



Evaluation of substrates in the germination of *Cannabis sativa* L. seeds, up to the V3 stage, Cotopaxi, Ecuador

Evaluación de sustratos en la germinación de semillas de *Cannabis sativa* L. hasta la etapa V3, Cotopaxi, Ecuador

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ABSTRACT

Cannabis sativa L. is distinguished by its ability to produce phytocannabinoids with high medicinal value, whose accumulation is strongly influenced by agronomic conditions in the early stages. Among these, seed germination and initial vegetative growth represent critical, yet little-explored, phases that determine the establishment of the plant and its subsequent productivity. The objective of this study was to evaluate the effect of substrate composition on seed germination and early vegetative development of *C. sativa* under controlled indoor conditions. A completely randomized design was used to evaluate four substrates (peat-based, coconut fiber, vermiculite, and humus) up to the V3 phenological stage. Germination percentage, plant height, root length, fresh biomass, and leaf area were quantified and analyzed using analysis of variance followed by Duncan's multiple range test ($p \leq 0.05$). Although all substrates promoted satisfactory germination, the peat-based substrate consistently promoted superior agronomic performance, including higher germination rates, greater biomass accumulation, and increased leaf area, while vermiculite significantly improved root development. Coconut fiber showed more variable responses, suggesting limitations related to nutrient availability. These findings demonstrate that substrate composition plays a decisive role during the early stages of *C. sativa* development, highlighting the importance of substrate selection in ensuring vigorous seedling establishment and providing practical guidance for optimizing nursery stage management in medical cannabis cultivation systems.

Keywords: *Cannabis sativa* L., substrate management, seed germination, seedling establishment, medicinal cannabis.

RESUMEN

El *Cannabis sativa* L. se distingue por su capacidad para producir fitocannabinoides con un alto valor medicinal, cuya acumulación está fuertemente influenciada por las condiciones agronómicas en las primeras etapas. Entre ellas, la germinación de las semillas y el crecimiento vegetativo inicial representan fases críticas, aunque poco exploradas, que determinan el establecimiento de la planta y su posterior productividad. El objetivo de este estudio fue evaluar el efecto de la composición del sustrato en la germinación de las semillas y el desarrollo vegetativo temprano de *C. sativa* en condiciones controladas en interior. Se utilizó un diseño completamente aleatorio para evaluar cuatro sustratos (a base de turba, fibra de coco, vermiculita, humus) hasta la fase fenológica V3. Se cuantificaron y analizaron el porcentaje de germinación, la altura de la planta, la longitud de la raíz, la biomasa fresca y el área foliar mediante un análisis de varianza seguido de la prueba de rangos múltiples de Duncan ($p \leq 0,05$). Aunque todos los sustratos favorecieron una germinación satisfactoria, el sustrato a base de turba promovió de manera constante un rendimiento agronómico superior, incluyendo mayores tasas de germinación, una mayor acumulación de biomasa y un aumento de la superficie foliar, mientras que la vermiculita mejoró significativamente el desarrollo radicular. La fibra de coco mostró respuestas más variables, lo que sugiere limitaciones relacionadas con la disponibilidad de nutrientes. Estos hallazgos demuestran que la composición del sustrato desempeña un papel decisivo durante las primeras etapas de desarrollo de *C. sativa*, lo que pone de relieve la importancia de la selección del sustrato para garantizar el establecimiento vigoroso de las plántulas y proporciona una guía práctica para optimizar la gestión de la etapa de vivero en los sistemas de cultivo de cannabis medicinal.

Palabras clave: *Cannabis sativa* L., manejo del sustrato, germinación de semillas, establecimiento de plántulas, cannabis medicinal.

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I. INTRODUCTION

Cannabis sativa L. is a herbaceous species belonging to the *Cannabaceae* family, historically used as a source of food, fiber, medicine, and psychoactive compounds since ancient times (Galzerano, 2019). Its multifunctional nature has positioned cannabis as a crop of growing agronomic, medicinal, and industrial relevance, particularly in scenarios where regulatory frameworks have evolved to allow for its controlled cultivation and use.

In Ecuador, the cultivation and commercialization of non-psychoactive cannabis have been legally permitted since 2019, marking a significant milestone in the regional development of hemp-based industries (Suárez-Jacobo et al., 2023). In this context, cannabidiol (CBD) is positioned as the main bioactive compound of interest, due to its widely recognized therapeutic and medicinal applications. Its pharmacological action is exerted on multiple molecular targets, including CB1 and CB2 receptors, GPR55, TRPV channels, 5-HT1A serotonergic receptors, and the PPAR γ nuclear receptor, giving it a neuroprotective, anti-inflammatory, antioxidant, anxiolytic, antipsychotic, anticonvulsant, and analgesic profile (Peng et al., 2022).

Importantly, CBD must be clearly distinguished from Δ^9 -tetrahydrocannabinol (THC), the psychoactive constituent responsible for narcotic effects, which remains prohibited under national legislation (Gálvez-Salazar, 2019; Heredia, 2024). This regulatory distinction has driven targeted interest in hemp varieties characterized by low THC and high CBD content.

In recent years, the global cannabidiol (CBD) market was estimated at approximately \$4.2 billion in 2021, and since then, global demand for products based on this compound has increased significantly. This growth has stimulated renewed interest in hemp cultivation systems primarily geared toward cannabidiol production (Trancoso et al., 2022). The expansion of this market has highlighted the need to optimize agronomic practices, given that cannabis cultivation involves several critical stages of development, each of which requires the precise

adjustment of specific environmental and management conditions, such as temperature, humidity, CO₂ concentration, light, nutrition, irrigation, and health. From seed germination to vegetative development and crop establishment, each phase has particular physiological requirements that directly affect plant performance and final production (Bafort et al., 2024; Schober et al., 2023; Trancoso et al., 2022).

Soil quality constitutes a fundamental factor in cannabis cultivation, as inadequate physical, chemical, or biological properties can severely limit plant development (Blandinières & Amaducci, 2022). Soils with low organic matter content, for instance, exhibit reduced water retention capacity, limited nutrient availability, and diminished microbial activity, all of which are essential for maintaining soil fertility and supporting vigorous plant growth (Lal, 2020b, 2020a). The incorporation of organic amendments such as earthworm humus and peat has been shown to significantly enhance soil structure and fertility, thereby improving the capacity of the substrate to sustain productive cannabis plants (Aytenew & Bore, 2020; Maticic et al., 2024).

In many cannabis production systems, a preliminary phase of plant material multiplication commonly referred to as plantlet propagation is required. During this stage, the selection of appropriate substrates is particularly critical, as asexual propagation is highly susceptible to sanitary losses. Likewise, under protected cultivation conditions, substrates such as rock wool or coconut fiber are frequently used during the transplanting phase, often in combination with soil and organic amendments, to promote uniform establishment and reduce biotic and abiotic stress (Patiño, 2022).

The use of substrates is widely adopted in modern agriculture, especially under controlled or experimental conditions, as substrate composition is a key factor influencing improvements in agronomic performance and production efficiency (Vargas, 2024). In cannabis cultivation, commercial substrates commonly consist of mixtures of blonde and black peat, earthworm humus, tree bark, coconut fiber, and volcanic materials, while inert components such

as perlite or vermiculite are incorporated to reduce compaction and enhance aeration (Collado, 2025).

Black soil and compost usually form the nutritional basis of substrate formulations, as they provide a high concentration of essential macro- and micronutrients that increase C mineralization and the availability of N, P, and Ca, producing highly nutritious vermicompost with lower heavy metal content (Nsiah-Gyambibi et al., 2021). These materials can be used independently or in combination, and compost provides a particularly rich nutritional profile derived from plant waste or animal manure. Substrate mixtures composed of equal proportions of black soil and compost have been shown to be suitable for cannabis cultivation in soil-based systems (Revistathc, 2025).

Beyond their physical and chemical properties, substrates also host diverse microbial communities that play a crucial role in plant health and productivity. Soil and substrate microorganisms contribute to nutrient cycling, disease suppression, and plant growth promotion through complex plant–microbe interactions (Vishwakarma et al., 2020). Consequently, understanding the composition and function of microbial communities in soils and substrates is essential for developing effective strategies for crop management, prevention, and biological control (Chernov & Semenov, 2021).

The agronomic management of hemp, as with other high-value crops, requires specific edaphic conditions to achieve optimal development and productivity (Visković et al., 2023). Recommended soils are typically loose and well-drained, with an approximate composition of 12% sand, 14% humus, and 1–3% clay, and a slightly acidic pH range between 5.6 and 6.6. Deviations from these conditions may adversely affect nutrient uptake and root development.

To mitigate soil compaction resulting from repeated irrigation, various substrates and inert materials are commonly employed to improve aeration. Components such as coconut fiber, vermiculite, perlite, volcanic lava rock, and similar materials enhance substrate porosity without significantly altering pH or electrical conductivity (EC), thereby maintaining compatibility between substrate conditions and the physiological requirements of cannabis plants (Malík

& Tlustoš, 2025; Shaheb et al., 2021). In this context, the objective of this study was to evaluate the effect of substrate composition on seed germination and early vegetative development of *Cannabis sativa* L. under controlled indoor conditions.

II. MATERIALS AND METHODS

Study site and experimental approach

The experimental study was conducted at PHOENICIAN FARM S.A.S., located in Poaló, Cotopaxi, Ecuador. The research followed an experimental design aimed at identifying and quantifying agronomic variables associated with seed germination and early vegetative development of *Cannabis sativa* up to the V3 phenological stage. The collected data were subjected to statistical analysis to test the proposed hypothesis and to determine the substrate treatments that most effectively promoted early plant growth.

Experimental management and growth conditions

The experiment was carried out under controlled indoor conditions. Lighting was provided using a TATU full-spectrum LED grow light designed for indoor cultivation, with a power output of 105 W, a frequency of 50–60 Hz, a 120° beam angle, and an AC input voltage of 85–265 V. The lighting panel consisted of 100 LEDs and measured 12.6 inches in length, 8.7 inches in width, and 1.9 inches in height.

A peat-based organic substrate (Stender S200) was used as the base growing medium. This substrate consists of stabilized white and black peat moss with a particle size of 0–8 mm, low electrical conductivity, and an NPK ratio of 14–10–18. The substrate is of German origin and contains perlite as a structural component.

Plants were grown under a photoperiod of 18 h light and 6 h darkness. Environmental conditions were maintained within a temperature range of 23–25 °C and relative humidity between 60% and 65%. Prior to sowing, all substrates evaluated in the study were sterilized. Subsequently, seeds were soaked and placed in seedbeds for germination. Approximately four days after sowing, cotyledon emergence was observed, indicating the need for transplanting due to spatial limitations of the seedbeds.

Plants were individually labeled according to treatment and replicate. A nutrient solution based on the Steiner formulation was applied during the experiment, as plants grown in Treatment 2 (coconut fiber) exhibited leaf yellowing symptoms indicative of macronutrient deficiency.

Substrate treatments

Four substrate treatments were evaluated:

T1 (Peat-based substrate)

T2 (Coconut fiber)

T3 (Vermiculite)

T4 (Humus)

During the course of the experiment, some plants exceeded the expected V3 phenological stage by the final evaluation.

Agronomic variables measured

Plant height (cm) was measured at seven-day intervals from the base of the substrate to the apical bud.

At the fourth week, root length (cm), fresh weight (g), and leaf area (cm²) were recorded. Root length was measured at the end of the experiment using a measuring tape or ruler. Fresh weight was determined by weighing all living plant tissue with a digital scale, corresponding to phenological stage V3. Leaf area was determined by scanning leaves from stages V0, V1, V2, and V3 and analyzing the images with ImageJ software. Measurements were expressed in square centimeters. Leaf area was considered a key variable due to its direct relationship with the plant's photosynthetic capacity.

The germination rate was calculated by counting the number of germinated seeds and expressing it as a percentage of the total number of seeds sown.

2.5. Experimental design and statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA) to evaluate the effects of substrate treatments on the measured agronomic variables. When significant differences were detected, means were compared using Duncan's multiple range test at a 5% significance level ($p \leq 0.05$). All statistical analyses were performed using Infostat software.

III. RESULTS

Agronomic performance of seedlings as affected by substrate type

The early vegetative growth of *Cannabis sativa* seedlings, assessed through plant height during the first four weeks, varied according to substrate type (Table 1). Differences among treatments were detected across the evaluation dates, and data dispersion decreased after Log10 transformation, as shown by the coefficients of variation (CV), which ranged from 29.36% to 36.47% in the original data and from 14.43% to 23.78% after transformation, indicating improved homogeneity following normalization. In the first week, seedlings grown in coconut fiber (T2) recorded the highest mean height (4.28 cm) and differed significantly from vermiculite (T3; 3.03 cm). Peat-based substrate (T1; 3.92 cm) and humus (T4; 3.93 cm) presented intermediate values and did not differ statistically from either T2 or T3, reflecting overlapping performance at this initial stage. A clearer separation emerged in the second week: T1 (5.36 cm), T2 (5.79 cm), and T4 (4.62 cm) formed the highest statistical group, whereas T3 remained significantly lower (3.73 cm). From the third week onward, the peat-based substrate (T1) became consistently superior, reaching 10.17 cm in week three and 15.78 cm in week four, significantly exceeding the other treatments at both time points. In contrast, coconut fiber (T2), vermiculite (T3), and humus (T4) remained in the same statistical group during weeks three and four ($p \leq 0.05$), despite numerical differences among their means. Overall, the results indicate that coconut fiber showed an initial numerical advantage during the first two weeks, but peat-based substrate promoted the greatest sustained height gain and final seedling stature by the end of the evaluation period (Table 1; Figure 1).

Table 1. Plant height (cm) of *Cannabis sativa* seedlings during the first four weeks of growth under different substrate treatments.

Treatment	H.P. 1st week	H.P. 2nd week	H.P. 3rd week	H.P. 4th week
T1 peat-based	3.92 a b	5.36 a	10.17 a	15.78 a
T2 Coconut fiber	4.28 a	5.79 a	8.58 b	11.39 b
T3 Vermiculite	3.03 b	3.73 b	6.60 b	11.64 b
T4 Humus	3.93 a b	4.62 a	7.85 b	7.85 b
CV (Original Data)	29.36 %	30.35%	32.15%	36.47%
CV (Log 10)	19.82%	23.78%	14.92%	14.43%

(Values followed by different letters within the same column differ significantly according to Duncan’s test at $p \leq 0.05$.)

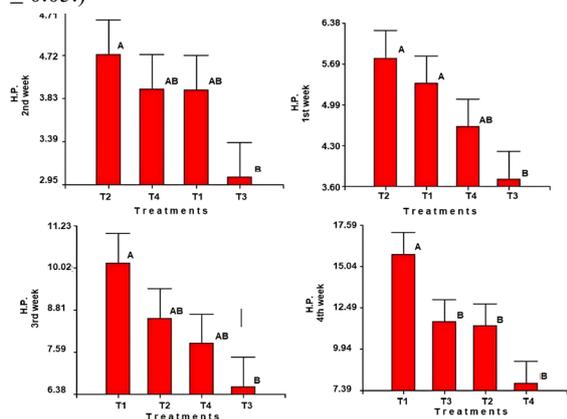


Figure 1. Comparison of plant height (cm) of *Cannabis sativa* seedlings grown on different substrates during weeks 1–4.

Leaf area development

Leaf area measured at phenological stages V0, V1, V2, and V3 showed significant differences among treatments according to the analysis of variance (Table 2). The coefficients of variation ranged from 35.83% to 53.11% for the original data and from 10.14% to 21.37% following Log10 transformation, indicating stage-dependent variability in leaf expansion.

Across all evaluated stages, treatment T1 (peat) consistently exhibited the highest leaf area values. At stage V0, seedlings grown in peat reached a mean

leaf area of 7.79 cm², which increased substantially through stages V1 and V2, reaching a maximum of 70.21 cm² at stage V2, before slightly decreasing at stage V3 (Table 2; Figure 2).

Treatments T2 (coconut fiber), T3 (vermiculite), and T4 (humus) showed significantly lower leaf area values at all stages. Among these, T2 generally ranked second during the early stages (V0–V2), whereas T3 and T4 exhibited the lowest values, with minor variations across stages. At stage V3, differences among these four treatments remained pronounced, confirming the superior leaf development associated with peat-based substrate.

Table 2. Leaf area (cm²) of *Cannabis sativa* seedlings at different phenological stages (V0–V3) under different substrate treatments.

Treatment	L.A. (V0)	L.A. (V1)	L.A. (V2)	L.A. (V3)
T1 peat-based	7.79 a	39.15 a	70.21 a	57.34 a
T2 Coconut fiber	6.16 ab	21.24 b	35.48 b	28.06 b
T3 Vermiculite	4.53 b	18.71 b	25.22 b	24.45 b
T4 Humus	5.67 b	17.54 b	33.73 b	28.39 b
CV (Original Data)	35.83%	37.78%	42.38%	53.11%
CV (Log 10)	21.37%	11.93%	10.14%	19.06 %

(Values followed by different letters within the same column differ significantly according to Duncan’s test at $p \leq 0.05$.)

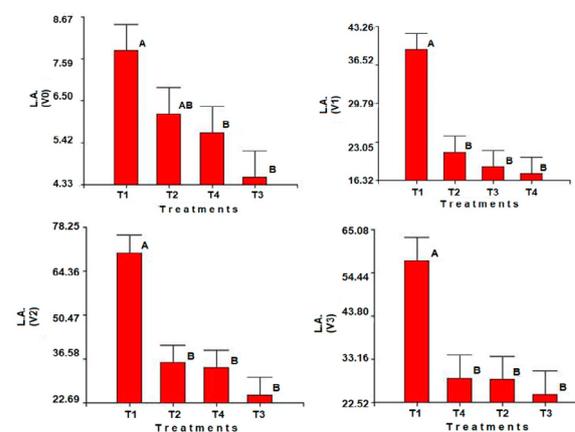


Figure 2. Leaf area comparison (cm²) of *Cannabis sativa* seedlings grown on different substrates at

stages V0–V3.

Fresh biomass accumulation

Fresh biomass weight measured at stage V3 showed significant differences among treatments (Table 3). The coefficient of variation was 34.75% for the original data and 15.10% after Log10 transformation, indicating moderate variability among experimental units.

Seedlings grown in peat-based substrate (T1) accumulated the highest fresh biomass, with a mean value of 20.64 g per plant (Figure 3). This value was significantly higher than those recorded for T3 (vermiculite) and T2 (coconut fiber), which showed intermediate biomass accumulation. The lowest fresh biomass was observed in T4 (humus), with a mean value of 7.30 g per plant.

These results highlight marked differences in biomass production among substrates during early vegetative development.

Table 3. Fresh biomass weight (g plant⁻¹) of *Cannabis sativa* seedlings at stage V3 under different substrate treatments.

Treatment	Weight / Plant (V3)
T1 peat-based	20.64 a
T2 Coconut fiber	13.12 b
T3 Vermiculite	15.01 b
T4 Humus	7.30 c
CV (Original Data)	34.75%
CV (Log 10)	15.10%

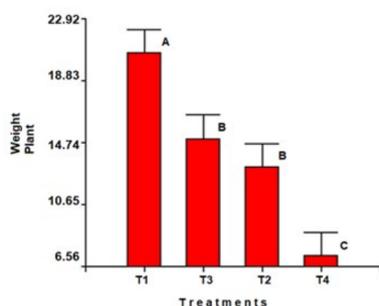


Figure 3. Comparison of fresh biomass weight (g) of *Cannabis sativa* seedlings grown on different substrates.

Root growth during the vegetative stage

Root length measured at stage V3 differed significantly

among treatments, as indicated by the analysis of variance (Table 4), with a coefficient of variation of 19.04%. The longest roots were observed in seedlings grown on vermiculite (T3), reaching a mean length of 31.00 cm (Figure 4). Treatments T1 (peat-based) and T2 (coconut fiber) showed intermediate root lengths, whereas T4 (humus) exhibited the shortest root systems. These results indicate substrate-dependent differences in root development during the vegetative stage.

Table 4. Root length (cm) of *Cannabis sativa* seedlings at stage V3 under different substrate treatments.

Treatment	Root length (V3)
T1 peat-based	28.38 ab
T2 Coconut fiber	27.82 ab
T3 Vermiculite	31.00 a
T4 Humus	23.90 b
CV (Coefficient of variation)	19.04%

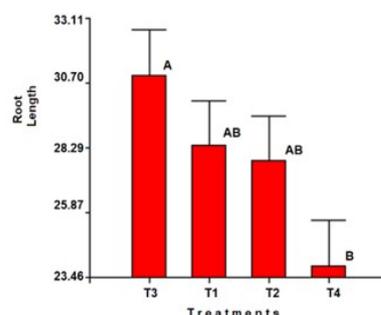


Figure 4. Root length (cm) of *Cannabis sativa* seedlings grown on different substrates at stage V3.

IV. DISCUSSION

The findings of the present study are consistent with and complementary to previous research evaluating the influence of substrate composition on the germination and early growth of *Cannabis sativa*. In particular, the results reported by García (2020) in Colombia and those obtained in Ecuador converge in their objective of optimizing cultivation conditions through substrate selection, highlighting the critical role of growth media during the nursery stage.

Peat consistently emerged as the most effective substrate for seed germination and early plant

development. In the Colombian study, peat achieved a germination rate of 100%, a result that aligns closely with the Ecuadorian findings, where peat also produced superior outcomes in germination percentage, plant height, biomass accumulation, and leaf area. These results reinforce the widely recognized suitability of peat-based substrates due to their favorable physical and chemical properties, including high water-holding capacity, adequate aeration, and nutrient availability during early developmental stages. Nevertheless, while García (2020) reported no statistically significant differences in plant height among treatments, the Ecuadorian study identified clear advantages of peat in this parameter, suggesting that local environmental conditions or substrate management practices may modulate plant growth responses.

Comparisons with the work of Burgel, Hartung, and Graeff-Hönninger (2022) further contextualize the present findings. Their study demonstrated a substantial increase in root length density when peat and green fiber were used, with genotype-specific responses observed for root development. While their results indicated no significant differences in root dry weight between peat and coconut fiber treatments, the present study identified vermiculite as the substrate promoting the greatest root length, followed by peat. These discrepancies may be attributed to differences in substrate composition, physical structure, or experimental conditions, emphasizing the sensitivity of root system development to substrate characteristics such as porosity, particle size distribution, and water availability.

A notable methodological distinction of the present research lies in the inclusion of four substrates peat, vermiculite, coconut fiber, and humus evaluated under a Completely Randomized Design (CRD) with Duncan's multiple range test. This approach enabled a more detailed differentiation among treatments. In contrast, García (2020) evaluated four substrates and relied on non-parametric statistical analyses for variables that did not meet normality assumptions. Although both studies concluded that all tested substrates were suitable for germination,

the differences in experimental design and statistical methodology underscore the importance of robust analytical frameworks for drawing precise agronomic conclusions.

The relevance of substrate selection is further supported by studies conducted by Curicho (2022), who assessed *Cannabis sativa* performance under controlled and open-field conditions using diverse substrate mixtures. Their findings demonstrated superior vegetative growth expressed as increased plant height, leaf number, and branching in treatments containing vermiculite and peat under controlled environments. These results are in strong agreement with those of the present study, which similarly highlight peat and vermiculite as key substrate components for optimal early-stage development. While the Cotopaxi study emphasized the interaction between substrate composition and environmental conditions, the present research conducted in Poaló focused on substrate performance at specific growth stages, thereby complementing existing knowledge on cannabis agronomic management.

Further evidence of the strong influence of substrate composition on cannabis growth is provided by Ortiz (2025), who reported enhanced vegetative development and biomass production in mixtures combining coconut fiber with peat and perlite. In contrast, the present study revealed a pronounced differential response among substrates, with peat and vermiculite exerting a stronger influence on plant development during the nursery stage. These contrasting outcomes suggest that substrate performance is highly context-dependent and may vary according to growth stage, environmental conditions, and substrate proportions.

V. CONCLUSIONS

The results of this study confirm that substrate composition plays a decisive role in the germination and early vegetative development of *Cannabis sativa*. While all evaluated substrates were suitable for seed germination, peat consistently promoted superior performance in terms of plant height, biomass accumulation, leaf area, and overall seedling

vigor, whereas vermiculite particularly enhanced root development. In contrast, coconut fiber and humus showed more variable responses, indicating a stronger dependence on substrate properties and management conditions. These findings underscore the importance of selecting substrates with adequate physical and chemical characteristics during early growth stages, when plant establishment is highly sensitive to cultivation conditions, and highlight the need for further research focused on optimizing substrate mixtures to improve early crop performance under diverse production systems.

ACKNOWLEDGEMENTS

The authors of this document would like to express their gratitude to PHOENICIAN FARM S.A.S., and especially to its directors, particularly its manager Michel Khoueiry, for their assistance and support in carrying out the experimental work.

SAMPLING AND STUDY PERMITS

PHOENICIAN FARM S.A.S. is authorised to grow flax and hemp, as established by the relevant regulatory bodies; similarly, the authors of this research are authorised to publish the results obtained. It should be noted that the work was carried out on the company's premises, in compliance with the necessary safety measures.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization: J.C.; C.C. Data curation: P.V.; J.C. Formal analysis: J.C.; C.C. Funding acquisition: J.C. Investigation: J.C.; C.C. Methodology: J.C.; C.C. Project administration: J.C.; C.C.; P.V. Resources: K.C.; J.C. Software: J.C.; K.C. Supervision: C.C.; P.V. Validation: C.C.; P.V. Visualization: J.C.; K.C. Writing – original draft: J.C. Writing – review & editing: J.C.; P.V.; C.C.

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