | 3.50 | 4.50 | 2.00 | 1.00 | -0.036 |
| T2 | 75 | 9.00 | 0.75 | 5.25 | 5.25 | 6.75 | 3.00 | 1.50 | -0.054 |
| T3 | 100 | 12.00 | 1.00 | 7.00 | 7.00 | 9.00 | 4.00 | 2.00 | -0.072 |
| T4 | 125 | 15.00 | 1.25 | 8.75 | 8.75 | 11.25 | 5.00 | 2.50 | -0.090 |
| T5 | 150 | 18.00 | 1.50 | 10.5 | 10.5 | 13.50 | 6.00 | 3.00 | -0.108 |

* EC = Electrical conductivity
** OS = Osmotic suction

**Table 2.** Chemical analysis of irrigation water.

| Analysis | Unit | Result |
|---|---|---|
| Nitrate ($NO_3$) | meq $L^{-1}$ | 0.00 |
| Phosphate ($PO_4$) | meq $L^{-1}$ | 0.00 |
| Sulfate ($SO_4$) | meq $L^{-1}$ | 0.02 |
| Potassium (K) | meq $L^{-1}$ | 0.06 |
| Magnesium (Mg) | meq $L^{-1}$ | 0.33 |
| Calcium (Ca) | meq $L^{-1}$ | 0.87 |

For the preparation of the nutrient solutions, five 200-liter containers were used, in which the corresponding doses of fertilizers for each treatment under study were added (Table 3). These were mixed and applied via fertigation, using between 0.2 L and 1 L per plant per day, from transplanting to harvest (17 weeks), according to the crop's phenological stage, five times a week, twice a day. During the last two days of the week, only water was applied. The electrical conductivity and pH of the irrigation water from the nutrient solutions were measured weekly. Frequent monitoring was carried out to determine the incidence of pests and diseases. Harvesting was done manually when the crop showed mature trichomes, identified using a pocket microscope with LED light.

**Table 3.** Fertilizers used in the treatments optimization of the Steiner nutrient solution at different concentrations in medicinal hemp flower (*Cannabis sativa* L.) var. Cherry Oregon, under greenhouse conditions.

| Fertilizer | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 |
| Potassium nitrate (mg $L^{-1}$) | 347.18 | 423.14 | 498.98 | 574.79 | 650.57 |
| Calcium nitrate (mg $L^{-1}$) | 362.88 | 628.18 | 893.47 | 1,158.90 | 1,424.36 |
| Potassium sulfate (mg $L^{-1}$) | 0.21 | 87.03 | 173.95 | 260.90 | 347.88 |
| Magnesium sulphate (mg $L^{-1}$) | 174.22 | 297.52 | 420.65 | 543.72 | 666.77 |
| Phosphoric acid (mL $L^{-1}$) | 0.03 | 0.05 | 0.07 | 0.08 | 0.10 |
| Sulfuric acid (mL $L^{-1}$) | 0.08 | 0.07 | 0.06 | 0.05 | 0.04 |
| Tradecorp AZ (mg $L^{-1}$)* | 13 | 26 | 39 | 52 | 65 |

* For microelements.

For the plant height variable, all plants in the experiment were measured using a measuring tape, in centimeters, from the base of the stem to the apex, once a month starting 15 days after transplanting until harvest. Stem diameter at the collar height was measured for all plants in the experiment using a digital caliper (in cm), at the same frequency as the plant height measurements. To determine dry biomass yield, three plants per treatment were taken to the Nutrition and Quality Laboratory of the EESC, where they were weighed and then placed in an oven at 65°C for 24 hours. Equation [1] was used to calculate dry matter, and Equation [2] was used to obtain yield (Velásquez et al., 2008).

$$DM = \frac{Wcpd - Cw}{Wcpw - Cw} \times 100 \quad [1]$$

where:

- *DM* = Percentage of dry matter.
- *Wcpd* = Weight of the container plus the dry sample.
- *Cw* = Container weight.
- *Wcpw* = Weight of the container plus the wet sample.

$$Y = DW \text{ x } D \quad [2]$$

where:

- *Y* = Yield (g $m^{-2}$).
- *DW* = dry weight (g).
- *D* = Plant density (number of plants per $m^2$).

The weights were averaged and summed to obtain the total weight per treatment, expressed in g $m^{-2}$, with conversion to t $ha^{-1}$. For the THC and CBD content analysis, a composite sample from three plants per treatment, consisting of 50 g of dry flower material, was used for analysis at the Nutrition and Quality Laboratory of the EESC. The samples were ground until a particle size of < 1 mm was obtained. Then, 10 ml of ethanol was added. The mixture was shaken for 1 minute at a speed of 6.5 m $s^{-1}$. The samples were placed in tubes and subjected to an ultrasonic bath, then centrifuged for 10 minutes at 4,500 rpm at 5°C. The extract was transferred to a 50 ml flask. An aliquot of the filtrate was passed through a 0.22 μm membrane and placed in a 2 ml vial for injection into the HPLC (High-Performance Liquid Chromatography) system. The injector operated under the following conditions:

1) Column: Restek, RAPTOR ARC-18, 150 mm × 4.6 mm ID, 2.7 μm;
2) Column temperature: 30°C;
3) DAD detector: wavelength 228 nm;
4) Mobile phase: A: aqueous solution of 5 mM ammonium formate with 0.1% formic acid; B: HPLC-grade acetonitrile with 0.1% formic acid;
5) Flow rate: 1.5 ml per minute (25% A and 75% B);
6) Injection volume: 10 μl; and
7) Chromatography time: 12 minutes

The quantification was carried out using a calibration curve, previously analyzed on the equipment, applying Equation [3] (Oficina de las Naciones Unidas contra la Droga y el Delito [UNODC], 2010).

$$PC = C \text{ x } TV \text{ x } (F \div W) \quad [3]$$

where:

- *PC* = Percentage of cannabinoids.
- *C* = Cannabinoid concentration from the calibration curve (μg $ml^{-1}$).
- *TV* = Total volume of extract (50 ml).
- *F* = Factor to transform units (0,0001).
- *W* = Sample weight (0,5 g).

To obtain the crop growth curve for the variables plant height and stem diameter at collar height, a normal logistic regression was applied (Equation [4]) (Alonso Báez et al., 2003).

$$Y = \frac{\alpha}{1 + \beta\, e^{-\gamma\, t}} \quad [4]$$

where:

- $Y$ = Value of the variable over time ($t$)
- α = Limit value of the variable.
- β = It has no biological meaning and only takes place in the initial time ($t$ = 0).
- ɣ = Rate constant that determines the amplitude of the curve.

The results of the analyzed variables were subjected to the corresponding statistical analysis (assumptions of normality and homogeneity). A Pearson correlation test was performed among the evaluated variables (plant height, stem diameter at collar height, biomass yield, and CBD content). An analysis of variance [ANOVA] was conducted, where the coefficient of variation [CV] was determined as a percentage, and Tukey's test (at 5%) was used to identify significant differences. Data analysis was carried out using the statistical software SAS, Version 9.4 (SAS Institute Inc., 2015).

## 3. Results and Discussion

For the plant height variable, significant differences were observed starting 15 days after transplanting and continuing until the fourth month of plant development (120 DAT), just before harvest (Table 4). After 15 days, plants that received 50% and 100% of the nutrient solution showed greater height, with an average of 22.71 cm. During the first month (30 DAT), corresponding to the vegetative growth stage, the treatment with 100% of the nutrient solution showed the highest average height, reaching 55.57 cm. However, during the second month (60 DAT), treatments with 100% and 125% Steiner nutrient solution reached greater heights, with 77.5 and 73.36 cm, respectively; the same trend was observed during the third month (90 DAT), with 87.36 and 83.86 cm, respectively covering both vegetative and reproductive stages. In the senescence stage, during the fourth month (120 DAT), just before harvest, the treatment with 100% of the nutrient solution recorded the greatest height, with an average of 90.43 cm. The logistic model for this variable indicated that the 100% concentration treatment resulted in the tallest plants (Figure 1). The heights obtained fall within the range reported by Gómez García et al. (2023), which ranged between 78.87 and 96.43 cm, in a study evaluating six genotypes of non-psychoactive *Cannabis* sp. under greenhouse conditions. Regarding the effects of nutrient concentrations in similar studies, Díaz-Vázquez et al. (2023) found that 100% and 125% Steiner nutrient solution concentrations produced the greatest plant heights, due to higher availability of nitrates, which act as precursors of amino acids and structural proteins—as was also observed in this study.

**Table 4.** Tukey's test at 5% for plant height, in the optimization of Steiner nutrient solution at different concentrations in medicinal hemp (*Cannabis sativa* L.) var. Cherry Oregon, under greenhouse conditions.

| Treatment | Concentration (%) | Plant height (cm)* | | | | |
|---|---|---|---|---|---|---|
| | | 15 DAT | 30 DAT | 60 DAT | 90 DAT | 120 DAT |
| T1 | 50 | 22.71 a | 46.07 ab | 67.64 ab | 75.64 ab | 77.14 ab |
| T2 | 75 | 19.36 ab | 43.57 b | 66.29 ab | 76.41 ab | 80.64 ab |
| T3 | 100 | 22.71 a | 55.57 a | 77.50 a | 87.36 a | 90.43 a |
| T4 | 125 | 18.79 ab | 50.50 ab | 73.36 a | 83.86 a | 85.79 ab |
| T5 | 150 | 17.54 b | 40.64 b | 54.79 b | 65.14 b | 69.36 b |

* DAT = days after transplanting. Values with the same letter do not differ statistically from each other, according to Tukey's test (p > 0.05).

For the stem diameter at collar height variable, no significant differences were observed between treatments at 15 days after transplanting; however, differences began to appear from the first month (30 DAT) onward (Table 5). In the first and second months (30 and 60 DAT), the 125% nutrient solution treatment stood out by showing the highest average stem diameter at collar height, with 0.79 and 1.39 cm, respectively. In the third and fourth months (90 and 120 DAT), the 100% and 125% Steiner nutrient solution treatments were significantly superior. In the third month (90 DAT), they reached 1.48 and 1.57 cm, and in the fourth month (120

DAT), 1.59 and 1.66 cm, respectively. The logistic model for this variable indicated that the 125% nutrient solution concentration resulted in the greatest stem diameter at collar height (Figure 2). Regarding the effects of nutrient concentrations in similar studies, Lazcano-Bello et al. (2021) and Díaz-Vázquez et al. (2023) found that 100% and 125% concentrations of Steiner's nutrient solution produced the greatest stem thickness, as was also observed in this study.

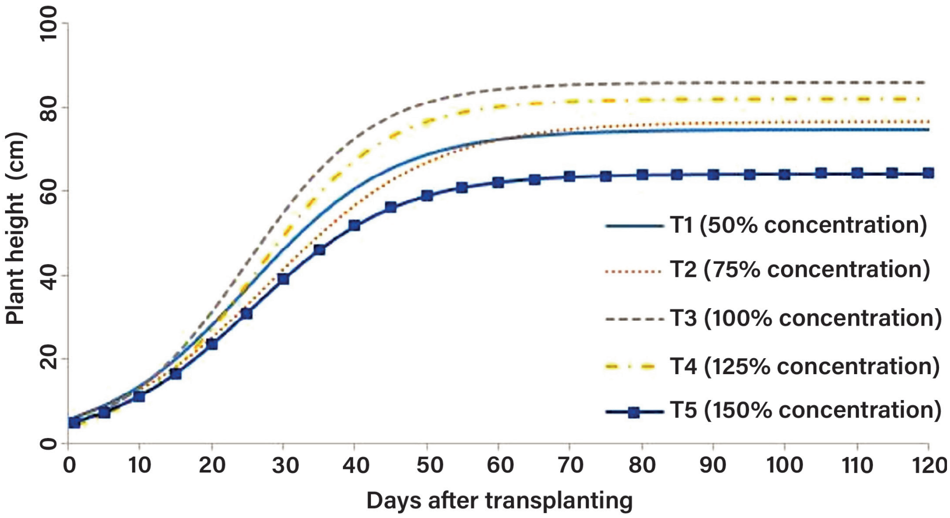


**Figure 1.** Effect of five concentrations of Steiner's nutrient solution on plant height in medicinal hemp (*Cannabis sativa* L.) var. Cherry Oregon, under greenhouse conditions.

**Table 5.** Tukey's test at 5% for the stem diameter at collar height variable in the optimization of the Steiner nutrient solution at different concentrations in medical hemp flower (*Cannabis sativa* L.) var. Cherry Oregon, under greenhouse conditions.

| Treatment | Concentration (%) | Diameter at collar height (cm)* | | | | |
|---|---|---|---|---|---|---|
| | | 15 DAT | 30 DAT | 60 DAT | 90 DAT | 120 DAT |
| T1 | 50 | 0.25 a | 0.54 b | 0.91 c | 1.09 b | 1.18 b |
| T2 | 75 | 0.25 a | 0.60 b | 1.06 bc | 1.29 ab | 1.39 ab |
| T3 | 100 | 0.25 a | 0.68 ab | 1.24 ab | 1.48 a | 1.59 a |
| T4 | 125 | 0.25 a | 0.79 a | 1.39 a | 1.57 a | 1.66 a |
| T5 | 150 | 0.23 a | 0.65 ab | 1.10 bc | 1.32 ab | 1.44 ab |

* DAT = days after transplanting. Values with the same letter do not differ statistically from each other, according to Tukey's test ($p > 0.05$).

For the biomass yield variable, a significant difference was observed among the treatments, with the 125% nutrient solution treatment standing out, reaching an average of 810 g $m^{-2}$ or 8.10 t $ha^{-1}$ (Table 6). This value aligns with the results reported by Díaz-Vázquez et al. (2023) in similar studies on the effects of nutrient concentrations, where the highest biomass yields were obtained with the 125% nutrient solution concentration.

**Table 6.** Tukey's test at 5% for the dry biomass yield variable and CBD content, in the optimization of Steiner nutrient solution at different concentrations in medicinal hemp flower (*Cannabis sativa* L.) var. Cherry Oregon, under greenhouse conditions.

| Treatment | Concentration (%) | Yield* | | CBD* |
|---|---|---|---|---|
| | | g $m^{-2}$ | t $ha^{-1}$ | % |
| T1 | 50 | 310 | 3.10 b | 13.83 ab |
| T2 | 75 | 515 | 5.15 ab | 15.30 a |
| T3 | 100 | 635 | 6.35 ab | 15.15 a |
| T4 | 125 | 810 | 8.10 a | 14.31 ab |
| T5 | 150 | 335 | 3.35 b | 12.87 b |

* Values with the same letter do not differ statistically from each other, according to Tukey's test ($p > 0.05$).

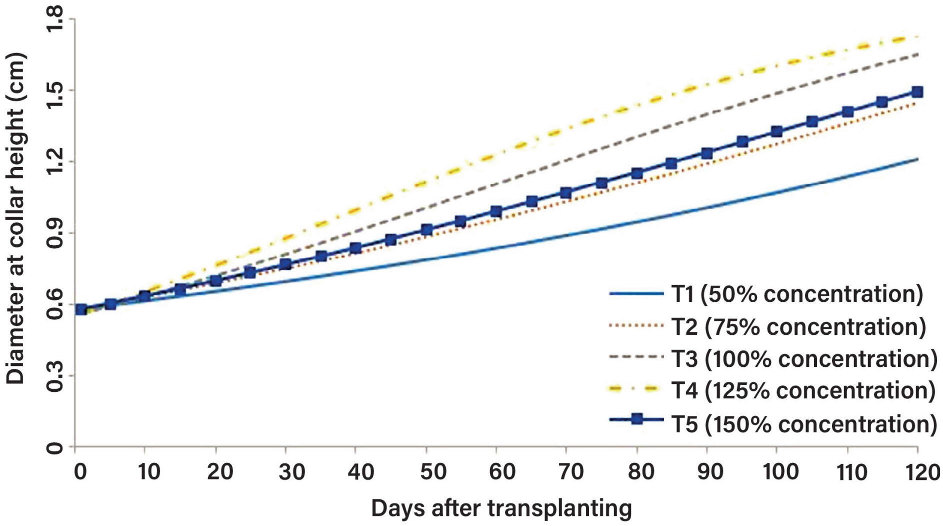


**Figure 2.** Effect of five concentrations of Steiner's nutrient solution on diameter at collar height in medicinal hemp (*Cannabis sativa* L.) var. Cherry Oregon, under greenhouse conditions.

For THC content, no significant differences were detected among treatments, with all concentrations remaining below 1%. This value confirmed that the THC content was within the legal limits established by the Regulation for the Therapeutic Use, Prescription, and Dispensation of Medicinal Cannabis and Pharmaceutical Products Containing Cannabinoids (Acuerdo Ministerial No. 148). On the other hand, for CBD content, significant differences were found among treatments, with the 75% and 100% nutrient solution treatments standing out, presenting average cannabinoid contents of 15.30% and 15.15%, respectively (Table 6). When compared with the findings of Aulestia Caiza (2022), who reported values ranging from 6.68% to 4.19%, CBD content was positively affected by nutrient distribution.

In the correlation tests (Table 7), the highest coefficients (close to 1) were observed between the variables: stem diameter at collar height vs. yield (0.87), plant height vs. CBD content (0.81), and plant height vs. yield (0.80). These results are consistent with the research by Delgado Cáceres (2022), who reported correlation coefficients above 0.55 when analyzing the relationship between plant vigor and biomass/inflorescence yield in response to cultivation technology in hemp production.

**Table 7.** Pearson's correlation test for the variables evaluated in the optimization of Steiner nutrient solution at different concentrations in medicinal hemp flower (*Cannabis sativa* L.) var. Cherry Oregon, under greenhouse conditions.

| Variable 1 | Variable 2 | Pearson* | p-valor** |
|---|---|---|---|
| Plant height (cm) | Stem diameter at collar height (cm) | 0.58 | 0.31 |
| Plant height (cm) | Yield (t $ha^{-1}$) | 0.80 | 0.1 |
| Plant height (cm) | CBD (%) | 0.81 | 0.1 |
| Stem collar diameter (cm) | Yield (t $ha^{-1}$) | 0.87 | 0.06 |
| Stem collar diameter (cm) | CBD (%) | 0.27 | 0.66 |
| Yield (t $ha^{-1}$) | CBD (%) | 0.55 | 0.34 |

* Correlation coefficient between the corresponding variables: a coefficient equal to zero indicates the lack of association; while when it is close to 1, the intensity of association is greater (Coyne & Thompson, 2006).
** Probability associated with the null correlation hypothesis test (Balzarini et al., 2008).

## 4. Conclusions

The optimal concentrations of the Steiner solution were 100% and 125% for the agronomic parameters of *Cannabis sativa* var. Cherry Oregon, including plant height and stem diameter at collar height, throughout the four months of cultivation. For dry biomass accumulation (yield), the optimal concentration was 125%. For CBD production in flowers, while adhering to the current regulations (< 1% THC), the optimal nutrient solution concentrations were 75% and 100%. The importance of nutritional management in Cannabis sativa cultivation is highlighted, as it optimizes cannabinoid production and ensures compliance with regulations, identifying nutrient solution concentrations that improve agronomic characteristics and dry biomass yield.

## Contributor roles

- Michelle Arcos: conceptualization, investigation, methodology, formal analysis, visualization, writing - original draft, writing - review & editing.
- Yamil Cartagena: investigation, project administration, supervision, validation, writing - review & editing.
- Jorge Merino: funding acquisition, supervision, validation, and project administration.
- Patricio Pérez Guerrero: supervision, validation, project administration, writing - review & editing.
- Rafael Parra: validation, supervision, investigation, data curation.
- Julio Moreno: data curation, software, visualization, writing - review & editing.

## Ethical implications

Ethics approval not applicable.

## Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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