AGRONOMIA COLOMBIANA

Doi: 10.15446/agron.colomb

VOLUME XLII, No. 2 MAY-AUGUST 2024 ISSN 0120-9965

Centro Editorial
Facultad de Ciencias Agrarias
Sede Bogotá

UNIVERSIDAD NACIONAL DE COLOMBIA

AGRONOMIA COLOMBIANA

VOLUME XLII | No. 2 | MAY-AUGUST 2024 | ISSN (print): 0120-9965 / ISSN (online): 2357-3732

PUBLICATION OF A SCIENTIFIC-TECHNICAL NATURE BY THE FACULTY OF AGRICULTURAL SCIENCES OF THE UNIVERSIDAD NACIONAL DE COLOMBIA, BOGOTA

Our cover (photo courtesy of Diego Fernando Romero Sánchez)

Publication registered at the Ministerio de Gobierno
Resolution No. 00862 of March 24, 1983

Information, correspondence, subscription and exchange:

Revista Agronomía Colombiana
Faculty of Agricultural Sciences,
Universidad Nacional de Colombia

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Electronic version available at:

http://www.revistas.unal.edu.co/index.php/agrocol

http://www.scielo.org.co/scielo.php?script=sci_serial&pid=0120-9965

ISSN: 2357-3732 (Online)

Published: Triannual

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In 2024, *Agronomía Colombiana* celebrates 41 years since its foundation, being an important member of the family of scientific journals in the area of Agronomy in the country. During this journey in time, many of our readers in Colombia and other parts of the world have been influenced by the publications of *Agronomía Colombiana*. This year, starting from issue 2, the journal launches a new section of Invited Editorial, where a guest researcher will propose a new theme that might have an impact on agronomic science at regional, national or global level. The first Invited Editorial in issue 2 of 2024 discusses the prospects of artificial intelligence in modern agriculture.

Agronomía Colombiana continues to encourage its readers to submit their original articles, with a particular interest in promising topics of agriculture.

En el año 2024 *Agronomía Colombiana* celebra 41 años desde su fundación, siendo un miembro importante de la familia de revistas científicas en el área agronómica en el país. Durante este recorrido en el tiempo, muchos de nuestros lectores en Colombia y otras partes del mundo han sido influenciados por las publicaciones de Agronomía Colombiana. Este año, desde el número 2 en adelante, la revista abre una nueva sección editorial, Editorial invitado, donde un(a) investigador(a) científico(a) invitado(a) proyectará un tema novedoso con posibles impactos en las ciencias agronómicas a nivel regional, nacional o global. El primer Editorial invitado publicado en el número 2 de 2024 habla sobre las perspectivas de la inteligencia artificial en la agricultura moderna.

Agronomía Colombiana continúa invitando a sus lectores a someter sus artículos originales, con especial interés en temas prometedores para la agricultura.

STANISLAV MAGNITSKIY
Editor-in-Chief

Opportunities and challenges of artificial intelligence in agriculture: Some brief reflections

Oportunidades y desafíos de la inteligencia artificial en la agricultura: algunas reflexiones breves

In recent years, agriculture sector has suffered significant transformation driven by the development of emerging technologies that form the basis approach known as Agriculture 4.0 (Alam *et al*., 2023). This new agricultural revolution is characterized by the integration of advanced technologies, such as the Internet of Things (IoT), robotics, Big Data, programming and computing, cloud computing, artificial intelligence (AI), among others (Erazo-Mesa *et al*., 2022). In this context, AI plays a crucial role by enabling the integration of these tools to efficiently analyze large volumes of data generated from traditional monitoring methods, proximal and remote sensors, and various platforms for plants, climate, soil, pest populations, and beneficial organism phenotyping.

AI facilitates evidence-based decision-making, optimizing resource use and enhancing productivity, competitiveness, and sustainability in agriculture. AI-based systems can integrate historical and real-time data to develop predictive models that assist farmers in planning their activities. For instance, AI algorithms can generate various innovative applications, such as comprehensive crop monitoring, detection of nutritional deficiencies and pests in plants, assessment of nutrient and soil moisture concentration, harvest forecasting, yield prediction, climate and irrigation forecasting, among others (Erazo-Mesa *et al*., 2022). Additionally, AI-equipped agricultural robots can perform repetitive tasks like planting, weeding, and harvesting with higher precision and efficiency than traditional methods (Wakchaure *et al*., 2023). Additionally, in the commercialization sector, AI can help predict market demand and agricultural product prices, enabling farmers to make informed decisions about when to harvest and sell their products to maximize profits. Furthermore, AI-based e-commerce platforms can connect producers with consumers, facilitating access to new markets and increasing the competitiveness of small-scale farmers (Junhui *et al*., 2021).

Despite its numerous applications and potential benefits, the implementation of AI in agriculture faces several challenges that need to be overcome to ensure its success and sustainability. One of the main challenges is the quality of the data used to train AI models (Rodríguez-Almonacid *et al*., 2023). In many cases, agricultural data is heterogeneous, incomplete, or of low quality, which can affect the accuracy and reliability of predictive models. It is crucial to develop robust methods for data collection, cleaning, and standardization to generate reliable information for decision-making (Rodríguez-Almonacid *et al*., 2023). Additionally, ensuring the reproducibility and transparency of AI models is essential. Often, AI algorithms function as “black boxes,” making it difficult to understand how decisions are made (Hu *et al*., 2023). There is also a need to adapt AI solutions to real field conditions, which are often more complex and diverse, involving multiple sources of variation.

Moreover, the applicability of AI in small-scale production systems, particularly in multicultural and economically diverse contexts, represents another significant challenge (Erazo-Mesa *et al*., 2022). In many developing countries, small-scale farmers lack the financial resources or access to advanced technologies needed to implement AI-based solutions (Alam *et al*., 2023). There is also a knowledge gap regarding the use and benefits of these technologies, limiting their adoption. This highlights the need to design accessible, affordable, and user-friendly AI tools (Erazo-Mesa *et al*., 2022). Furthermore, to fully realize AI’s potential in agriculture, widespread adoption across value chains is necessary, involving not only the development of accessible technologies but also the training of farmers, companies, technical assistants, and students in their use, along with the creation of public policies supporting the adoption of digital technologies in the agricultural sector (Cáceres-Zambrano *et al*., 2022). These programs should focus on demonstrating the benefits of AI-based solutions and providing the skills necessary for their implementation. Additionally, the development of open-source and low-cost software platforms can facilitate access to AI tools for small-scale farmers, helping reduce economic and technical barriers to adoption.

Undoubtedly, we are observing a significant technological revolution where AI plays a essential role in human history, and the agricultural sector is no exception. This invites us to harness its vast potential to enhance multiple processes and provide more decision-making elements, promoting the correct, responsible, and honest use of AI. Additionally, to fully capitalize on these opportunities, several challenges must be addressed, such as data quality, model transparency, and applicability in different production contexts. Through overcoming these multiple challenges, AI can become a key tool to address the agricultural sector's challenges and enhance its long-term competitiveness and sustainability, particularly in the changing environments associated with variability and climate change.

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Improvement of growth and productivity in potato (*Solanum tuberosum* L.) crop by using biostimulants

Mejora del crecimiento y productividad del cultivo de papa (*Solanum tuberosum* L.) mediante el uso de bioestimulantes

Jenifer Dayanne Medina Avendaño[1], Elberth Hernando Pinzón-Sandoval[1*], and David Fernando Torres-Hernández[1]

ABSTRACT

In Colombia, potato cultivation has significant social and economic importance for the population; however, rising input costs and low yields have led to a decline in the areas planted in the country. Biostimulants are substances or microorganisms that can enhance yield by improving the physiological processes of the plants. In Colombia, there are few studies evaluating their efficiency on potato productivity. Therefore, the aim of this research was to assess the effect of biostimulant applications on the growth and productivity of potato (*Solanum tuberosum* L.) variety 'CIP 39' under the conditions of the municipality of Paipa, Boyacá department. A completely randomized design was used, with four treatments corresponding to commercially registered biostimulants with an active hormonal ingredient, seaweed extract (SWE), or carboxylic acids, and a control. Variables such as fresh and dry weight of roots, shoots, and total biomass, leaf area index (LAI), yield by quality, and total yield were evaluated. The application of biostimulants resulted in improved physiological response of the plants. The SWE-based biostimulant exhibited a better balance in terms of fresh and dry biomass, as well as in LAI, leading to a significant increase in quality and yield. This indicates that the application of biostimulants can be an alternative to increase productivity in this production system.

Key words: seaweed extracts, carboxylic acids, plant biostimulation, sustainable production.

RESUMEN

En Colombia el cultivo de papa tiene gran importancia social y económica para la población; sin embargo, el aumento de los precios de los insumos y los bajos rendimientos han generado una caída en las áreas sembradas del país. Los bioestimulantes son sustancias o microorganismos que pueden mejorar el rendimiento, a través de la mejora de los procesos fisiológicos de la planta. En Colombia hay escasos estudios que evalúen la eficiencia de aquellos sobre la productividad en el cultivo de papa; por esto el objetivo de esta investigación fue evaluar el efecto de las aplicaciones de bioestimulantes en el crecimiento y productividad del cultivo de papa (*Solanum tuberosum* L.) variedad 'CIP 39' bajo condiciones del municipio de Paipa, departamento de Boyacá. Se utilizó un diseño completamente al azar, con cuatro tratamientos que correspondieron a bioestimulantes comerciales registrados cuyo compuesto activo fuera de tipo hormonal, extracto de algas marinas (EAM) o ácidos carboxílicos, y un control. Se evaluaron las variables de peso fresco y seco de raíz, parte aérea y total, índice de área foliar (IAF), rendimiento por calidades y total. La aplicación de bioestimulantes resultó en una mejor respuesta fisiológica de la planta. El bioestimulante a base de EAM mostró un mejor balance en cuanto a la biomasa fresca y seca, así como en el IAF; esto generó un aumento significativo de la calidad y el rendimiento. Esto indica que la aplicación de bioestimulantes puede ser una alternativa para aumentar la productividad en este sistema productivo.

Palabras clave: extractos de algas marinas, ácidos carboxílicos, bioestimulación de plantas, producción sostenible.

Introduction

The potato (*Solanum tuberosum* L.) is one of the most important crops worldwide for both human consumption and industrial use (Sebnie *et al*., 2021). It is considered a versatile food with high nutritional and energetic value due to its high content of starch and antioxidants such as polyphenols, amino acids, essential minerals, and vitamins B6, B3, and C (Van Dingenen *et al*., 2019).

The potato supply chain in Colombia generates approximately 264,000 jobs annually, of which around 75,000 are direct jobs and about 189,000 are indirect. Annual variations are due to changes in the planted area. It is estimated that potato cultivation alone generates around 20 million workdays per year, with nearly 100,000 families dedicated to potato farming across 9 departments and 283 municipalities (Vélez, 2020). However, the high cost of agricultural inputs, along with the low response of crops

Received for publication: February 28, 2024. Accepted for publication: June 10, 2024.

Doi: 10.15446/agron.colomb.v42n2.114683

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to fertilization (Hailu *et al*., 2017), has increased interest in the development of technologies and practices that improve agricultural efficiency and productivity (Torres-Hernández *et al*., 2023). Among these, the application of biostimulants is becoming a sustainable agricultural practice with positive effects on crop yields (Brown & Saa, 2015).

Currently, the production of sustainable crops is focused on obtaining high-value products, where biostimulants have been gaining increasing importance (Bulgari *et al*., 2019). According to du Jardin (2015), a plant biostimulant is any substance or microorganism applied to plants with the aim of enhancing nutrient efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of its nutrient content. These substances are not considered mineral nutrients but facilitate nutrient uptake or beneficially contribute to growth promotion and tolerance to abiotic and biotic stress (Brown & Saa, 2015). Biostimulants can be classified as humic substances (humic and fulvic acids), protein hydrolysates (peptides and free amino acids (FAAs)), seaweed extracts and botanicals, chitosan and other biopolymers, inorganic compounds (beneficial and essential mineral elements), beneficial fungi and bacteria (du Jardin, 2015).

According to Li *et al*. (2022), plant biostimulants are products that stimulate plant growth, improve nutrient use efficiency, enhance abiotic stress tolerance, improve crop quality, and increase the availability of nutrients in the soil or plant rhizosphere. This is achieved by inducing both structural and physiological changes in plants related to nutrient absorption, assimilation, and distribution, as well as changes in the primary and secondary metabolism of the plants (Canellas & Olivares, 2014). Consequently, vegetable, cereal, and ornamental crops exhibit greater vigor, higher yields, and improved harvest quality (Kisvarga *et al*., 2022). Plant biostimulants can be applied either through soil (edaphically) or by foliar application, promoting positive effects on plant growth, nutrition, and quality traits in crops (Van Oosten *et al*., 2017).

Research conducted on crops such as beans (Martínez *et al*., 2017), soybeans (Santos *et al*., 2017), maize (Lephatsi *et al*., 2022), and potatoes (Lazzarini *et al*., 2022; Wadas & Dziugieł, 2020) in other countries indicated that biostimulants can improve nutritional efficiency, tolerance to abiotic stress, and crop quality. In Colombia, the use of biostimulants has been increasing substantially; however, there are few studies evaluating the effect of biostimulants on the productivity of crops such as potatoes, which are of great social and economic importance to the country.

According to the above, various studies indicate the beneficial effects of using biostimulants. However, research on the use of these types of products in Colombia is limited, particularly for species where the organ of commercial interest is roots or tubers. This study was conducted to understand the response of potato (*Solanum tuberosum* L.) plants variety CIP 39 in terms of growth, biomass gain, and productivity when subjected to the application of different types of biostimulants during their growth under field conditions.

Materials and methods

Location

The research was conducted at the Tunguavita Experimental Farm of the Pedagogical and Technological University of Colombia (UPTC) located in the municipality of Paipa (Boyacá, Colombia) in the Salitre district. The geographical coordinates are 5°45’ N and 73°45’ W at an altitude of 2470 m a.s.l. The site has an average annual temperature of 14.1°C, a bimodal rainfall pattern with an average annual precipitation of 966 mm, and a relative air humidity of 75%.

Plant material

The plant material used was seed-tubers of potato variety CIP 39, with the following morphological characteristics: predominant yellow skin color, white flesh, oblong tuber shape, white flowers, early to semi-early vegetative period (120 to 130 d), and industrial uses (French fries) (Instituto Nacional de Innovación Agraria, 2012).

The fertilization plan was adjusted according to the results of soil analysis (Tab. 1) and following the recommendations for potato cultivation by Guerrero-Riascos (1995). The nutrient requirements in terms of nitrogen, phosphorus, and potassium were adjusted using simple fertilizer sources such as urea, diammonium phosphate, and KCl. In addition, micronutrients were applied using B-Zn (8% boron + 4% zinc).

For the control of pest insects and diseases, protective and systemic action products were used. The most limiting disease was late blight caused by the fungus *Phytophthora infestans*, which was managed using protective and systemic fungicides such as chlorothalonil, dimethomorph, mancozeb, cymoxanil, and metalaxyl. For the control of the most limiting pest insect, the Guatemalan moth (*Tecia solanivora*), applications of thiamethoxam, cyantraniliprole, and imidacloprid were carried out. Irrigation was adjusted based on the percentage of allowed depletion according

TABLE 1. Physical and chemical properties of soil at the experimental site.

Texture			pH	Organic matter %	P mg kg^{-1}	Ca	Mg	K	Na	Electric conductivity
Sand	Clay	Silt								
23	38	39				meq 100 g^{-1}				dS m^{-1}
Clay Loam			5.27	7.33	18.8	6.02	1.07	1.02	0.06	0.15

to the phenological state, following the methodology employed by Guerrero-Guio *et al*. (2019), and was supplied through a sprinkler system.

A completely randomized design (CRD) was employed, with four treatments corresponding to registered commercial biostimulants in Colombia, clearly labeled with the type of active compound. The treatments were as follows: T1: Hormonal (Hormonal), corresponding to the commercial product Stimulate® (Stoller Colombia S.A.), which contains a mixture of kinetin, indole-3-butyric acid, and gibberellic acid (GA_3); T2: Seaweed extract (SWE), corresponding to the product Radifarm™ (Valagro) composed of proteins, amino acids, betaines, alginates, and polysaccharides extracted from *Ascophyllum nodosum* algae; T3: Carboxylic acids (Carboxylic A), corresponding to the product Radigrow® (Innovak Global, S.A.) composed of Carboxy acids® expressed as total oxidizable organic carbon; and T4: No application (water). Each treatment had three replicates, totaling 12 experimental units which corresponded to plots of 72 m^2 with a planting distance of 0.3 m between plants and 1 m between rows, with 240 plants per plot.

The treatments were applied by soil spraying at two times with a solution volume of 1.5 L per plot. The first application was performed at phenological stage 08, which corresponds to stems growing towards the soil surface (Hack *et al*., 1993). The second application was performed 15 d after the first application. The dosage for all treatments was 10 ml L^{-1} of commercial product, following the technical recommendation for ground spraying. Water was used as the diluent for preparing the solutions.

Growth parameters

Growth parameters were evaluated 94 d after planting, when the plants were at phenological stage 69, corresponding to full bloom (Hack *et al*., 1993). At this stage, plants achieve their maximum fresh and dry mass gain as well as the largest leaf area.

To determine the leaf area index (LAI), leaf area was measured using an electronic leaf area meter (Area Meter CI-202, CID Bio-Science, Inc., USA). For this, two plants per experimental unit were selected, and all leaves from each plant were removed for measurement. Leaf area was expressed in square meters (m^2), and the number of plants per 1 m^2 was also determined. The obtained values were used to calculate the LAI using Equation 1 proposed by Reis *et al*. (2013):

$$\text{LAI} = \frac{(\text{LA} \times \text{NP})}{\text{TA}} \tag{1}$$

where LAI is in m^2 m^{-2}, LA is the average leaf area of two plants (m^2), NP is the number of plants per m^2, and TA is the total area considered (1 m^2).

Fresh weight gain was evaluated on four plants from the center of each experimental unit. These plants were placed in paper bags with a capacity of 10 kg, properly labeled according to treatment. The plants were then separated into root and above-ground parts (stems + leaves) and weighed using an Acculab VIC 612 electronic balance with a precision of 0.01 g. Subsequently, the samples were dried in a Memmert oven at 65°C until reaching a constant weight (approximately 96 h) to determine dry weight gain. The analyses were conducted at the Plant Physiology Laboratory of the Pedagogical and Technological University of Colombia.

Productivity parameters

For the productivity evaluation, all tubers were collected from each experimental unit and then placed in white fiber bags with a capacity of 50 kg. They were commercially classified into two categories according to the Colombian technical standard NTC 341 (ICONTEC, 1996). The category of first quality (Quality 1) corresponds to tubers with a diameter of 65 to 90 mm, and the category of second quality (Quality 2) corresponds to tubers with a diameter of 45 to 64 mm. The fresh weight data of tubers obtained from each experimental unit was extrapolated to obtain productivity expressed as yield in tons per ha (t ha^{-1}).

Statistical analysis

The obtained data were tested for normal distribution of residuals and homogeneity of variances using the Shapiro-Wilk test ($P \geq 0.05$) and Bartlett test ($P \geq 0.05$), respectively. Once the assumptions were confirmed, hypotheses were evaluated for each of the variables assessed through an analysis of variance (ANOVA). Finally, a multiple mean comparison test was conducted using the Tukey test ($P \leq 0.05$). The analyses were performed using the 'agricolae' package of the statistical software R Core Team (2022).

Results and discussion

Growth variables

The variables of fresh and dry weight were significantly affected ($P \leq 0.05$) by the application of biostimulants (Fig. 1). In the Hormonal treatment, the fresh weight of the roots, the aerial part, and the total were 124.8±3.4 g, 675.7±17.6 g, and 800.5±18.7 g, respectively, with gains of 65%, 14%, and 16%, respectively, compared to the control treatment, which had values of 75.4±2.3 g, 595.2±12.6 g, and 690.1±8.3 g, respectively (Fig. 1A).

In the carboxylic acids treatment (Carboxylic A), the dry weight of the roots, the aerial part, and the total plant weight showed values of 35.8±0.9 g, 116.2±2.9 g, and 152.0±3.8 g, respectively, with gains of 2%, 41%, and 29%, respectively, compared to the control treatment, which had values of 35.3±1.6 g, 82.3±1.0 g, and 117.6±1.1 g, respectively (Fig. 1B).

Fresh weight is considered a good estimator of plant volume, as water is the main component of all organs and tissues, while dry weight is a good estimator of the total carbon content of the plant, allowing for the analysis of plant physiology (Di Benedetto & Tognetti, 2016). Increases in fresh weight may be related to changes occurring in the organization and cellular metabolism of plants grown under the influence of biologically active substances or products, as these substances regulate nutrient absorption and translocation and alter the phytohormone levels (Falcón Rodríguez *et al*., 2015).

The results with the hormonal treatment are attributed to the ratio between kinetin, indole-3-butyric acid, and GA_3 in

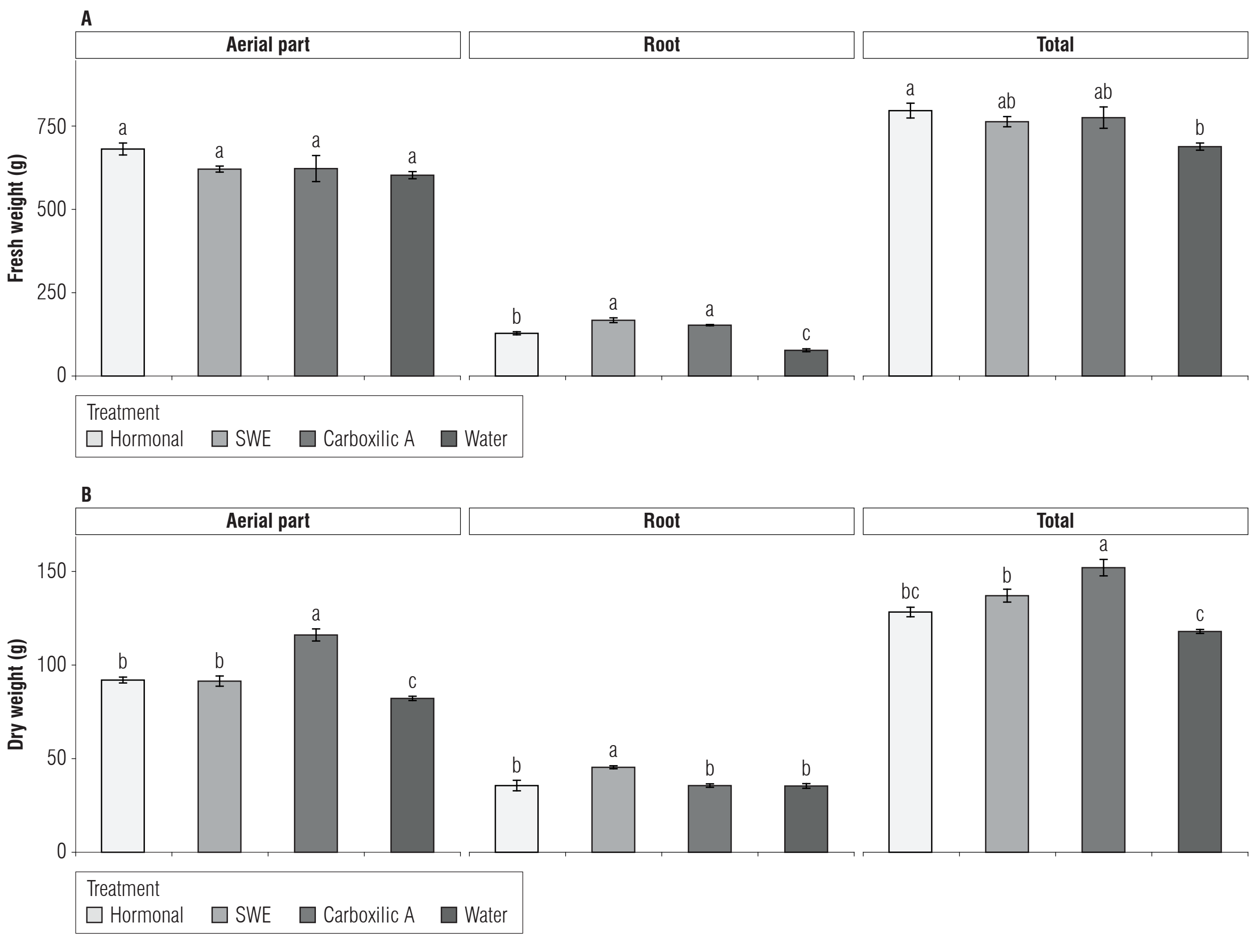

FIGURE 1. Fresh weight (A) and dry weight (B) of potato plants variety CIP 39 under the application of different types of biostimulants. Different letters indicate significant differences between treatments according to the Tukey mean test ($P \leq 0.05$). Vertical bars represent standard error (n=3). SWE – seaweed extract, Carboxylic A – carboxylic acids.

the applied product, which preferentially stimulates growth of roots, leaves, and stems. This could be related to its action on hormonal balance (Asari *et al*., 2017). One of the most widespread uses of commercial biostimulants is as growth promoters. These morphological responses are frequently attributed to the activity of endogenous auxins and cytokinins in treated plants (Sharma *et al*., 2014; Wally *et al*., 2013) or exogenous ones present in the extracts (Vinoth *et al*., 2019), which often contain fractions of polysaccharides that could induce root growth similarly to synthetic auxins (Hernández-Herrera *et al*., 2016).

Biostimulants are primarily derived from a variety of organic materials, including humic, fulvic, and carboxylic acids, among others (Drobek *et al*., 2019; Zuzunaga-Rosas *et al*., 2023). The treatment with the application of carboxylic acids favored both root and shoot growth (Fig. 1). The weight gain obtained with the application of organic biostimulants may be attributed to their promotion of the absorption of macro and micronutrients, boosting metabolic activity. This makes root absorption more efficient and regulates the nutrient absorption activity of the rhizosphere by stimulating activity of H^+ ATPases in the plasma membrane. These enzymes convert the free energy released by ATP hydrolysis into an electrochemical potential across the membrane, which is used for the uptake of nitrate and other nutrients by roots (Canellas *et al*., 2015; Drobek *et al*., 2019; du Jardin, 2015).

Leaf area index (LAI)

The application of a biostimulants resulted in significant differences ($P \leq 0.05$) compared to the control treatment (Fig. 2). The treatments with seaweed extract (SWE), carboxylic acids (Carboxylic A), and hormonal treatment resulted in mean values of 2.4±0.04 m^2 m^{-2}, 2.3±0.12 m^2 m^{-2}, and 2.2±0.06 m^2 m^{-2}, respectively. In contrast, the control treatment (water) presented the lowest LAI with a mean value of 1.6±0.02 m^2 m^{-2}.

The plant is essentially a capturer of solar energy, which is stored in the form of carbohydrates. This process takes place in leaves, from which carbohydrates are then mobilized towards the tubers (storage organs). The LAI is closely related to the plant's ability to intercept solar radiation, directly associated with the processes of photosynthesis and transpiration. These processes are directly linked to biomass accumulation and productivity (Hernández-Hernández *et al*., 2011). LAI is a fundamental parameter for evaluating crop growth and development, as it can be

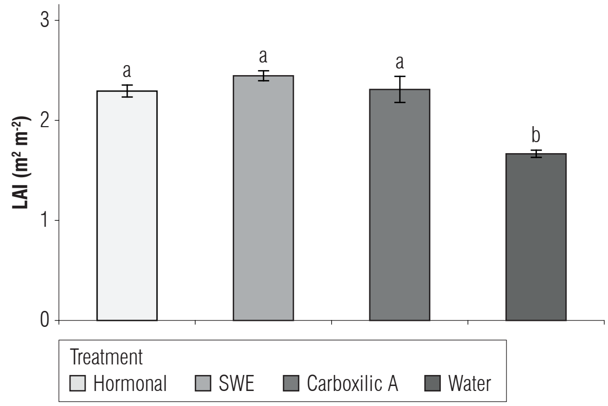

FIGURE 2. Leaf area index (LAI) of potato plants variety CIP 39 under the application of biostimulants. Different letters indicate significant differences according to the Tukey test ($P \leq 0.05$). Vertical bars represent standard error (n=3). SWE – seaweed extract, Carboxylic A – carboxylic acids.

used to estimate water and nutrient requirements as well as bioenergetic efficiency (Reis *et al*., 2013). Therefore, LAI is a useful variable for quantifying crop growth and agronomic performance (Mendoza-Pérez *et al*., 2017).

Dry matter production is commonly related to the plant's capacity to increase its leaf area; therefore, a larger leaf area will result in greater dry matter accumulation. However, this is not always the case, as the optimal leaf area index is the one that maximizes the dry matter accumulation rate. This is achieved when the crop intercepts nearly all available photosynthetically active radiation (PAR) and, consequently, the lower leaf layers are still capable of maintaining a positive carbon balance (Bergamaschi *et al*., 2010; Hunt, 2016).

Polysaccharides extracted from macroalgae cell walls and their derived oligosaccharides can enhance growth (Zou *et al*., 2019). This is because seaweeds affect plant metabolism and physiology, as their extracts possess growth inducers and/or trigger differential expression of genes involved in the synthesis of endogenous phytohormones and other primary metabolism pathways (Ghaderiardakani *et al*., 2019).

According to Santos *et al*. (2010), who evaluated four potato cultivars, the highest LAI values were registered during flowering, with values of 2.8 m^2 m^{-2}. The application of SWA resulted in an LAI of 2.4±0.04 m^2 m^{-2}, which generated a better balance between the plant's ability to intercept light and photosynthesis processes, leading to increased crop productivity (Figs. 3 and 4).

Productivity variables

Yield by commercial grades showed statistical differences between the treatments ($P \leq 0.05$). For Quality 1 tubers, with diameters of 65 to 90 mm, the SWE treatment exhibited a 17.6% increase with a mean value of 40.7±0.36 t ha^{-1}, compared to the control which had a value of 34.6±0.52 t ha^{-1} (Fig. 3). For Quality 2 tubers, with a diameter of 45 to 64 mm, the treatment based on carboxylic acids showed an 80% increase with a mean value of 7.4±0.6 t ha^{-1}, compared to the control, which had a value of 4.07±0.6 t ha^{-1} (Fig. 3).

Total yield analysis had a significant increase ($P \leq 0.05$) compared to the control treatment. SWE treatment had an 18.2% rise in total yield, averaging 46.7±0.75 t ha^{-1}, compared to the control yield of 39.4±1.6 t ha^{-1} (Fig. 4).

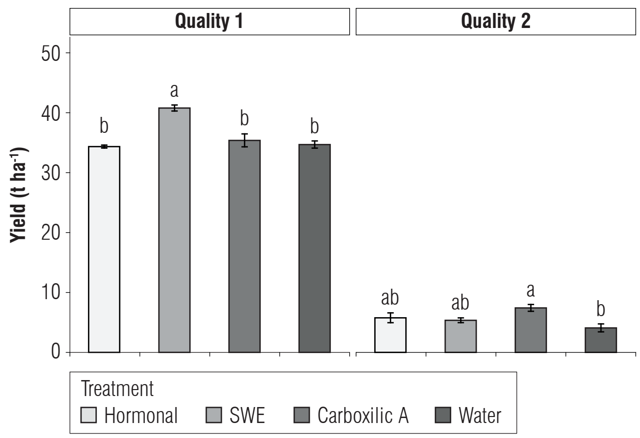

FIGURE 3. Yield by grades in potato plants variety CIP 39 subjected to different types of biostimulants. Identical letters among treatments indicate no significant differences according to the Tukey mean test ($P \leq 0.05$). Vertical bars represent standard error (n=3). Quality 1: tubers with a diameter of 65 to 90 mm; Quality 2: tubers with a diameter of 45 to 64 mm (ICONTEC, 1996). SWE – seaweed extract, Carboxylic A – carboxylic acids.

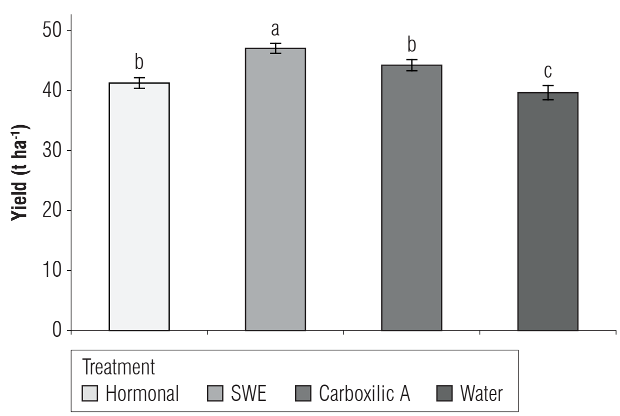

FIGURE 4. Total yield in potato plants (*Solanum tuberosum* L.) variety CIP 39 subjected to different types of biostimulants. Identical letters among treatments indicate no significant differences according to the Tukey mean test ($P \leq 0.05$). Vertical bars represent standard error (n=3). SWE – seaweed extract, Carboxylic A – carboxylic acids.

The application of biostimulants at different stages of crop growth can improve yield per plant by triggering a series of physiological and biochemical events in plants that result in increased production (Martínez *et al*., 2017).

Extract-based biostimulants promote many physiological processes in the plants, including photosynthesis. These biostimulants contain amino acids such as alanine and glycine, which enhance photosynthesis and also play a role in the synthesis of porphyrins, structural pillars of chlorophyll and cytochromes. This enhances plant activity, increasing reserve substances that are translocated to different parts of the plants, such as storage organs (Díaz *et al*., 2020; Ertani *et al*., 2018) .

Various studies confirm the positive effects of seaweed-based biostimulants on crop performance and post-harvest quality. According to Abbas *et al*. (2020), foliar applications significantly increased bulb and neck diameter as well as yield per hectare in four onion cultivars and improved the contents of total soluble solids, ascorbic acid, nitrogen, potassium, and phosphorus. Similarly, Yao *et al*. (2020) indicate that seaweed-based products significantly increased the net yield of *Solanum lycopersicum* L. by 6.9% compared to the control and positively affected fruit firmness and soluble sugar content.

However, the effects of biostimulants are not always consistent, as they depend on the plant species, the sensitivity thresholds to one or more bioactive molecules, as well as the different extraction procedures that ensure the purity and quality of the bioactive compounds in the products.

Conclusions

The application of biostimulants based on seaweed generated a positive effect on plant growth, improving the accumulation of both fresh and dry biomass, as well as the leaf area index (LAI). This led to an increase in both the quantity and quality of yield components. This suggests that this practice is an alternative worth considering within agronomic management plans for potato cultivation in different production zones of the country.

Acknowledgments

We would like to thank the agricultural engineers Ana Aydeé Figuero, Javier Martinez Balcazar, Herlyn Giovanni Martinez Anzola for their support in the development of this research, and the Pedagogical and Technological University of Colombia for their logistical support, infrastructure, and laboratory resources. The authors do not

endorse, nor do they disapprove the use of the biostimulant formulations and/or chemical products mentioned in this article. The authors have no affiliation with the biostimulant manufacturing companies or organizations whose products they review as treatments in the experiment.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

JDMA: research, writing - original draft, visualization, writing, and editing. EHPS: conceptualization, writing, formal analysis, data curation, and supervision. DFTH: writing, editing, and supervision. All authors have read and approved the final version of the manuscript.

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Photosynthesis in fruit crops of the high tropical Andes: A systematic review

La fotosíntesis en los cultivos frutales de trópico alto de los Andes: una revisión sistemática

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ABSTRACT

Commercially grown fruit crops in the high tropical Andes zones from 1,600 to 3,200 m a.s.l. are increasingly important in the world market, mainly because they are exotic fruits, and also because they are produced by hundreds of small growers. Photosynthesis is one of the most important physiological processes involved in the production and quality of fruit crops. However, many aspects of this process are unknown in fruit species grown in the Andean highlands. This systematic review presents the main themes and advances in research on photosynthesis of Andean fruit crops. A systematic literature search was carried out in the Scopus and Web of Science databases using the RStudio Bibliometrix package tool and VOSviewer version 1.6.16 software. Research on this topic has focused on high tropical Andean countries with climatic conditions for the growth of fruit species. Notably, the research addresses themes related to the photosynthesis of Andean highland fruit crops in Brazil and Colombia. The authors cover research topics from horticulture and plant physiology to photosynthesis and leaf anatomy and acclimation, where most research literature currently focuses. In most of the analyzed fruit crops, photosynthetic parameters such as maximum photosynthesis (A_{max}), light compensation point, light saturation point, and apparent quantum yield are known. These are important advances in the knowledge of the fluorescence of chlorophyll *a*, which is mainly used as a tool to characterize the eco-physiological response of these fruit species to different environments.

Key words: gas exchange, chlorophyll fluorescence, light, chloroplasts, ecophysiology.

RESUMEN

Los frutales cultivados comercialmente en las zonas de trópico alto andino, de 1.600 a 3.200 m s.n.m., son cada vez más importantes en el mercado mundial, principalmente porque se consideran frutas exóticas nutritivas, y también porque son producidas por cientos de pequeños cultivadores. La fotosíntesis se considera uno de los procesos fisiológicos más importantes involucrados en la producción y calidad de los cultivos de frutales, pero muchos aspectos de este proceso son desconocidos en las especies cultivadas en los Andes. Esta revisión sistemática presenta los principales temas y avances en la investigación sobre la fotosíntesis de cultivos de frutas andinas. Se realizó una búsqueda sistemática de literatura en las bases de datos Scopus y Web of Science utilizando la herramienta RStudio Bibliometrix y el software VOSviewer versión 1.6.16. La investigación sobre este tema se ha centrado en países de trópico alto andino con condiciones climáticas apropiadas para el crecimiento de estas especies frutales. Se abordan temas relacionados con la fotosíntesis de cultivos de frutas de alta montaña andina centrados en Brasil y Colombia. Los autores cubren temas desde la horticultura y la fisiología vegetal hasta la fotosíntesis, y tópicos como la anatomía foliar y la aclimatación, donde la mayor parte de la investigación se ha realizado recientemente. En la mayoría de los cultivos de frutas analizados, se conocen parámetros fotosintéticos como fotosíntesis máxima (A_{max}), punto de compensación de luz, punto de saturación de luz y rendimiento cuántico aparente, que son avances importantes en el conocimiento de la fluorescencia de la clorofila *a*, la cual se utiliza principalmente como herramienta para caracterizar la respuesta ecofisiológica de estas especies frutales en diferentes ambientes.

Palabras clave: intercambio de gases, fluorescencia de la clorofila, luz, cloroplastos, ecofisiología.

Introduction

Tropical altitude ecosystems are characterized by significant climatic changes in a single day, which can be much more pronounced than between the seasons throughout the year. Due to global warming, these ecosystems are attracting increasing attention (Fischer *et al*., 2024; Körner, 2007). As an area of primary diversity and richness of plant species, tropical countries provide a great range for the growth of fruit crops, and high tropical Andean regions, especially the Eastern Andes (Barthlott *et al*., 2005), provide adequate ecological niches for their cultivation (Ligarreto, 2012). The South American Andes are a mountain chain that extends from Chile and the north of Argentina to Venezuela, with an average altitude of 3,000 to 4,000 m a.s.l. (Guerrero *et al*., 2011). The tropical Andes contain

Received for publication: April 10, 2024. Accepted for publication: July 29, 2024. Doi: 10.15446/agron.colomb.v42n2.113887

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15% of the planet's biodiversity (Peyre *et al.*, 2019) due to their very particular environmental conditions which are influenced by their proximity to the equator, unlike other mountain ranges such as the Himalayan mountains. This review focuses on the tropical Andean fruit species which grow well between 1,600 and 3,200 m a.s.l.

Climatic conditions are the main factor in the adaptation of fruit crops to higher elevations, specifically between 1,600 and 3,200 m a.s.l. At these altitudes, the temperature and partial pressure of gases decrease and solar radiation, especially ultraviolet (UV), increases (Fischer, Orduz-Rodríguez *et al.*, 2022; Fischer, Parra-Coronado *et al.*, 2022). Within the spectrum of daylight, UV-B radiation (280-315 nm) has the greatest energy, and its high incidence can damage macromolecules, including DNA (Jenkins *et al.*, 2009). In addition, as reported by Terfa *et al.* (2014), UV-B impacts the morphology, biochemical composition, and molecular response of high tropical Andean plants. For each increase of 100 m of tropical altitude, the temperature descends in a range of 0.6 to 0.7°C (Benavides *et al.*, 2017). This extends the phenological cycles of the fruit crops, allowing the cultivation of other fruit species from subtropical and temperate zones.

The commercially grown fruit species that can adapt to tropical altitude conditions generally originate from the same Andean zone. According to Fischer *et al.* (2024), these include the Solanaceae species, such as tree tomato (*Solanum betaceum*; 1,700-2,600 m a.s.l.), cape gooseberry (*Physalis peruviana*; 1,800-2,800 m a.s.l.), and lulo or naranjilla (*Solanum quitoense*; 1,600-2,400 m a.s.l.) as well as the Passifloraceae fruit species, which includes gulupa or purple passion fruit (*Passiflora edulis* f. *edulis* Sims; 1,600-2,300 m a.s.l.), sweet granadilla (*Passiflora ligularis*; 1,800-2,600 m a.s.l.) and curuba or banana passion fruit (*Passiflora tripartita* var. *mollissima*; 1,800-3,200 m a.s.l.); the Myrtaceae species feijoa (*Acca sellowiana*; 1,800-2,700 m a.s.l.); the Rosaceae species mora or Andean blackberry (*Rubus glaucus*; 1,500-2,600 m a.s.l.); and the Ericaceae species agraz or Andean blueberry (*Vaccinium meridionale*; 2,200-3,200 m a.s.l.). These fruit species, produced mainly in Brazil and the Andes of Colombia, Peru, and Ecuador, are projected to be an important and healthy contribution to global food consumption (Fischer & Miranda, 2021; Viera *et al.*, 2019). They are known as exotic fruits in other parts of the world and are classified as important functional foods (Campos *et al.*, 2018; Moreno *et al.*, 2014). Exported in significant quantities, they are increasingly important for the economy of small producers in the Andean countries, who are mainly responsible for their production (Moreno-Miranda *et al.*, 2019; National Research Council, 1989).

Research on Andean highland fruit crops is less advanced compared to studies on tree fruits from lowland tropical valleys, subtropical, and temperate regions. It has mainly focused on agronomic aspects related to production and quality (Fischer, 2012; Fischer *et al.*, 2016; Fischer, Orduz-Rodríguez *et al.*, 2022; Fischer, Parra-Coronado *et al.*, 2022). Research on physiology, especially photosynthesis, has focused on the improvement of productive systems and abiotic stress (Castañeda-Murillo *et al.*, 2022; Ramírez-Soler *et al.*, 2021; Sánchez-Reinoso *et al.*, 2019). However, research on photosynthesis and related topics on fruit crops from the high tropical Andean has not been consolidated in an article that allows detailed analysis of the advances achieved and the aspects that have still not been investigated.

Photosynthesis is the defining physiological process that determines the maximum achievable yield of crops and drives life on the planet (Vishwakarma *et al.*, 2023). More than 90% of biomass and crop yield derives from photosynthesis, with the rest coming from absorbed and assimilated mineral nutrients (Vishwakarma *et al.*, 2023). This process is vital for the growth and survival of practically all plants during most of their growth cycle (Lambers & Oliveira, 2019). Photosynthesis involves processes of light reactions and carbon fixation. In the first part of the process, light reactions take place in the complexes present in the chloroplast thylakoid. There, photosynthetically active radiation (PAR, 400-700 nm) is absorbed to boost the transport of electrons derived from water photolysis, releasing O_2 and producing NADPH and ATP (Taiz *et al.*, 2017). These molecules are then used in the second part of the process, when the carbon photosynthetic reduction cycle, or Calvin-Benson cycle, takes place in the stroma of the chloroplasts (Silva *et al.*, 2020; Taiz *et al.*, 2017). This synthesizes triose phosphate (Fig. 1), compounds of 3 carbons that are fundamental for producing hundreds of biomolecules in plants.

The photosynthetic process requires an adequate supply of water, nutrients, and CO_2, as well as favorable temperature and light conditions (Lambers & Oliveira, 2019; Silva *et al.*, 2020). When these conditions are unfavorable for the photosynthesis process, photochemical quenching (QP) occurs, and the plant must activate several mechanisms to protect the photosynthetic device (Castañeda-Murillo *et al.*, 2022). Several of these mechanisms involve the functioning of photosystem (PSII); these include the dissipation of excess energy in heat or non-photochemical quenching

(NPQ), or dissipation in the form of fluorescence, with a greater wavelength and lower energy than is dissipated by chlorophyll *a* (Lambers & Oliveira, 2019). Knowledge of these energy dissipation mechanisms has been fundamental in elucidating the physiological status of plants and is considered a key tool for the diagnosis and management of biotic and abiotic stress in different crops, including fruit plants (Castañeda-Murillo *et al*., 2022; Chávez-Arias *et al*., 2019, 2020; Sánchez-Reinoso *et al*., 2019).

Given the above, this review aims to systematically analyze the state of knowledge of the process of photosynthesis in high tropical Andean fruit crops, enabling the consolidation of research progress on this subject and the determination of priorities for future studies. This approach will facilitate continued advancement in the improvement of the productive systems of these fruit species. Moreover, according to Li *et al*. (2018), the regulation of photosynthesis can provide novel solutions to increase yields.

Bibliometric analysis

A systematic review was carried out in the Scopus and Web of Science (WoS) databases. The search equation was defined as TITLE-ABS-KEY ("cape gooseberry" OR "passion fruit" OR "lulo" OR "tree tomato" OR "feijoa" AND photosynthesis AND NOT "yellow passion fruit", following Pullin and Stewart (2006). Yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) was omitted due to its growth in the Colombian tropical warm climate of the Andean lowlands. The search terms were then evaluated. The downloaded Scopus and WoS databases were combined into a single Excel file and, using the RStudio Bibliometrix package tool, the bibliometric parameters were analyzed. The timespan with the most scientific contributions in photosynthesis and development of high tropical Andean fruits was 2000-2023. The VOSviewer version 1.6.16 software types were used to determine bibliometric networks such as co-occurrence maps.

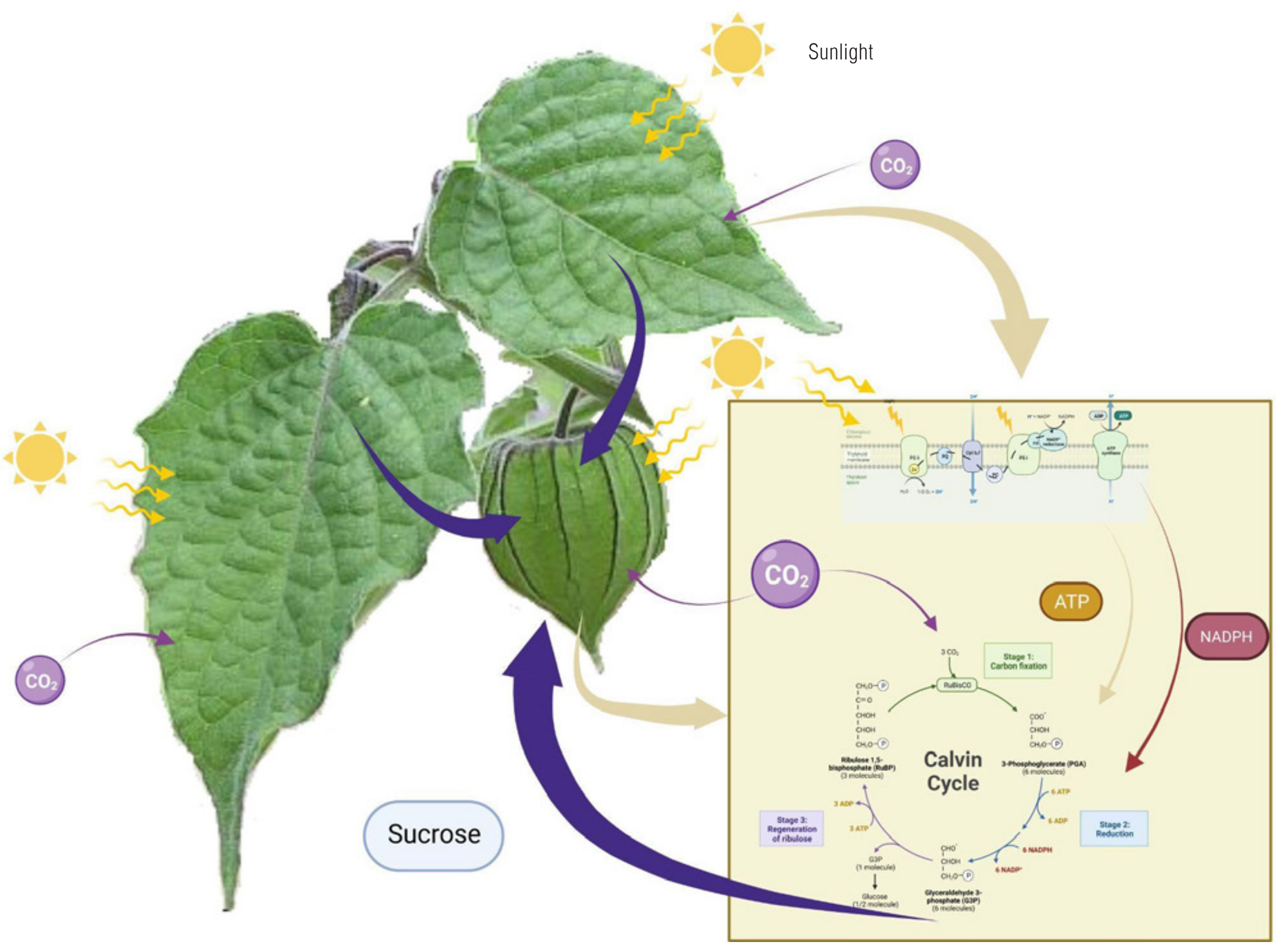

FIGURE 1. General schematic photosynthesis process illustrated for cape gooseberry (*Physalis peruviana* L.), a high-altitude tropical fruit plant. In this species, the calix covers the fruit during development and can perform photosynthesis. In the photo phase, NADPH and ATP are produced, which will then be used in the Calvin cycle to synthesize sugars.

Scientific publications per country

Figure 2 shows Brazil as the most significant producer of scientific publications on photosynthesis in the Andean fruit crops. The blue colors indicate that the People's Republic of China ranks second in research on this topic. Colombia was third place in scientific publications on the Andean highland fruits. Finally, Germany and Egypt have been important contributors to research in foliar gas exchange in Andean fruit species. It is interesting to note that Ecuador, an important country in the production of Andean fruits, is not included in this statistic, suggesting that the results of their studies are not frequently published in international journals.

Co-occurrence and centrality analysis

Network analysis helps to depict and graphically visualize the structure of a relationship in social networks, natural phenomena, and biological systems (Bilen *et al*., 2022). In this study, the network map shows three groups of interrelated keywords. The blue node groups themes concerning the leaf anatomy, native fruits, and acclimatization. The green node associates themes related to water absorption, solar radiation, roots, and water use efficiency. Finally, the red node associates themes related to plant physiology, horticulture, and salt stress (Figs. 3 and 4).

Research on leaf photosynthesis of Andean fruit species was subjected to bibliometric analysis for the period from 2000 to 2023 (Fig. 5). The themes of plant physiology, horticulture, and salt stress first appeared in early 2014. By 2022, the focus had shifted to solar radiation, mulching, water use efficiency, water absorption and roots, while for 2023 the scientific focus was related to leaf anatomy, native fruits, and acclimatization.

The ranks of centrality and density are important tools for analyzing keywords of authors (Herrera-Viedma *et al*., 2016). Centrality is the degree of interaction of the topic of interest with other research topics, while density is the internal strength of the research topic (Salleh & Bushroa, 2022). Figure 5 shows the author keywords divided into four main clusters. The first cluster, namely plant leaf, genetic variation, and plant hormone, had a low density and high centrality (Basic Themes Quadrant), which indicates weak development; however, these were important topics in the research field. The second cluster, photosynthesis, article, and biosynthesis had high centrality and density (Motor Themes Quadrant). These are the most developed and important topics in the field of photosynthesis in Andean fruit crops. The third cluster, *Passiflora*, metabolism, and light, had developed internal links (high density) but low centrality, that is, of limited importance for the field (the Niche Themes quadrant) (Della Corte *et al*., 2019). Finally, the fourth cluster, *Passiflora edulis*, physiological response, and fruit, had low centrality and density, indicating weak and marginal development in the field.

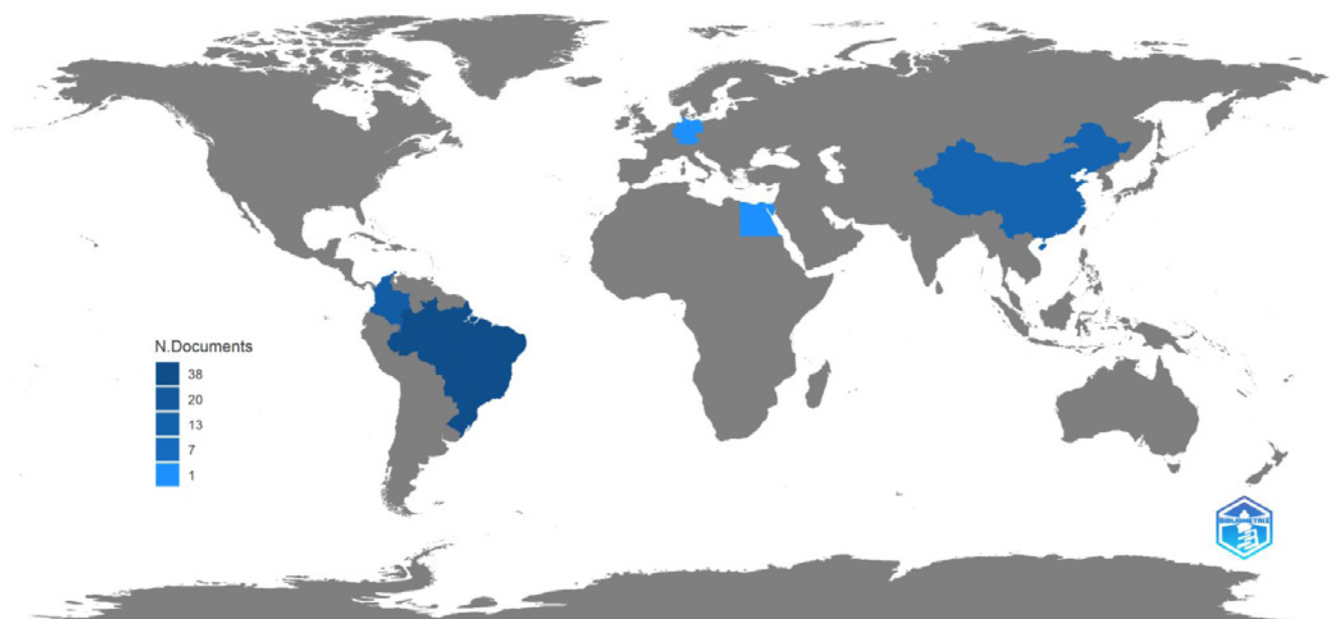

FIGURE 2. Country scientific production in topics related to photosynthesis in high tropical Andean fruits. The intensity of the blue color indicates a higher number of articles published during the 2000-2023 period.

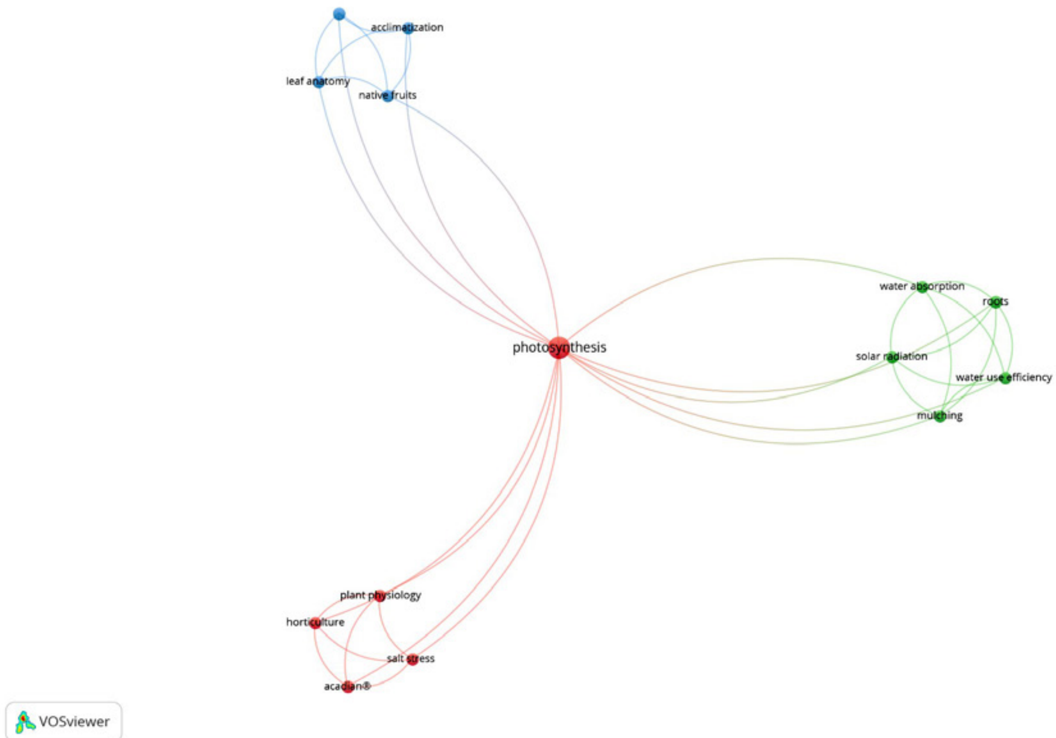

FIGURE 3. Network map based on co-occurrence of terms on titles and abstracts related to photosynthesis in high tropical Andean fruits. Colors indicate clusters of related terms identified by VOSviewer.

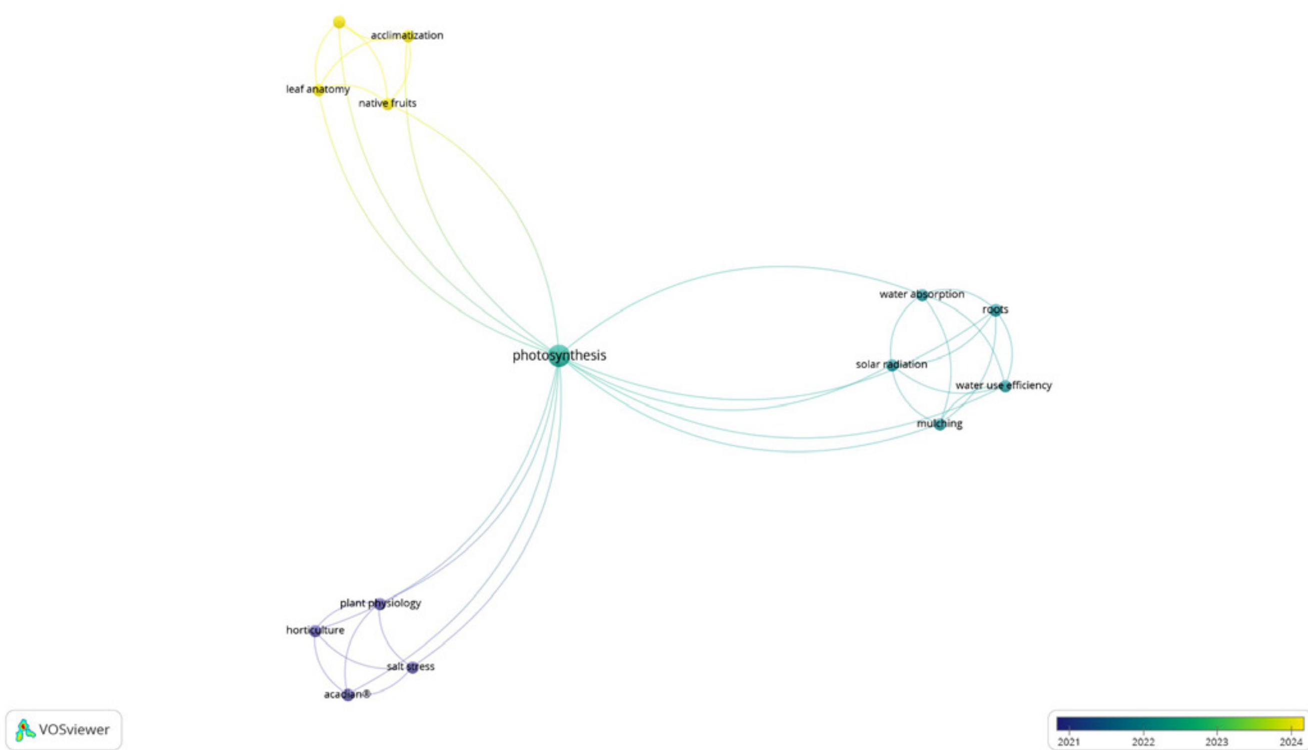

FIGURE 4. Network map based on co-occurrence over time of the terms presented on titles and abstracts related to photosynthesis in high tropical Andean fruits.

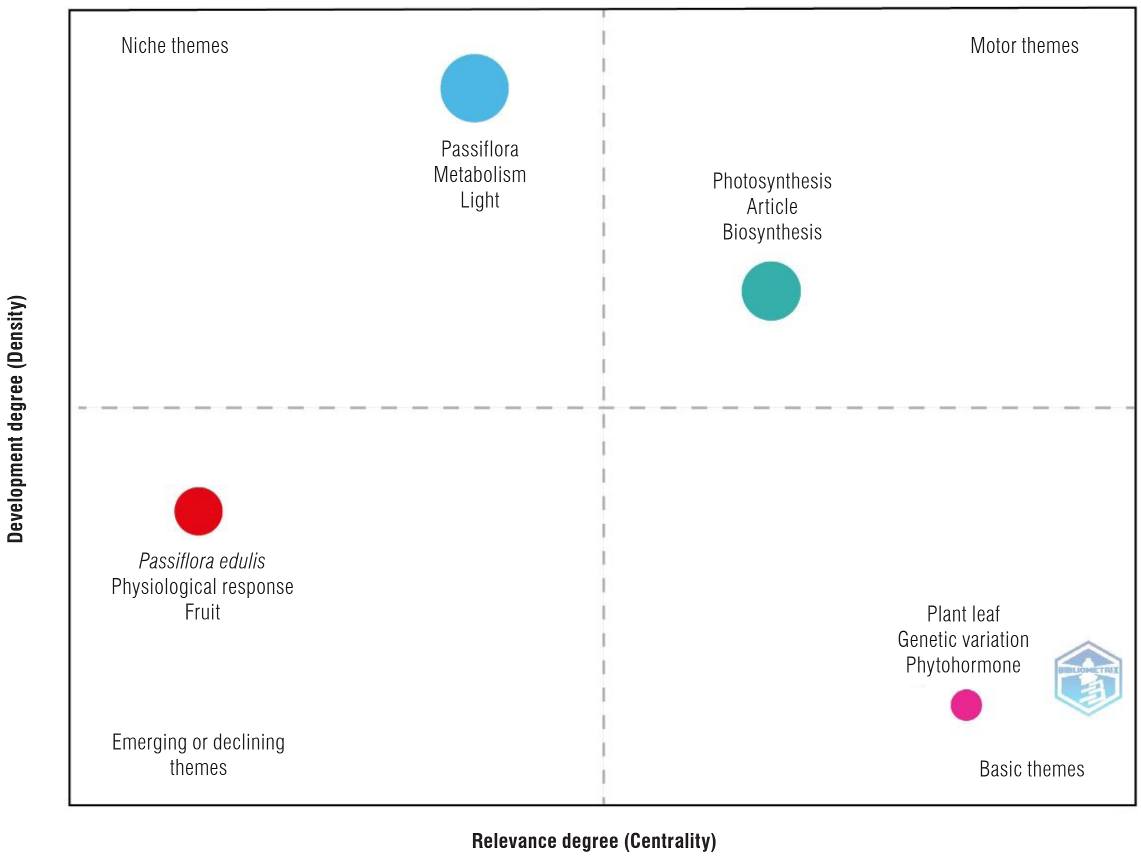

FIGURE 5. Cluster of the author keywords related to photosynthesis in high tropical Andean fruit species. Each bubble represents a network cluster, and the bubble position is set according to the cluster's Callon centrality and density.

Main aspects of photosynthesis in Andean highland fruit crops

Solanaceae fruits

Cape gooseberry

The cape gooseberry (*Physalis peruviana* L.) is native to the South American Andes. In Colombia, the widely planted material is the regional ecotype known as Ecotipo Colombia. Two important commercial varieties are also reported, Agrosavia Dorada and Agrosavia Andina (Nuñez-Zarantes *et al*., 2024). The cape gooseberry is a C3 photosynthetic metabolism plant. It belongs to the Solanaceae family (nightshade) but also develops and is produced under direct sun, as is the case for commercial plantations in Colombia (Fischer *et al*., 2024; Fischer & Melgarejo, 2020). For this reason, Carrillo-Perdomo *et al*. (2015) cataloged it as a light-demanding plant. A special characteristic of this fruit is the calyx that covers it throughout its development and in its green state, together with the two adjacent leaves; these are the most important sources for the production and translocation of carbohydrates to the fruit during its development (Fischer *et al*., 2015). As tropical altitude increases, cape gooseberry plants develop a greater number of leaf stomata per leaf area to better compensate for the reduced partial gas pressure (CO_2, O_2) at higher elevations. In Colombia, this plant grows between 1,800 and 2,800 m a.s.l. (Fischer *et al*., 2024; Fischer, Parra-Coronado *et al*., 2022) and, in Ecuador, it grows at elevations up to 3,300 m. Its temperature and precipitation range are 13°C - 16°C and 1,000-1,800 mm per year, respectively (Fischer & Melgarejo, 2020).

Under open field conditions in Bogotá, Colombia (2,556 m a.s.l.), Fischer and Melgarejo (2020) reported an average rate of maximum photosynthesis A_{max} = 10.545 µmol CO_2 m^{-2} s^{-1}. This rate was measured within the range of 0 to 1,500 µmol photons m^{-2} s^{-1} (to 18°C and 400 ppm CO_2), using the Light Curve methodology (Light response curve methodology *A*/PFFD). Multiple measurements were made on leaves close to the source-sink, with an A_{max} between 8 and 15 µmol CO_2 m^{-2} s^{-1}; light compensation point I*c* = 13.645 µmol CO_2 m^{-2} s^{-1}; light saturation constant = 416 µmol photons m^{-2} s^{-1}; dark respiration Rd = 0.6496 µmol

CO_2 m^{-2} s^{-1}; and photosynthetic efficiency φ = 0.03011 µmol CO_2 µmol^{-1} photons (Tab. 1). Therefore, areas with solar radiation from 900 to 1,500 µmol photons m^{-2} s^{-1} are suitable for the cultivation of cape gooseberry, allowing high photosynthesis rates and, with optimal crop management, high yields and fruit quality (Fischer & Melgarejo, 2020).

In the above-mentioned study, the plants and leaves of the cape gooseberry in Bogotá (Colombia), with response curves of net photosynthesis to CO_2 concentrations between 0 to 600 Ci, had a maximum carboxylation rate of Rubisco V_{cmax} = 75.70 µmol CO_2 m^{-2} s^{-1} and a maximum regeneration rate of ribulose-1.5-bisphosphate, controlled by electron transport J_{max} = 288.09 µmol CO_2 m^{-2} s^{-1} (Fischer & Melgarejo, 2020). Additionally, through the measurements of the fluorescence of chlorophyll *a* under the same conditions, the cape gooseberry had a maximum potential quantum efficiency of PSII (*Fv/Fm*) of 0.82. This shows that there is no damage to photosystems and that the plant performed well in energy transduction, with no photoinhibition (Maxwell & Johnson, 2000). Other values of chlorophyll *a* fluorescence parameters include an electron transport rate (ETR) around 8.3 µmol electrons m^{-2} s^{-1} and QP close to 0.77 (Tab. 2) (Chávez *et al*., 2019).

Several studies on the cape gooseberry measured photosynthesis and/or fluorescence of chlorophyll to elucidate its response to stress conditions. Barbosa *et al*. (2019), applying irrigation indices of 50, 100, 125 and 150% of the reference evapotranspiration (ETo) in cape gooseberry plants, found that 125% and 150% of the ETo led to a lower stomatal restriction with a higher assimilation rate of CO_2, foliar transpiration and intrinsic water use (WUEi) than with a lower volume of irrigation. Segura-Monroy *et al*. (2015) reported for cape gooseberry that water deficit caused reductions in the chlorophyll index and stomatal density and an increase in the trichome density, while foliar application sprays with kaolin improved *Fv/Fm* and water use efficiency by reducing the leaf transpiration rate and the leaf temperature (Segura-Monroy *et al*., 2015).

TABLE 1. Photosynthetic parameters of various high tropical Andean fruit crops. Light compensation point (I*c*) (µmol photons m^{-2} s^{-1}), light saturation point (I*s*) (µmol photons m^{-2} s^{-1}), photosynthetic rate at light saturation (A_{max}) (µmol CO_2 m^{-2} s^{-1}), and efficiency of photosynthesis (ΦPPFD) measured under Colombian high-altitude conditions.

Species and conditions	I*c*	I*s*	A_{max}	ΦPPFD	Reference
Physalis peruviana, vegetative phase, Bogotá (2556 m a.s.l.)	13.65	~416	10.55	0.03011	Fischer & Melgarejo (2020)
Solanum betaceum, vegetative phase, Bogotá (2556 m a.s.l.)	35.1	-	6.8	-	Ramírez-Soler *et al*. (2021)
Passiflora edulis Sims, flowering, Tena municipality (2090 m a.s.l.)	13.37	419.54	19.93	0.042	Pérez Martínez & Melgarejo Muñoz (2015)
Passiflora edulis Sims, flowering, Granada municipality (2230 m a.s.l.)	15.31	1161.44	15.84	0.024	Pérez Martínez & Melgarejo Muñoz (2015)
Passiflora ligularis Juss., vegetative phase, Santa María municipality (2060 m a.s.l.)	39.63	285.04	19.44	0.036	Rodríguez-Castillo & Melgarejo (2015)
Passiflora ligularis Juss., reproductive phase, Santa María municipality (2060 m a.s.l.)	34.66	661.38	23.61	0.044	Fernández *et al*. (2014)
Passiflora tripartita var. *mollissima*, reproductive phase, Pasca municipality (2498 m a.s.l.)	19.07	584.78	16.09	0.031	Mayorga (2016)

TABLE 2. Parameters of chlorophyll *a* fluorescence of different high tropical Andean fruit crops measured in Colombia. *Fv/Fm*: maximum potential quantum efficiency of PSII, QP: photochemical quenching, NPQ: non-photochemical quenching, ETR: electron transport rate, ϕPSII: PSII operating efficiency.

Species and conditions	*Fv/Fm*	QP	NPQ	ETR (µmol electrons m^{-2} s^{-1})	PSII operating efficiency (ϕPSII)	Reference
Physalis peruviana	0.82	-	-	-	-	Fischer and Melgarejo (2020)
Physalis peruviana, vegetative phase, Bogotá (2556 m a.s.l.)	~0.77	~0.77	~1	~8.3	-	Chávez-Arias *et al*. (2019)
Solanum betaceum, vegetative phase, Bogotá (2556 m a.s.l.)	0.81	-	-	-	-	Ramírez *et al*. (2021)
Solanum quitoense, vegetative phase, Bogotá (2556 m a.s.l.)	~0.79	~0.47	~1.7	-	-	Castañeda-Murillo *et al*. (2022)
Passiflora edulis Sims, vegetative phase, Bogotá (2556 m a.s.l.)	0.81	-	-	-	~0.5-0.8	Cárdenas-Pira *et al*. (2021)
Passiflora ligularis Juss., reproductive phase, Santa María municipality (2060 m a.s.l.)	0.87	-	-	-	~0.35-0.55	Fernández *et al*. (2014)
Passiflora tripartita var. *mollissima*, reproductive phase, Pasca municipality (2498 m a.s.l.)	0.825	-	-	-	0.580-0.600	Mayorga (2016)

In cape gooseberry plants flooded for 4, 6 or 8 d, stomatal conductance (*gs*), photosynthetic pigments, chlorophyll fluorescence parameters (*Fv/Fm*, ETR, and QP) decreased as the waterlogging period increased. These effects were more pronounced in plants inoculated with *Fusarium oxysporum* f. sp. *physali*, indicating a low acclimatization to flooding conditions greater than 6 d in soils with *Fusarium* (Chávez-Arias *et al*., 2019). In another study on cape gooseberry stressed by inoculation with *Fusarium*, Chávez-Arias *et al*. (2020) observed that three applications of brassinosteroids, salicylic acid, and jasmonic acid promoted chlorophyll fluorescence, contents of photosynthetic pigments, *gs*, water potential (Ψw), proline synthesis, and plant growth.

Lulo

To date, two varieties of this plant have been reported. The first is *Solanum quitoense* var. *septentrionale*, which is a variety with thorns adapted to understory conditions. The second is *Solanum quitoense* var. *quitoense*, a thornless variety that adapts to areas with greater sun exposure (Ardila *et al*., 2015; Jaime-Guerrero *et al*., 2022). According to Jaime-Guerrero *et al*. (2022), the lulo (or naranjilla) grows better under shading and can present modifications in photosynthetic capacity when exposed to full sun. Excess solar radiation can cause photoinhibition (Fig. 6) (Sogamoso Alape, 2020). Under temperate conditions of Central Europe, supplemental lighting in winter increased flower and fruit numbers but not yield (Messinger & Lauerer, 2015). Under tropical Andean conditions, lulo plants grow well in ranges of 1,600-2,400 m a.s.l., temperatures between 16 and 24°C and precipitation ranging from 1,000 to 2,800 mm per year (Jaime-Guerrero *et al*., 2022; Paull & Duarte, 2012).

The lulo is a C3 photosynthetic plant that derives minimal benefit from additional light above 500 µmol m^{-2} s^{-1} of photonic flow (Ardila *et al*., 2015). Medina Cano *et al*. (2006) obtained net photosynthesis averages close to 8 µmol CO_2 m^{-2} s^{-1} during the first 84 d after transplanting. These same authors reported that the contents of chlorophyll

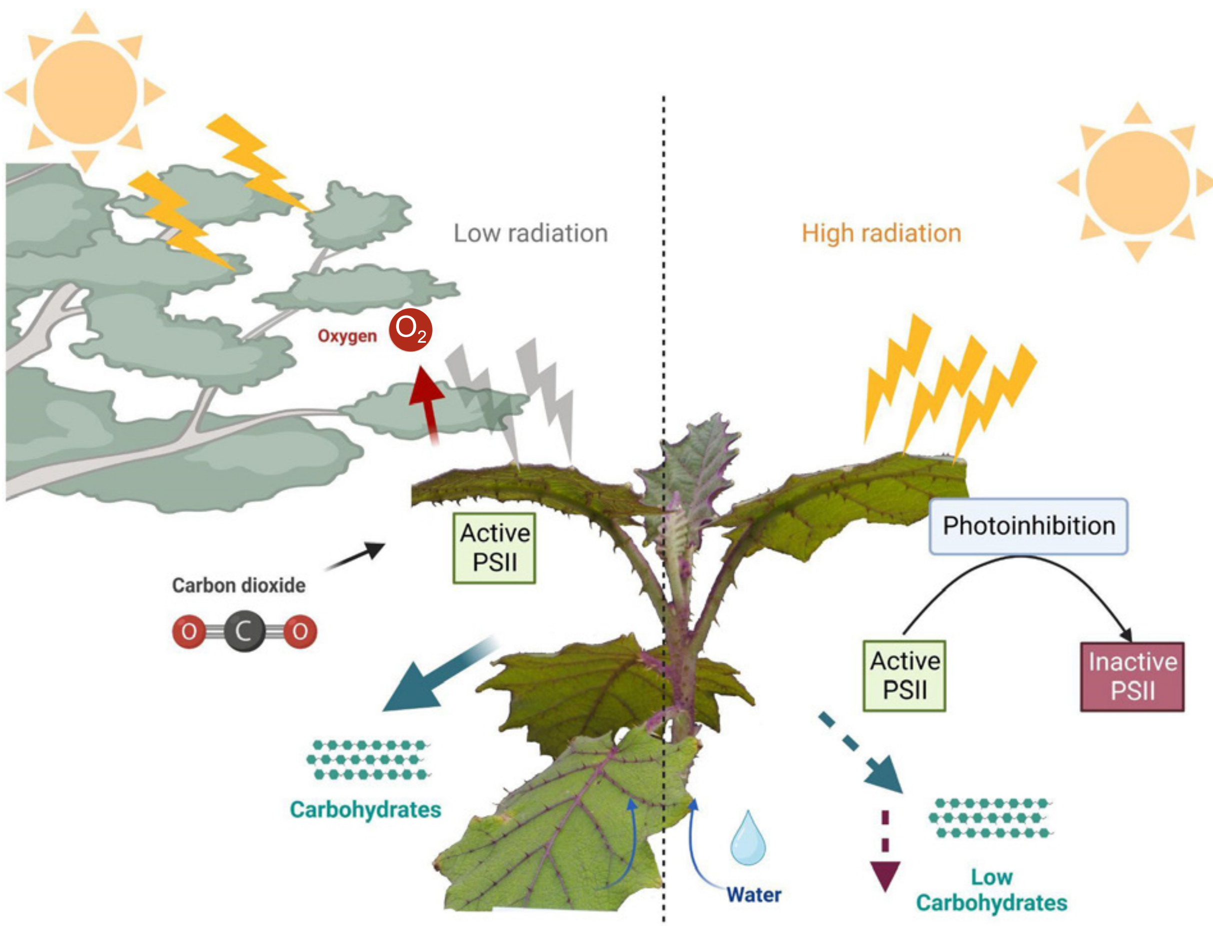

FIGURE 6. Schematic overview of photosynthesis process in lulo or naranjilla (*Solanum quitoense*) plants. Left side: Shadow condition available photosynthesis process, active PSII drives electron to ATP and NADPH for Calvin cycle reactions and carbohydrate synthesis. Right side: Light excess in non-shading conditions causes photoinhibition and reduces photosynthetic rates.

a, chlorophyll *b*, and total chlorophyll in lulo plants are higher in the transplanting stage (4.24, 1.14, and 4.33 mg g^{-1} of fresh weight (FW), respectively) than in production (1.66, 0.58, and 2.98 mg g^{-1} FW, respectively). This higher chlorophyll content during transplanting is attributed to the plants having a small photosynthetic area with high chlorophyll concentrations. Shade-grown plants, when transferred to high-radiation conditions, decrease their chlorophyll *a* content (Medina Cano *et al*., 2006).

Lulo with thorns presents higher contents of total protein and greater activity of Rubisco and PEP carboxylase compared to lulo without thorns (Medina Cano *et al*., 2006). During the development of these two types of lulo, the photosynthesis rates were similar. Greater photosynthesis rates were observed up to 84 d after transplanting to the field. These rates then decreased dramatically, followed by small increases. However, interestingly, photosynthesis was greater in the two upper leaf strata of the plants, which received more PAR, than the lower strata of the plants (Medina Cano *et al*., 2006). However, there are no reported photosynthetic parameters obtained from light curves in lulo.

The use of algae-based bio-stimulants in lulo plants is recommended. These treatments, both to the soil and soil + foliar, can increase the net photosynthesis rate to close to 6 µmol CO_2 m^{-2} s^{-1}, almost twice that reported for control plants. Bio-stimulants also generated a positive effect on *gs*, transpiration rate, efficient use of water (WUE), *Fv/Fm*, and chlorophyll content in leaves (Díaz-Leguizamón *et al*., 2016).

Regarding the light phase of photosynthesis, Sánchez-Reinoso *et al*. (2019) found that measuring chlorophyll *a* fluorescence is a useful tool to characterize the lulo seedlings under stress conditions in terms of maximum quantum efficiency of the PSII, the effective photochemical quantum yield of PSII and the QP. Castañeda-Murillo *et al*. (2022) report control values of *Fv/Fm* close to 0.79, QP~0.47 and NPQ~1.7 for lulo plants (Tab. 2).

Several factors affect photosynthesis in lulo plants, mainly those related to availability of soil water and light. Lulo is susceptible to waterlogging conditions, and this stress condition generates reductions in shoot length, stomatal conductance, plant transpiration, and leaf chlorophyll pigments (Flórez-Velasco *et al*., 2015). In addition, lulo plants are more susceptible to waterlogging stress than to shading as waterlogging leads to damage at the level of PSII and a decrease in the chlorophyll content. Plants with waterlogging stress under shading tolerated the stress more than those cultivated in full light (Sánchez-Reinoso *et al*., 2019). Interestingly, foliar N applications helped to mitigate the negative effects of waterlogging by increasing leaf chlorophyll concentration, *Fv/Fm* value, and nitrogen use efficiency (Flórez-Velasco *et al*., 2015).

Under water deficit conditions, for example, during the dry seasons of the year, lulo plants present a reduction in *Fv/Fm* ratio, leaf gas exchange properties, total biomass, and relative water content (Castañeda-Murillo *et al*., 2022). This indicates that water stress is a condition that negatively affects lulo plants, necessitating alternatives that mitigate these effects. In this regard, DI-31 (brassinosteroid analog) sprays enhance the photochemical efficiency of PSII, plant growth, and the concentration of photosynthetic pigments, and reduce lipid peroxidation of membranes under drought conditions (Castañeda-Murillo *et al*., 2022).

Tree tomato

The Solanaceae tree tomato (*Solanum betaceum* Cav.) is an important crop native to South America (Ramírez & Kalla-rackal, 2019). The best conditions for its commercial cultivation in Colombia are altitudes between 1,800 and 2,600 m a.s.l., temperatures between 13 and 20°C, and precipitation between 1,500–2,000 mm per year (Bonnet & Cárdenas, 2012). In subtropical zones, the crop can be grown down to sea level (Blancke, 2016). The plant does not resist prolonged drought, which particularly affects flowering due to its very superficial root system (Carrillo-Perdomo *et al*., 2015; Ramírez & Kallarackal, 2019). Additionally, warm temperatures affect the reproductive phases of this plant (Carrillo-Perdomo *et al*., 2015). Ramirez and Kallarackal (2019) mentioned different cultivars of tomato tree, including cv. Mora, cv. Mango, cv. Common, and cv. Common crossed with cucubo (*Solanum ovalifolium*).

A study conducted in Pasca (Cundinamarca, Colombia) at 2,452 m a.s.l. with tree tomato plants in the juvenile phase of growth using light curve methodology found a maximum photosynthesis rate of 17.477 µmol CO_2 m^{-2} s^{-1}, a light compensation point of 54.42 µmol photons m^{-2} s^{-1}, a saturation constant (K) of 613.08 µmol photons m^{-2} s^{-1}, and dark respiration of -0.0008 µmol CO_2 m^{-2} s^{-1} (Niño & Cotrino, 2015). Lower values in some of these parameters were observed in plants grown under the conditions of Bogotá (Colombia), with an A_{max} of 6.8 and a light compensation point of 35.1 µmol photons m^{-2} s^{-1}. However, in the photo phase, an *Fv/Fm* of 0.81 was reported (Tabs. 1 and 2) (Ramírez-Soler *et al*., 2021), indicating ecophysiological factors that affect the photosynthetic responses, which remain largely unexplored in tree tomato plants.

The growth of tree tomato plants could be affected by low nitrogen levels. A nitrogen concentration of 10 mg N L^{-1} results in an *Fv/Fm* ratio of 0.5 and a chlorophyll index (in SPAD units) of 12.7, while a level of 150 mg N L^{-1} results in a higher *Fv/Fm* ratio of 0.62 and a SPAD value of 37.5 (Betancourt-Osorio *et al*., 2016). In this same study, 5-d flood periods caused a 75% reduction in the leaf area and a 50% reduction in the nitrogen use efficiency; however, the flooding increased the partition of photoassimilates to the stem.

Potassium (K) is the second most important mineral nutrient absorbed by the tree tomato plants after nitrogen (N) (Clark & Richardson, 2002). Clavijo-Sánchez *et al*. (2015) found that tomato tree plants maintained with an optimal nutritional level of K^+ (applying 2.5 mm KCl) had better acclimatization to drought conditions since their WUE did not fall drastically despite having stomatal conductance and low transpiration. Also, Ramírez-Soler *et al*. (2021) confirmed the importance of K for the correct physiological functioning of the tree tomato plants, as deficient K (without KCl and KNO_3 in the nutrient solution) reduced the A_{max} (66%), I*c*, transpiration rate (E), *Fv/Fm*, and the contents of chlorophylls *a*, *b*, and total chlorophyll, but increased the stomatal resistance and the thickness of the upper and lower epidermis of the leaves. Lu *et al*. (2016) suggest that K deficiency decreases the photosynthetic rate due to the lower activity of Rubisco and the activation of the pyruvate kinase.

Passifloraceae fruits

Gulupa

Several ecophysiological studies in gulupa (purple passion fruit, *Passiflora edulis* f. *edulis* Sims) and other passion fruits confirm the adaptability of these to the climatic conditions of tropical regions (Mayorga *et al*., 2020; Rodríguez *et al*., 2019). In Colombia, this fruit species is well adapted to 1,600–2,300 m a.s.l., 15-22°C temperature and 1,800–2,300 mm precipitation per year (Ocampo & Posada, 2012; Ocampo *et al*., 2020).

Sánchez *et al*. (2013) found a positive correlation in gulupa between stomatal opening and temperature and solar radiation, while observing a negative correlation between relative humidity and stomatal opening. On average, they found 107 stomata per mm^2 leaf surface. The stomata are responsible not only for the control of the entry of CO_2 for photosynthesis but also for the optimization of WUE (Bergmann & Sack, 2007). In another study on the purple passion fruit "maypop" (*P. incarnata*), García-Castro *et al*. (2017) observed an exponential decrease in the photosynthetic rate when soil water potential becomes more negative than -1.0 MPa, although the plants promptly reestablished their gas exchange after being watered at 100%. Lozano-Montaña *et al*. (2021) showed that the gulupa plant prevents water loss under progressive drought stress by stomata closure, modulation of growth, and accumulation of proline and sugars in leaves, while promoting root growth, although total chlorophyll content in leaves decreased. These authors suggest that their results should be complemented by future studies on gas exchange analysis and measurements of fluorescence of chlorophyll *a*. The gulupa is classified as moderately tolerant to water stress (Crane *et al*., 2019) and displays isohydric behavior, avoiding water loss as a response strategy (Lozano-Montaña *et al*., 2021). Also, Jiménez-Bohorquez *et al*. (2024) found a reduction in *gs* in gulupa grafted on *Passiflora maliformis* when irrigation was reduced to 50% and 25%, while *Fv/Fm* was not affected.

Mineral nutrition is one of the most important factors that affect photosynthesis (Lambers & Oliveira, 2019; Rengel *et al*., 2023). Cárdenas-Pira *et al*. (2021) conducted a study using the missing element methodology on gulupa seedlings. They found that the seedlings subjected to Fe deficiency had the lowest A_{max} with 1.72 µmol CO_2 m^{-2} s^{-1}. They also found that a deficiency of Mg generated the least apparent quantum efficiency (0.008 µmol CO_2 µmol $photons^{-1}$), while the lack of P induced the lowest maximum photochemical efficiency values of photosystem II, *Fv/Fm* (0.69). The nutrient deficiencies, mainly of Fe or Mg, negatively affected photosynthesis in gulupa plants at the vegetative stage of growth (Cárdenas-Pira *et al*., 2021).

Salinity considerably affects the growth of *P. edulis* plants. With a dose of 150 mm of NaCl, growth was reduced due to negative effects on gaseous exchange parameters such as net photosynthetic rate (*A*), E and *gs*, and the synthesis of chlorophyll (Lima *et al*., 2020). Interestingly, *P. mucronata* exhibited better salt tolerance and maintenance of photosynthesis, conductance and stomatal functionality (Lima *et al*., 2020). Therefore, this species has a potential for use in improvement programs or as a rootstock for commercial passion fruit crops.

Rodríguez Castillo *et al*. (2020) described landraces of gulupa in Colombia characterized by minimal branching and longer internodes, requiring less pruning. In addition, the location of their leaves, with longer internodes, above the trellis system, provides better exposure of the canopy to sunlight and, thus, greater photosynthetic capacity. Also, in Cundinamarca, some gulupa landraces showed better adaptation to conditions in the high-elevation municipality of Susacón (2,500 m a.s.l.) than those in Pasca (1,800 m a.s.l.). Photosynthesis measurements confirmed these varieties' broad adaptive response to the highest zone, an important result given the need to cultivate gulupa at higher altitudes due to global warming (Rodríguez *et al*., 2019). Likewise, the authors concluded that these genotypes, under high-altitude tropical conditions, must present not only high efficiency in the use of water but also in photosynthesis, so that they adapt better to these colder and drier sites, compared to those of medium-sized elevations that are more humid and warmer.

Pérez Martínez and Melgarejo Muñoz (2015) compared three sites of gulupa cultivation in Cundinamarca (Colombia) and concluded that the municipality of Granada (2,230 m a.s.l., 15°C average temperature) offers conditions for optimal physiological performance due to differences in soil moisture content, vapor-pressure deficit (VPD) and temperature (day/night 18/13°C), as well as solar radiation ≤1,000 µmol photons m^{-2} and 0.5 kPa VPD. These conditions support the recovery of the foliar water status and photosystems, particularly when combined with a low VPD in the daytime. The cultivation sites affected the photosynthetic parameters (Tabs. 1 and 2), indicating the great importance of the environment for the physiology of gulupa. However, photosynthetic parameters also vary depending on the phenological stage of this fruit species (Pérez Martínez & Melgarejo Muñoz, 2015).

In addition, Tominaga *et al*. (2018) evaluated the overestimation of calculated $C_{i(c)}$ without stimulating stomatal closure. The researchers measured gas exchange and $C_{\mathrm{i(m)}}$ simultaneously in hypostomatous leaves of gulupa. They concluded that direct measurement of C_{i} is a more accurate estimate than the calculation when stomatal gas transport is restricted (Tominaga *et al*., 2018).

Sweet granadilla

Miranda (2020) reported that sweet granadilla (*Passiflora ligularis*), native to tropical America, has various ecotypes, including Criolla, Pecosa, Valluna, Urrao, Cascara de huevo, and Huila. In Colombia, sweet granadilla best adapts to elevations of 1,800-2,600 m a.s.l. (Miranda, 2020), with growth temperatures of 15-23°C and precipitation between 800 and 1,500 mm per year. Research on sweet granadilla aiming to characterize its ecophysiology at different altitudes in the Huila department (Colombia) found A_{max} of 23.6 µmol CO_2 m^{-2} s^{-1}, I*c* of 34.6 µmol m^{-2} s^{-1} (Tab. 1) and dark respiration of 2.24 µmol CO_2 m^{-2} s^{-1} with average temperature conditions of 17.15°C, and photosynthetically active radiation (PAR) of 1,186.2 µmol photons m^{-2} s^{-1} at altitudes of 2,060 m a.s.l. However, an altitude of 2,270 m a.s.l. reduced the photosynthetic performance of sweet granadilla, with A_{max} of 17.5 µmol CO_2 m^{-2} s^{-1}, dark respiration of 1.34 µmol CO_2 m^{-2} s^{-1}, and I*c* of 21 µmol m^{-2} s^{-1}, observed in plants without stress (Fernández *et al*., 2014).

These photosynthetic parameters are not only affected by the area in which the sweet granadilla is cultivated but also by the phenological state as well as the time of day. For more details on this, refer to Rodríguez and Melgarejo (2015).

Banana passion fruit

In Colombia, commercial banana passion fruit (*Passiflora tripartita* var. *mollissima*) cultivation is found in zones of 1,800–3,200 m a.s.l., with temperatures of 13-16°C, and precipitation between 1,000 and 1,500 mm per year. The main variety is Castilla (Campos & Quintero, 2012; Fischer *et al*., 2020a). The ecophysiology of banana passion fruit is not well studied (Mayorga, 2016). As in several other fruit species, the banana passion fruit shows an increase in total soluble solids in the fruits with the increase in altitude, which could be related to the higher photosynthetic activity in the leaves adjacent to the fruits due to the high luminosity at these elevations (Mayorga *et al*., 2020). Therefore, the temperature must be within the optimal range of the species and/or the respiratory loss of carbohydrates must be lower due to the lower night temperature at these altitudes (Fischer *et al*., 2016).

Mayorga (2016) compared photosynthesis and efficient water use in banana passion fruit plants at two altitudes in Pasca (Cundinamarca) of 2,498 m a.s.l. (13.9°C, PAR of 680 µmol photons m^{-2} s^{-1}) and 2,006 m a.s.l. (17.8°C, PAR of 620 µmol photons m^{-2} s^{-1}). This study found that the banana passion fruit in the vegetative phase developed greater photosynthetic rates, while during flowering the plants showed high water-use efficiency, low transpiration, large water potentials, and high *Fv/Fm* (Tab. 2) compared to the fructification phase, which had lower photosynthetic rates and lower water-use efficiency. At 2,006 m a.s.l., the *Fv/Fm* was higher in plants in a vegetative stage of growth, and at 2,498 m a.s.l. it was higher during fructification. In the higher zone, the transpiration rate of the plants was reduced, which increased the WUE and water potentials. Due to the higher temperature in the lower zone, the banana passion fruit reached each of its phenological stages in less time compared to those of the higher zone, but with smaller fruits. These results suggest that the high zone better favors the development of the plants; however, each of the two sites affected the phenology and physiology differently due to climatic factors. The parameters of the light curve reported by Mayorga (2016) are included in Table 1.

Applications of N, K, and Mg to banana passion fruit at the amounts zero, low, and high resulted in the accumulation of plant dry matter (DW) only at a high amount of N. This finding aligns with the Thornley model, with accumulation of DW in the roots and its subsequent partition to above-ground plant parts (Lizarazo *et al*., 2013). This result confirms the importance of N and its foliar level, which is closely related to the content of chlorophyll, Rubisco activity, the quantum performance of photosynthesis, and the electron transport rate, processes that directly affect photosynthetic efficiency (Sanclemente & Peña, 2008).

Myrtaceae fruits

Feijoa

Feijoa (*Acca sellowiana*), native to South America, has a high degree of adaptation to the agroecological conditions of the tropical Andean area (Naizaque *et al*., 2014). Different varieties and clones have been reported, as detailed in Parra-Coronado and Fischer (2013). In Colombia, it is commercially grown at altitudes between 1,800 and 2,700 m a.s.l., with average temperatures between 13 and 21°C and precipitation of 700-1,200 mm per year (Fischer *et al*., 2020; Fischer & Parra-Coronado, 2020). Feijoa requires a base temperature of only 1.76°C from fruit set to harvest (Fischer & Parra-Coronado, 2020; Parra-Coronado, Fischer *et al*., 2015). In temperate and subtropical regions of the world, feijoa restarts its growth, sprouting of branches, and formation of floral buttons when temperatures increase in spring, but in the tropics it grows and produces fruits throughout the year, favored by the rainy season or irrigation (Fischer, 2003).

Solar radiation is a very important factor in the development, production, and quality of feijoa fruits. In Colombia (Cundinamarca), a study during two harvest seasons comparing average radiation of 11,082 W m^{-2} at 2,580 m a.s.l. and 8,918 W m^{-2} at 1,800 m a.s.l. found that the higher elevation generated fruits of greater weight and higher content of soluble solids (Parra-Coronado, Fischer, Camacho *et al*., 2015) and sucrose (Parra-Coronado *et al*., 2022). The authors concluded that the higher temperature at the lower site led to greater respiration and loss of sugars in these fruits.

This differential reaction of the feijoa to solar radiation was also found by Silva *et al*. (2024) in feijoa saplings. These saplings had optimal growth under direct light or shade up to 30%, but when exposed to a shade of 80%, they developed larger leaves with lower thickness, reduced rate of CO_2 assimilation and electron transport and greater quantum performance of photosystem II. As in the other Andean fruit crops, the fruits unprotected by foliage suffer sunburn, stomatal closure, and photoinhibition, especially if the high-radiation periods, particularly of UV light, are extended (Fischer, Orduz-Rodríguez *et al*., 2022).

Naizaque *et al*. (2014) studied the reception of light by the different strata of the canopy of feijoa trees and observed, in the leaves of the upper stratum, a higher rate of transpiration and higher number of stomata per unit of leaf area (91 stomata/mm^2) than in the lower stratum (78 stomata/mm^2). In their study, foliar transpiration directly increased with the temperature (being higher in the upper stratum than in the lower one) and with a higher irradiance, leading to increased transpiration as relative humidity decreased. When comparing the incident solar radiation in the two strata of the feijoa, Martínez-Vega *et al*. (2008) measured an average of 90% radiation in the upper stratum, while in the lower interior stratum only 35% of the incident radiation was found. This shows that pruning and training of this cone-shaped plant with horizontal side branches guarantee maximum photosynthesis throughout the canopy (Fischer *et al*., 2020).

Germanà and Continella (2004) found that feijoa grown under the climatic conditions of Sicily had a low photosynthetic rate (on average between 4 and 6 μmol CO_2 m^{-2} s^{-1}), with a high energy demand during the bloom. Under these conditions, the feijoa plants, due to the high stomatal transpiration and low stomatal resistance, presented a WUE of only 1/3 that of the avocado and the custard apple.

Observing the behavior of several varieties of feijoa in southern Russia with subtropical climate, where the development of the fruits coincides with the dry season, Omarova *et al*. (2020) found that, in the Dagomyskaja variety, the intensity of respiration exceeds photosynthesis. This finding suggests that adaptation to drought abiotic stress depends considerably on the genotype. Also, Peña Baracaldo and Cabezas Gutiérrez (2014) reported in a study in Colombia at 2,450 m a.s.l. that feijoa plants without additional irrigation developed lower leaf area index, compared to the largest index with 50% irrigation. This reduced leaf area index would greatly limit the ability of the plants to capture light and restrict the production of photoassimilates in these non-irrigated plants.

Despite the advances mentioned in feijoa research, we did not find reports that indicate the characterization of parameters of photosynthesis in this species, such as fluorescence of chlorophyll *a* and gas exchange.

General discussion

This review consolidates the main advances in the current knowledge of photosynthesis in high tropical Andean fruit crops. This knowledge of photosynthesis should be used as a priority to generate strategies focused on increasing yield (Li *et al*. 2018) and quality at harvest. In the high tropical Andean areas, an increase in photosynthesis due to the higher solar radiation can favor fruit quality parameters, such as soluble solid contents, as has been observed in feijoa and banana passion fruit (Fischer *et al*., 2024; Mayorga *et al*., 2020).

The reviewed studies report important advances in the understanding of fluorescence of chlorophyll *a* and gas exchange, with a good part of these studies carried out in the main fruit producing countries, Brazil and Colombia (Fig. 2). However, these advances lag behind those achieved in species of the low tropics, subtropical, and temperate areas. This is evidenced in the modest number of articles published on these topics. The greatest number of studies have been done on cape gooseberry and gulupa, high-altitude species that are most exported. The above shows that the progress in research is related to the economic importance of fruit crops.

Fluorescence of chlorophyll *a*

As expected, the most frequently characterized parameter in the high tropical Andean fruit crops has been *Fv/Fm* (Tab. 2), mainly because it is an indicator widely used to determine the physiological status of the plants. In the reported fruit crops, *Fv/Fm* ranges from ~0.77 to 0.87 in non-stress conditions (Tab. 2). To a lesser extent, parameters such as QP, NPQ, ϕPSII, and ETR have been used. These parameters have been used as indicators of the physiological behavior of fruit plants in different agroecological zones, providing insight into the most appropriate areas for crop, as mainly reported for sweet granadilla and banana passion fruit (Fernández *et al*., 2014; Mayorga, 2016). However, these indicators have been most utilized in studies of physiological response of the fruit crops to biotic stress conditions (e.g., *Fusarium*) and abiotic stress conditions. In the latter case, experiments have focused on characteristic conditions of climatic variability, such as water deficit, waterlogging, nutrient deficit, and even overshading (Cárdenas-Pira *et al*., 2021; Castañeda-Murillo *et al*., 2022; Chávez-Arias *et al*., 2019; Sánchez-Reinoso *et al*., 2019; Segura-Monroy *et al*., 2015). The trend indicates that the fluorescence of chlorophyll *a* has been mainly used as a tool to characterize the ecophysiological response of these fruit crops.

It is important to investigate further the response of the photo-phase of the photosynthesis in the high tropical Andean fruit crops under conditions of salinity stress, one of the most important types of stress in agriculture (Eswar *et al*., 2021). It should also be noted that there is very little information on the tree tomato, a fruit crop with an important cultivated area (Ramírez *et al*., 2021), and feijoa, a fruit crop that is grown in several countries in the world (Fischer & Parra-Coronado, 2020).

Gas exchange

Significant contributions are evidenced through light response curves (Tab. 1) for cape gooseberry, tree tomato, gulupa, sweet granadilla, and banana passion fruit (Fernández *et al*., 2014; Figueiredo *et al*., 2021; Fischer & Melgarejo, 2020; Mayorga, 2016; Pérez Martínez & Melgarejo Muñoz, 2015, Ramírez-Soler *et al*., 2021). The main purpose of this research has been to identify the photosynthetic performance of the plants in different ecophysiological conditions to select optimal cultivation areas. It is recommended to extend these studies of light response curves to lulo and feijoa. These studies have also included gaseous exchange measurement across times of day and in several phenological stages and are frequently complemented with measurements of fluorescence of chlorophyll *a* and plant water potential (Fernández *et al*., 2014; Pérez Martínez & Melgarejo Muñoz, 2015).

Gas exchange parameters (*A*, *E*, *gs*, *C*i (intracellular carbon concentration)) have generally been evaluated in the high tropical Andean fruit species (Fernández *et al*., 2014; Lima *et al*., 2020; Pérez Martínez & Melgarejo Muñoz, 2015; Ramírez-Soler *et al*., 2021), but these studies are scarce and do not yet provide enough detailed understanding of the photosynthetic performance of the fruit species in the various edaphoclimatic conditions of the high Andean areas. There is also a lack of CO_2 response curves of photosynthesis, with few reports available (Fischer & Melgarejo, 2020). Such curves are necessary for the current conditions of climatic variability. In addition, as atmospheric concentration of CO_2 rises in tropical highlands, plants living in 'thinner' air (low partial pressure) may benefit from increased CO_2 since leaf photosynthesis in plants rises when supplied with extra CO_2, potentially enhancing growth (Körner, 2023).

Finally, it is important to mention that there is almost no research on the morphological, biochemical, and molecular levels of the photosynthetic processes of these fruit crop species in the high tropics of the Andes. The effects of UV light, wind, relative air humidity, and temperature on the indirect features associated with photosynthesis – density of stomata, size of stomata, morphology and size of leaves, number of leaves – should be studied with priority.

Conclusions and recommendations for future research

The fruit crops produced in Andes, considered exotic fruits and important functional foods, are gaining increasing global importance. Most research into these fruit species has been carried out by producing countries to improve their productive systems. Photosynthesis is one of the most important physiological processes that determines the production and quality of crops. This review presented the main aspects and advances of research in photosynthesis of the high tropical Andean fruit crops. Bibliometric analysis shows that research on the topic has focused on the countries with the climatic conditions for the growth of these fruit species, namely Brazil and Colombia. In the past, research topics focused on horticulture and plant physiology, evolving to photosynthesis, and more recently, leaf anatomy and the acclimation process. In aspects of the photosynthetic process, in most of the analyzed species, the photosynthetic parameters such as A_{max}, I_c, I_s, and ΦPPFD are derived from light curves. Important advances in the knowledge of the fluorescence of chlorophyll *a* have been made, which are mainly used as a tool to characterize the ecophysiological response of these fruit crops.

Despite the progress reported, the understanding of the photosynthetic process in the high tropical Andean fruit species remains limited. The main findings are related to instrumental measurement under specific ecophysiological conditions, often focusing on climatic variability.

We suggest that future research be focused on elucidating the biochemical, genetic, and molecular aspects of photosynthesis. We also recommend using photosynthesis as a tool to evaluate improvements in the production system including fertilization, irrigation, pruning and training, planting densities and arrangements, bioregulators, protected cultivation, and shading nets, among others. In addition, the evaluation of new genetic materials for varieties and rootstocks is crucial. It is also necessary to study photosynthesis related to climate change, ideally in controlled conditions (phytotrons, growth chambers or free air CO_2 enrichment (FACE) chambers), varying temperature of the air and soil, solar radiation, concentration of CO_2, relative air humidity, atmospheric pressure, and other variables. Currently, there are no published reports on these aspects of photosynthesis for the high tropical Andean fruit species.

Acknowledgments

The authors wish to recognize the important contribution of Professor Luz Marina Melgarejo Muñoz (Laboratory of Plant Physiology and Biochemistry, Department of Biology, Universidad Nacional de Colombia), for the great advances in the study of the ecophysiological aspects of the high tropical Andean fruit crops. Professor Melgarejo has carried out extensive research on the photosynthesis of these fruit plants in Colombia, addressing aspects of fluorescence of chlorophyll *a* and of gas exchange.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

NFV: conceptualization, research, writing - original draft, visualization, writing, and editing. GF: conceptualization, writing, and supervision editing. HEBL: conceptualization, visualization, writing, and editing. All authors have read and approved the final version of the manuscript.

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Invasive aquatic plants as a mixed substrate with Red Ferralitic soil in vegetable seedbeds

Plantas acuáticas invasoras como un sustrato mezclado con suelo Ferralítico Rojo en semilleros de hortalizas

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ABSTRACT

The composition of the substrates in the vegetable seedbed phase is important for subsequent transplanting. The aim of this study was to evaluate the use of dry mass of the invasive aquatic plants *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms as a substrate mixed with Red Ferralitic soil for seedbeds of tomato and pepper. To plant tomato and pepper seeds, 300 alveoli were prepared with a mixture of Red Ferralitic soil with different proportions of dry mass of *P. stratiotes* (0.5% and 1.0%) and *E. crassipes* (2.0% and 4.0%). At 28 and 40 d after sowing, 30 seedlings per treatment were selected and the average length and diameter of the stem and primary root (cm) were measured. The Dickson quality index was determined in order to select the best treatment. The average stem length was greater in seedlings treated with *P. stratiotes* (0.5%) and *E. crassipes* (2.0%) and the diameter was greater in tomato seedlings treated with *P. stratiotes* (1.0%) and pepper seedlings with *P. stratiotes* (0.5%); this showed significant differences from the rest of the treatments. The maximum length and diameter of the primary root varied between treatments for both vegetables. The best treatments for the initial growth of tomato and pepper were those when Red Ferralitic soil and dry mass of *P. stratiotes* (0.5% and 1.0%) and *E. crassipes* (2.0%) were used as a mixed substrate.

Key words: *Eichhornia crassipes*, *Pistia stratiotes*, seedlings, Dickson quality index, tomato, pepper.

RESUMEN

La composición de los sustratos, en la fase de semillero de hortalizas, es importante para su posterior trasplante. El objetivo de este estudio fue evaluar el uso de masa seca de las plantas acuáticas invasoras *Pistia stratiotes* L. y *Eichhornia crassipes* (Mart.) Solms como sustrato mezclado con suelo Ferralítico Rojo en semilleros de tomate y pimiento. Para plantar semillas de tomate y pimiento se prepararon 300 alvéolos con una mezcla de suelo Ferralítico Rojo y diferentes proporciones de masa seca de *P. stratiotes* (0,5% y 1,0%) y *E. crassipes* (2,0% y 4,0%). Luego de 28 y 40 d de siembra respectivamente, se seleccionaron 30 plántulas por tratamiento y se les midieron la longitud y diámetro promedio del tallo y de la raíz primaria (cm). Se determinó el índice de calidad de Dickson para seleccionar el mejor tratamiento. La longitud promedio del tallo fue mayor en las plántulas tratadas con *P. stratiotes* (0,5%) y *E. crassipes* (2,0%) y el diámetro fue mayor en las plántulas de tomate tratadas con *P. stratiotes* (1,0%) y de pimiento con *P. stratiotes* (0,5%), mostrando diferencias significativas con el resto de los tratamientos. La longitud máxima y el diámetro de la raíz primaria variaron entre tratamientos para ambas hortalizas. Los mejores tratamientos para el crecimiento inicial de tomate y pimiento fueron aquellos donde se utilizó suelo Ferralítico Rojo y masa seca de *P. stratiotes* (0,5% y 1,0%) y *E. crassipes* (2,0%) como sustrato mezclado.

Palabras clave: *Eichhornia crassipes*, *Pistia stratiotes*, plántulas, Dickson quality index, tomate, pimiento.

Introduction

The consumption of vegetables is associated with the quality of human health due to their high contribution of vitamins and minerals that support adequate blood pressure, body mass, and prevent the risk of strokes and certain types of cancer (Quiñones-López, 2023; Reyes, 2023). Tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) are among the most important vegetable crops (Reyes-Pérez *et al*., 2018; Rodríguez-Delgado *et al*., 2021) for human health.

The tomato is native to the Andean mountains. The ancestors of this species grew in the Colombian wilderness, Ecuador, Bolivia, Peru, and the Atacama region of Chile, on both slopes of the Andes and in the Galapagos Islands

Received for publication: July 9, 2024. Accepted for publication: August 26, 2024. Doi: 10.15446/agron.colomb.v42n2.115670

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(Sims, 1980). Also, pepper is one of the vegetables most in demand by consumers. It is the one with the highest economic value (Reyes-Pérez *et al*., 2018). China, the USA, and Turkey are the largest producers of pepper worldwide (Alarcón-Zayas, 2013). In Cuba, it is the most important crop and is grown in all provinces for local consumption and export. The Amalia tomato variety produces numerous large fruits and is highly valued (Moya *et al*., 2009).

The genus *Capsicum* is native to the tropical zone of Central and South America and is made up of several species (Barboza & Bianchetti, 2005; Del Valle-Echevarría *et al*., 2019). Five of these species are used as vegetables, including *C. annuum* (Ibiza *et al*., 2012; Pino *et al*., 2011), and several varieties, including variety True Heart (*i.e*., *C. annuum* var. True Heart). The pepper is recognized worldwide as one of the most important vegetables because of its high nutritional content and the profits it yields to the producer (Rodríguez-Delgado *et al*., 2021). In several countries of the Americas, including Cuba, pepper is the second most important vegetable (after tomato) and widely accepted among the population of the continent (Barrios *et al*., 2005).

Globally, the demand and production cost of inorganic fertilizers has increased (Tang *et al*., 2022), obligating farmers to search for alternatives to obtain the necessary organic and/or environmentally friendly fertilizers. An interesting organic option for an organic fertilizer substrate could be the use of invasive aquatic plants *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms found in artificial freshwater lagoons in the province of Ciego de Ávila, Cuba (Hernández-Fernández *et al*., 2023).

Although these aquatic plants cannot be used for large-scale crops, they could benefit seedbeds and crops grown in urban, family, and community agriculture (Honmura, 2000; Sondang *et al*., 2021; Wamba *et al*., 2012). Today, in Ciego de Ávila, *P. stratiotes* is not used for anything; and, although it does not directly affect human populations, its excessive proliferation in the lagoon must affect the flora and fauna that inhabit it. This plant also has a negative impact on migratory water birds, which used the lagoon when it was not yet covered by *P. stratiotes* (personal observation). Only the leaves of *E. crassipes* are used in crafts, which means that their roots remain in the water, thus ensuring their immediate reproduction. These plants constitute an accessible raw material, since they are extracted and deposited on the shores of La Turbina lagoon (affecting the aesthetic value of the site) or, as a final destination, they are sent to landfills without prior treatment, putting nearby groundwater or surface water at risk. The aim of this research was to evaluate the use of the dry mass of the invasive aquatic plants *P. stratiotes* L. and *E. crassipes* (Mart.) Solms as a substrate mixed with Red Ferralitic soil for the seedbeds of tomato and pepper.

Materials and methods

This research was carried out at the Center of Bioplants of the Maximo Gomez Baez University in the province Ciego de Avila, located between the provinces of Sancti Spíritus and Camagüey in central Cuba. The soil used was Ferralitic Red soil according to the Cuban soil classification (Hernández-Jiménez *et al*., 2015). This soil is the predominant soil in the province of Ciego de Ávila (González-Domínguez *et al*., 2019). The soil has low phosphorus (P) and potassium (K) content (González-Domínguez *et al*., 2019) and has the following concentration of chemical elements determined by energy-dispersive X-ray fluorescence spectrometry (EDXRF) (Tab. 1).

TABLE 1. Chemical elements present in the Ferralitic Red soil used in the seedbeds where the tomato (var. Amalia) and pepper (var. True Heart) seeds were sown.

Chemical elements	Soil (%)	Soil (mg kg^{-1})
Al	6.23 ± 0.77	
As		6.08 ± 0.76
Br		17.7± 3.3
Ca	2.58 ± 0.13	
Cr		> 280 (487)
Cu		115 ± 21
Fe	> 6.74 (7.1)	
K	0.350 ± 0.050	
Mg	1.17 ± 0.16	
Mn		1670 ± 120
Mo		2.83 ± 0.84
Na	< 0.15	
Ni		> 130 (480)
P		> 1100 (1130)
Pb		15.2 ± 2.3
S		< 83,5
Si	23.4 ± 1.6	
Ti		6450 ± 400
Zn		105.0 ± 7.3

Experiment preparation and design

To obtain dry mass (DM), *P. stratiotes* plants were collected in the Vista Alegre artificial freshwater lagoon (21°51'9" N, 78°46'39" W, 0.013 km^2 area), and *E. crassipes* in La Turbina lagoon (21°50'51" N, 78°45'43" W, 0.086 km^2 area), in the municipality of Ciego de Ávila. Leaf and root samples were first washed with tap water and then distilled water. Afterwards, they were placed in a drying house for 30 d at an average temperature of 30 ± 1°C. The plant samples were then placed in a Boxun oven at 70 ± 1°C for a period of 48 h. The constant DM obtained was crushed to 2 µm particle size. The phosphorus (P) and potassium (K) concentrations of *P. stratiotes* and *E. crassipes* were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES) (iCAP 6000 ICP Spectrometer, Thermo Fisher Scientific, USA). Total nitrogen (TN) was determined in the plant samples by the salicylic acid-thiosulfate modification of the Kjeldahl method and included determination of $N\text{-}NO_3$ and $N\text{-}NO_2$ (Dhaliwal *et al*., 2014) (Tab. 2). The heavy metals present in the DM of *P. stratiotes* and *E. crassipes* were below the maximum permissible limits established by EU regulations (Serra, 2019). To determine the DM doses, the criterion of Rodríguez-Chow and Trujillo-Mendoza (2013) on the proportions of N, P and K for tomato seedlings was used. The criteria of Álvarez and Pino (2018) were also considered, regarding the absorption of macronutrients, in the first 35 d of pepper cultivation.

Three hundred alveoli (volume of each alveolus 30 cm^3) were prepared in four polystyrene trays, each divided into three sections, filled with a mixture of Ferralitic Red soil and different amounts of dry mass (DM) of *P. stratiotes* and *E. crassipes* (Tab. 2). Five sections with 30 alveoli per section were considered. On May 1, 2023, two seeds of tomato were sown in each of the 150 alveoli, and two seeds of *C. annuum* were sown in each of the 150 alveoli in another group. The viability of tomato seeds was 94%. The viability of pepper seeds was 98%. Seed viability was determined by flotation separation (Solís-Sandoval *et al*., 2019).

During the day, the average temperature was 29.9°C, the maximum temperature was 36.0°C, and the minimum was 20.8°C. During the night, the average ambient temperature was 24.5°C, the maximum was 28.2°C, and the minimum was 20.4°C. During the study, the experimental units received an average photosynthetic photon flux density of 326.57 µmol m^{-2} s^{-1} with a maximum of 2548.90 µmol m^{-2} s^{-1} and a minimum of 0.199 µmol m^{-2} s^{-1}, depending on the time of the day.

Analysis of the experimental units

The percentage of emergence of the tomato and pepper plants was analyzed, taking into account the number of seeds sown and those that germinated per day. For this purpose, each emerging seedling was counted from the first day of emergence until the number of emerged seedlings was constant. For tomatoes, it was from May 9 to May 20, 2023, while, for peppers, it was from May 12 to May 26, 2023. After 28 d (tomato) and 40 d (pepper) of seed sowing, 15 experimental units were randomly selected until reaching 30 seedlings per treatment. The seedlings were selected from the central part of each treatment area to avoid the border effect (Fig. 1).

Once the roots of seedlings were washed to remove traces of soil and each seedling had been drained, the length of the stem (cm) and the primary root (cm) were measured with a ruler. Stem diameter was measured with an OWT 30311 digital caliper (mm). The seedlings were then placed in an oven (IF-3D, Cuba) at 60±1°C until a constant dry mass weight (g) was obtained. To determine the dry mass of the aerial part and the root of each tomato and pepper seedling, an analytical digital balance was used.

The Dickson quality index (DQI) (Eq. 1) of the data was determined (Acevedo-Alcalá *et al*., 2020; Dickson *et al*., 1960; Domínguez-Liévano & Espinosa-Zaragoza, 2021). The highest DQI matches the best treatment.

$$\text{DQI} = \frac{\text{Total dry mass (g)}}{\dfrac{\text{Stem height (cm)}}{\text{Stem diameter (mm)}} + \dfrac{\text{Aerial part dry mass (g)}}{\text{Root dry mass (g)}}} \qquad (1)$$

TABLE 2. Treatments applied using a mixture of Ferralitic Red soil with dry mass (DM) of *Pistia stratiotes* and *Eichornia crassipes*.

Number of alveoli	Preparation of the alveoli	Soil (g)	Concentration of N, P, and K in the dry mass of *P. stratioites* and *E. crassipes* (mg g^{-1})		
			N	P	K
30	Soil without plant addition (control)	19.0			
30	Soil + *P. stratiotes* (0.1 g DM) (0.5%)	18.9	24.9 ± 2.0	5.0 ± 0.1	22.5 ± 0.2
30	Soil + *P. stratiotes* (0.2 g DM) (1.0%)	18.8			
30	Soil + *E. crassipes* (0.4 g DM) (2.0%)	18.6	19.5 ± 0.8	1.9 ± 0.1	14.6 ± 1.2
30	Soil + *E. crassipes* (0.8 g DM) (4.0%)	18.2			

Soil + *P. stratiotes* (0.1 g DM) (0.5%): 0.1 g DM of *P. stratiotes* represent 0.5% of substrate; Soil + *P. stratiotes* (0.2 g DM) (1.0%): 0.2 g DM of *P. stratiotes* represent 1.0% of substrate; Soil + *E. crassipes* (0.4 g DM) (2.0%): 0.4 g DM of *E. crassipes* represent 2.0% of substrate; Soil + *E. crassipes* (0.8 g DM) (4.0%): 0.8 g DM of *P. stratiotes*, represent 4.0% of substrate.

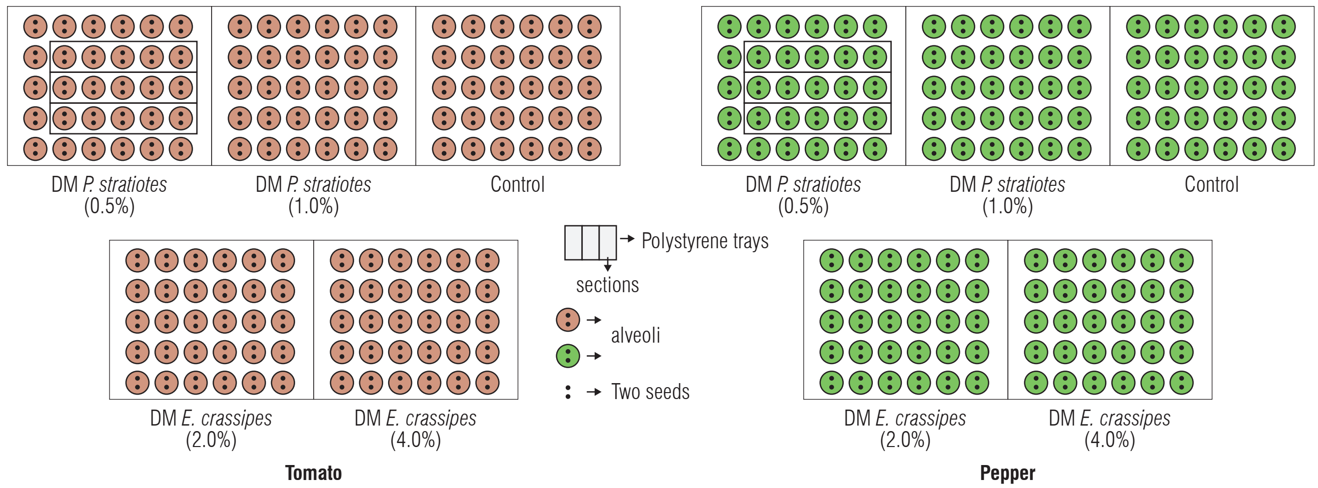

FIGURE 1. Experimental design in tomato and pepper seedbeds. DM: dry mass.

Statistical analysis

Since the data did not have a normal distribution (Shapiro-Wilk test), the non-parametric Kruskal-Wallis test was performed to detect differences in the morphological characteristics of the tomato and pepper seedlings from one treatment to another and to determine the most feasible treatment. When significant differences were present, the Wilcoxon test was applied to determine with which treatments the differences were obtained. Statistical analyses were performed using the R software version 3.1.2 (R Core Team, 2018) and the Vegan package (Oksanen *et al*., 2005).

Results and discussion

The emergence of tomato seedlings was approximately at 5 d, and that of pepper at 12 d (Fig. 2). The percentage emergence of the tomatoes ranged from 65.0 to 85.0% and that of pepper from 40.0 to 80.0%. No significant differences between the control and the treatments with DM of *P. stratiotes* (0.5% and 1.0%) and DM of *E. crassipes* (2.0% and 4.0%) were observed.

The different treatments with DM of *P. stratiotes* and *E. crassipes* used as substrate in the seedbeds did not directly affect the percentage of emergence, since there were no significant differences among the treatments used for the tomatoes nor among those used for the pepper plants. However, the emergence of seedlings is one of the key stages for the success of plant establishment. Temperature, moisture, aeration, and compaction of the substrate are the determining factors for a successful emergence (Porta-Siota *et al*., 2021). In this research, it is assumed that the

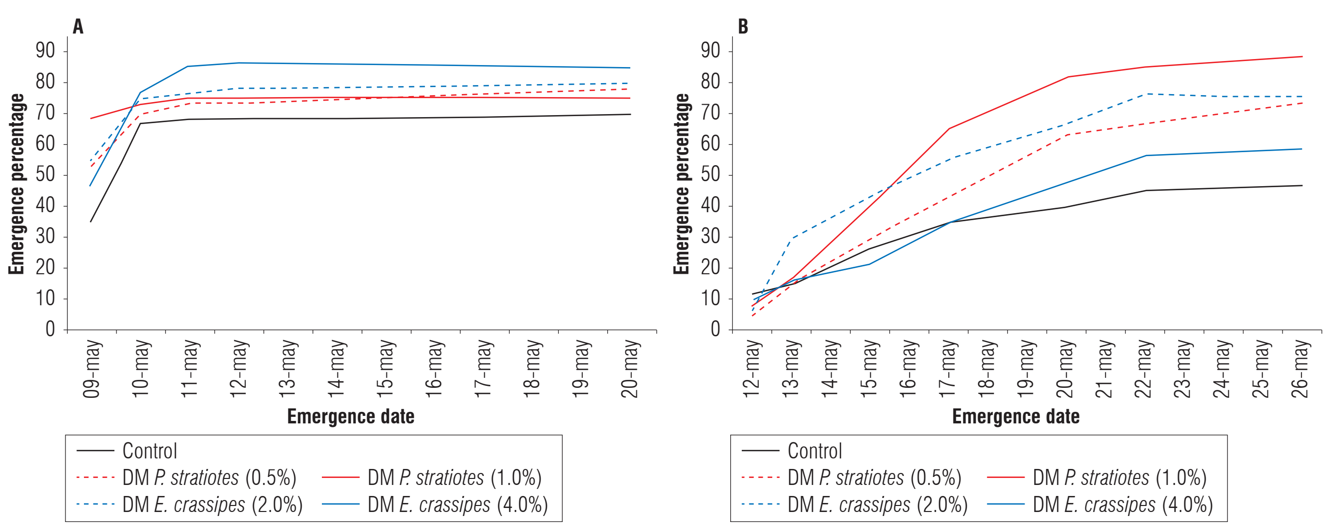

FIGURE 2. A) Percentage emergence of tomato seedlings (var. Amalia) (*P-value*=0.1947). B) Percentage emergence of pepper seedlings (var. True Heart) (*P-value*=0.06849). The *P-value* was determined according to the Kruskal-Wallis test. DM: dry mass.

characteristics of the substrates applied were different for the DM of *P. stratiotes* and *E. crassipe*s with respect to those of the control substrate; and these could influence the physiological processes of the seedlings that increased their emergence percentage, but without significant differences between them.

The optimal air temperature for tomato is between 16°C and 28°C, and that emergence occurs within 5 to 8 d if exposed to sunlight (López-Marín, 2017). For the germination of *Capsicum spp*., the optimal air temperature is 24°C and the optimal photosynthetic photon flux 230 µmol m^{-2} s^{-1} (Pine *et al*., 2018). The *C. annuum* seed takes a fairly long time to germinate and emerge, during which light is not decisive, and the optimal air temperature to achieve good results is around 30°C (Saavedra del Real, 2019). In our study, the tomato seeds emerged in the optimal range of air temperature and time (Lopez-Martín, 2017), while those of pepper emerged at an average air temperature above the one suggested by Pino (2018) and below the one stated by Saavedra del Real (2019). The average photosynthetic photon flux density was above the one suggested by Pino (2018).

Morphological characters of tomato seedlings

After 28 d of culture, the average height of tomato seedlings was 9.8 ± 0.9 cm in the control treatment, while that of the treatments where the substrate of the invasive aquatic plants was used varied between 10.5 ± 0.5 cm and 12.1 ± 0.6 cm. The highest value was obtained in the treatment with DM from *P. stratiotes* (0.5%) and *E. crassipes* (2.0%) (Figs. 3A and 4). The average stem diameter varied between 1.9 ± 0.2 mm and 2.5 ± 0.1 mm. The highest value was obtained in the treatment with DM of *P. stratiotes* (1.0%) (Figs. 3B and 4).

Tomato seedlings also had morphological variations in the length and diameter of the primary root between treatments. The average root length ranged from 8.6 ± 1.0 cm to 10.6 ± 0.8 cm. The highest value was from the treatment with DM of *E. crassipes* (2.0%) (Fig. 3C). The average root diameter was between 1.5 ± 0.1 mm and 2.1 ± 0.2 mm. The highest value was obtained in the treatment with DM of *P. stratiotes* (1.0%) (Fig. 3D).

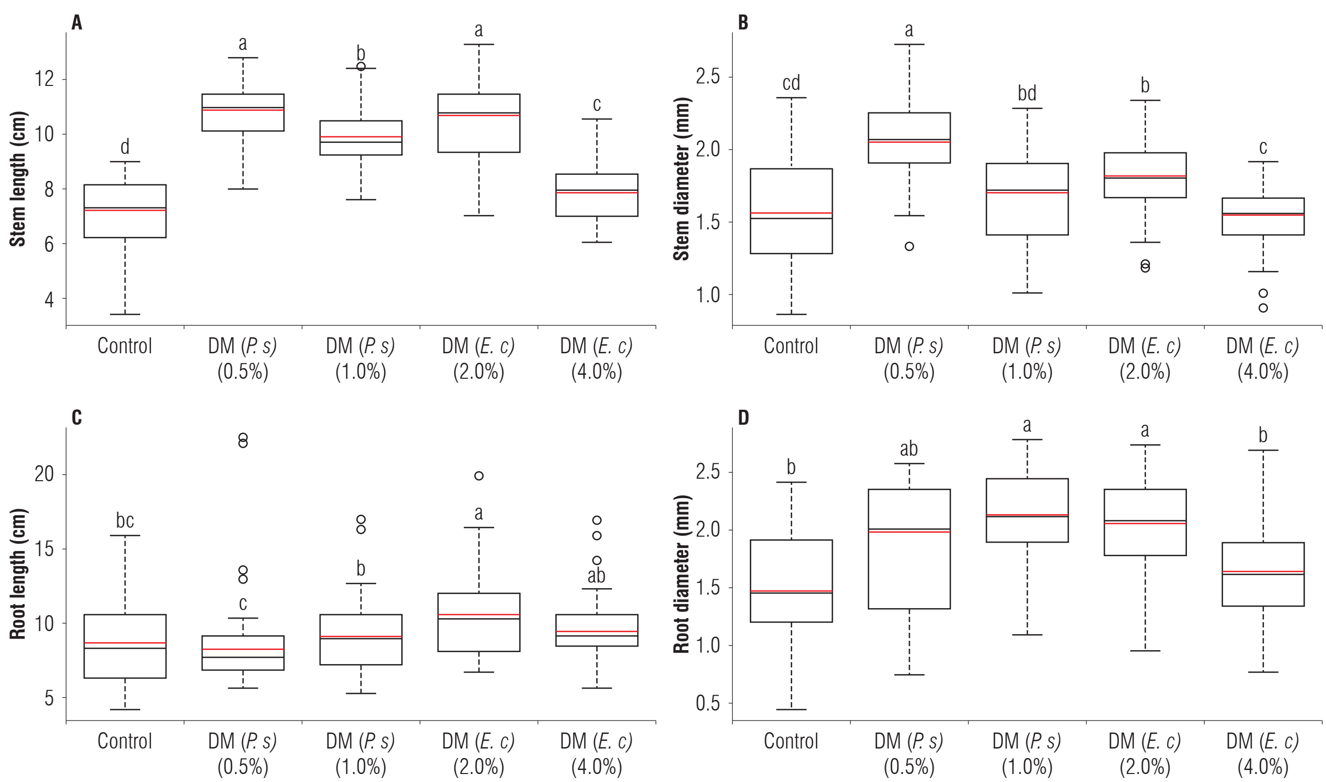

FIGURE 3. A) Stem length after tomato (var. Amalia) (*P-value*=7.247e^{-05}); B) stem diameter (*P-value*=9.377e^{-11}); C) root length (*P-value*=0.004532); D) root diameter (*P-value*=4.889e^{-05}). DM: dry mass, *P.s: P. stratiotes*, *E.c: E. crassipes*. *P*-values were determined according to the Kruskal-Wallis test. Each box represents 50% of the data. Black lines inside boxes represent a median. Red lines inside the boxes represent a mean. Dotted line at the ends=maximum and minimum values. Circles=atypical values. Different letters indicate significant differences between treatments according to the Wilcoxon test. Confidence interval was 95%.

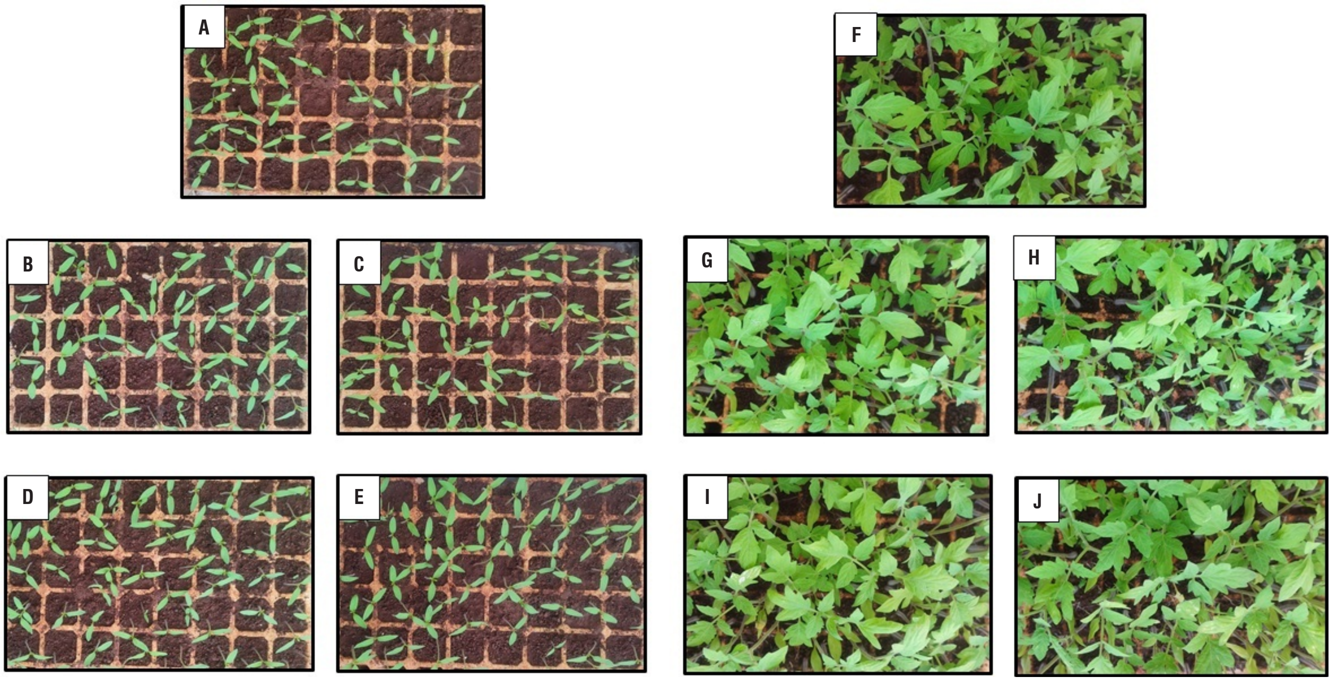

FIGURE 4. Tomato seedbeds (var. Amalia). A-E: 7 d after sowing. F-J: 28 d after sowing. (A and F) control=Ferralitic Red soil (RFS); (B and G) substrate=RFS+*P. stratiotes* DM 0.5%; (C and H) substrate=RFS + *P. stratiotes* DM 1.0%; (D and I) substrate=RFS + *E. crassipes* DM 2.0%; (E and J) substrate=RFS + *E. crassipes* DM 4.0%. DM: dry mass.

The dry mass (g) of the aerial parts of the tomato seedlings with *E. crassipes* DM treatment (2.0%) was the one with the highest mean value when compared to the control and the rest of the treatments. The dry mass (g) of the root was greater in the treatments with DM of *P. stratiotes* (0.5%) and with DM of *E. crassipes* (2.0%) (Tab. 3).

TABLE 3. Average dry mass (DM) of the aerial parts and the roots of tomato seedlings (var. Amalia) with the different treatments. Aerial part (*P-value*=3.284e-^{07}). Roots (*P-value*=1.231e^{-06}).

Treatments	Dry mass of the aerial part (g)	Dry mass of the roots (g)
Soil without MS (Control)	0.07±0.01^{d}	0.01±0.002^{c}
Soil + DM of *P. stratiotes* (0.5%)	0.15±0.02^{b}	0.03±0.009^{a}
Soil + DM of *P. stratiotes* (1.0%)	0.15±0.01^{b}	0.02±0.002^{b}
Soil + DM of *E. crassipes* (2.0%)	0.18±0.01^{a}	0.03±0.003^{a}
Soil + DM of *E. crassipes* (4.0%)	0.13±0.01^{c}	0.02±0.002^{b}

The *P-values* were determined according to the Kruskal-Wallis test. Different letters indicate significant differences between the treatments according to the Wilcoxon test.

The stem length of the tomatoes showed significant differences for all treatments when compared to the control. The values achieved with the DM of *E. crassipes* were similar to those obtained by Luna-Murillo *et al.* (2015) after 30 d of culture; they used, among other treatments, *E. crassipes* as organic fertilizer. However, the values for both stem length and diameter were below those of Fernández-Delgado *et al.* (2021) using different treatments. Specifically, stem diameter was greater in the treatments with DM of *P. stratiotes* (1.0%) and *E. crassipes* (2.0%). The average maximum length of the primary root was obtained with the treatment of DM of *E. crassipes* (2.0%) and did not show significant differences with respect to the treatment with DM of *E. crassipes* (4.0%) and with the rest of the treatments and the control. Root diameter was greater in the treatment with DM of *P. stratiotes* (1.0%), showing significant differences when compared to the control and the treatment with DM of *E. crassipes* (4.0%).

Morphological characters of pepper seedlings

After 40 d of culture, the average height of the control pepper seedlings was 7.0 ± 0.5 cm, while that of the treatments varied between 7.8 ± 0.3 cm and 10.8 ± 0.4 cm. The greatest value was obtained in the treatment with *P. stratiotes* DM (0.5%) and with *E. crassipes* DM (2.0%) (Figs. 5A and 6). The average stem diameter varied between 1.5 ± 0.1 mm and 2.0 ± 0.1 mm. The greatest value was obtained in the treatment with *P. stratiotes* DM (0.5%) (Figs. 5B and 6).

Pepper seedlings also had morphological variations between treatments for the length and thickness of the primary roots. The mean root length ranged from 6.0 ± 0.7 cm to 9.4 ± 0.8 cm. The highest value was obtained in the treatment with *P. stratiotes* DM (1.0%) (Fig. 5C).

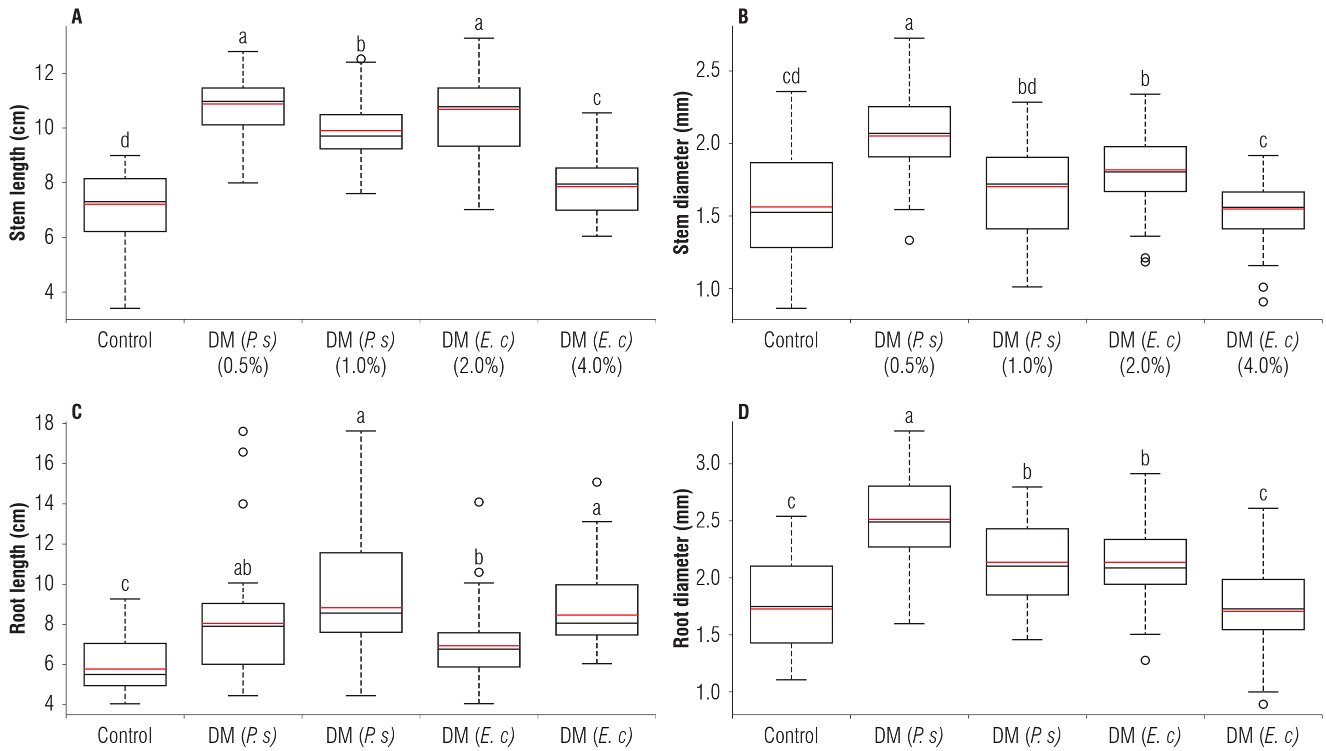

FIGURE 5. A) Stem length of pepper seedlings var. True Heart (*P-value* $<2.2e^{-16}$); B) stem diameter (*P-value* $=9.284e^{-09}$); C) root length (*P-value* $=9.724e^{-08}$); D) root diameter (*P-value* $=3.487e^{-10}$). DM: dry mass; *P.s*: *P. stratiotes*, *E.c*: *E. crassipes*. *P-values* were determined according to the Kruskal-Wallis test. Different letters indicate significant differences between treatments according to the Wilcoxon test; the box represents 50% of the data; black line inside the box=median; red line inside the box=mean; dotted line ends=maximum and minimum values; circles=atypical values; confidence interval was 95%.

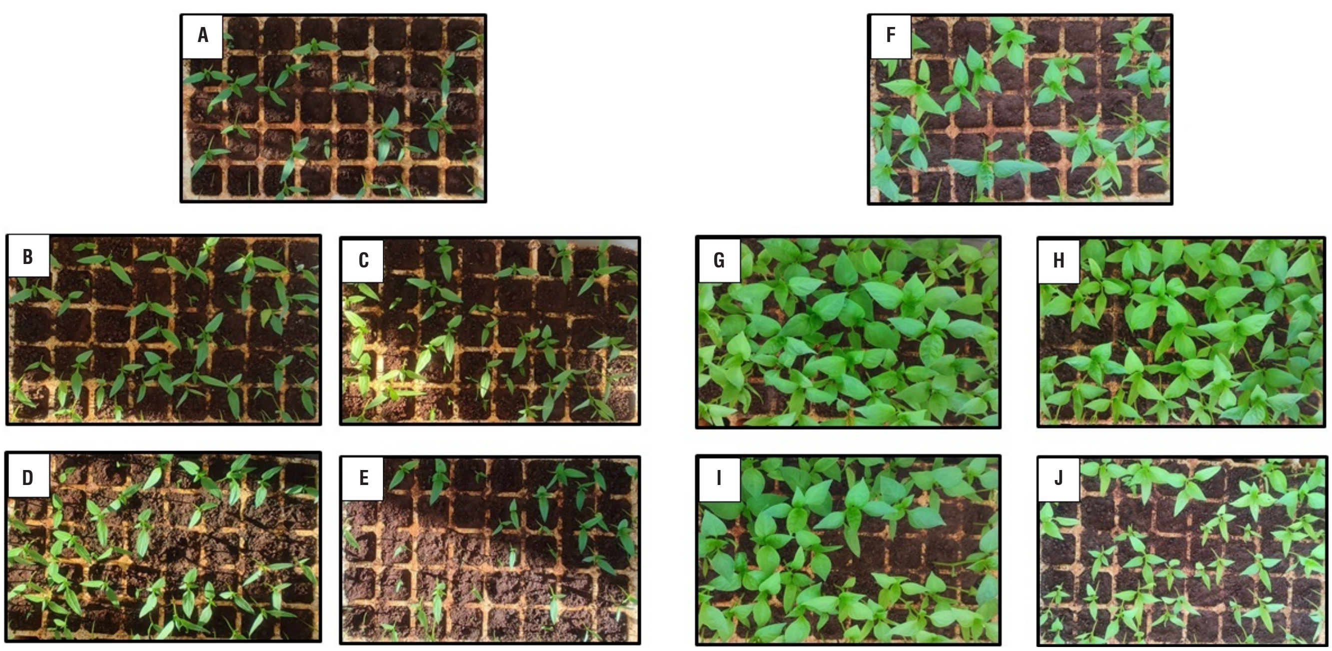

FIGURE 6. Pepper seedbeds var. True Heart. (A-E) 14 d of sowing; (F-J) 40 d after sowing; (A and F) (control=Ferralitic Red soil (RFS); (B and G) substrate=RFS + DM of *P. stratiotes* 0.5%; (C and H) substrate=RFS+DM of *P. stratiotes* 1.0%; (D and I) substrate=RFS+DM of *E. crassipes* 2.0%; (E and J) substrate=RFS+DM of *E. crassipes* 4.0%.

The mean root thickness was between 1.7 ± 0.2 mm and 2.5 ± 0.2 mm. The highest value was obtained in the treatment with *P. stratiotes* DM (0.5%) (Fig. 5D).

The dry mass (g) of the aerial part of the *C. annuum* seedlings for the treatment with DM of *P. stratiotes* (1.0%) and for DM of *E. crassipes* (2.0%) had the highest mean values with respect to the control and the remaining treatments. The treatments with DM of *P. stratiotes* (0.5% and 1.0%) and with DM of *E. crassipes* (2.0%) showed the same values in terms of the dry mass (g) of the root of *C. annuum* seedlings (Tab. 4).

TABLE 4. Average dry mass (DM) of the aerial part and the roots of pepper (var. True Heart) with different treatments. Aerial part (*P-value*= $1.743e^{-08}$). Roots (*P-value*= $2.029e^{-06}$).

Treatments	Dry mass of the aerial part (g)	Dry mass of the roots (g)
Soil without MS (Control)	0.07 ± 0.01^{c}	0.01 ± 0.002^{c}
Soil + DM of *P. stratiotes* (0.5%)	0.11 ± 0.01^{b}	0.03 ± 0.005^{a}
Soil + DM of *P. stratiotes* (1.0%)	0.13 ± 0.01^{a}	0.03 ± 0.005^{a}
Soil + DM of *E. crassipes* (2.0%)	0.13 ± 0.01^{a}	0.03 ± 0.005^{a}
Soil + DM of *E. crassipes* (4.0%)	0.07 ± 0.01^{c}	0.02 ± 0.003^{b}

The *P-values* were determined according to the Kruskal-Wallis test. Different letters indicate significant differences between treatments according to the Wilcoxon test.

The stem length of pepper for all treatments showed significant differences when compared to the control. These values were higher than those obtained by Silva *et al.* (2018) in pepper seedlings with commercial Plantmax and PlantHort II substrate (between 6.38 and 8.25 cm) and lower than those obtained with PlantHort III substrate (12.13 cm). However, the values were above those recorded by Álvarez-Romero *et al.* (2022) between 4.07 cm and 4.37 cm for pepper seedlings grown with natural light (Painita, Yolo, and 13LR62100 varieties). However, Hernández-Huerta *et al.* (2023) obtain higher values of pepper stem length (var. Jalapeño) in all their treatments. Regarding stem diameter, the values were similar to those obtained by Silva *et al.* (2018) (1.4 mm and 2.5 mm), Álvarez-Romero *et al.* (2022) (1.9 mm and 2.0 mm), and Hernández-Huerta *et al.* (2023) (1.1 mm and 2.5 mm). For the lengths of the primary root, all treatments had significant differences when compared to the control, with values similar to those achieved by Hernández-Huerta *et al.* (2023) of 6.8 cm and 9.1 cm. For root thickness, treatments with DM of *P. stratiotes* (0.5% and 1.0%) and with *E. crassipes* (2.0%) resulted in the highest values, with significant differences with respect to the treatment with DM of *E. crassipes* (4.0%) and the control.

The dry mass values of the aerial parts and the roots of pepper seedlings were within the range recorded by Silva *et al.* (2018) in pepper seedlings using commercial substrates Plantmax, PlantHort II, and PlantHort III (between 0.02 g and 0.11 g aerial part and between 0.01 g and 0.04 g root). However, Hernández-Huerta *et al.* (2023) obtained higher values of dry mass from the aerial parts and roots of pepper seedlings.

Dickson quality index (DQI) for tomatoes and peppers

Regarding the DQI for tomatoes, we obtained average values between 0.008 and 0.02. We obtained the highest values with the treatment with DM of *E. crassipes* (2.0%), not significantly different from the treatments with DM of *P. stratiotes* (0.5% and 1.0%) (Fig. 7A). Regarding the DQI for pepper, we obtained average values between 0.008 and 0.017. The highest values for the treatments with *P. stratiotes* DM (0.5%) that did not show significant differences were *P. stratiotes* (1.0%) and *E. crassipes* (2.0%) DM treatments (Fig. 7B).

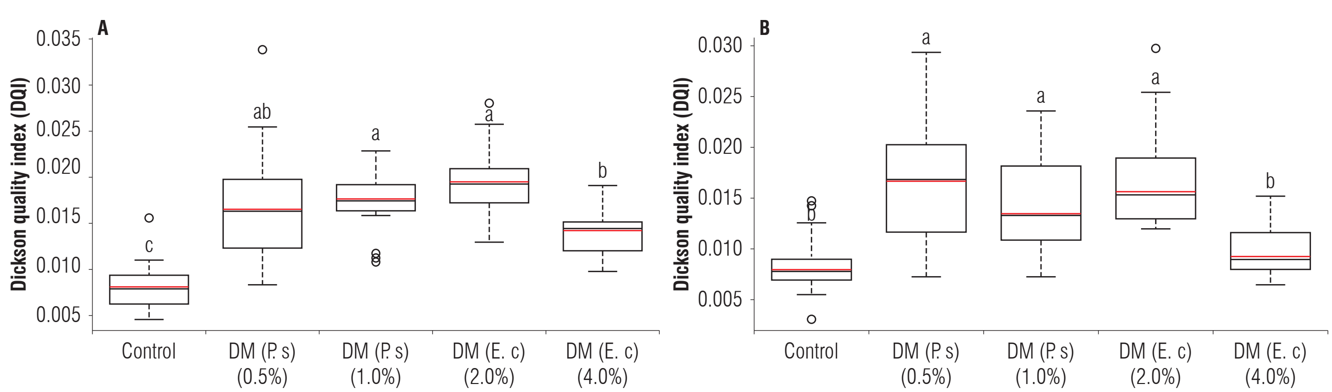

FIGURE 7. Dickson quality index (DQI). A) tomato (var. Amalia) (*P-value*= $2.343e^{-08}$); B) pepper (var. True Heart) (*P-value*= $2.21e^{-06}$). DM: dry mass, *P.s*: *P. stratiotes*, *E.c*: *E. crassipes*. *P-values* were determined according to the Kruskal-Wallis test. Different letters indicate significant differences between treatments according to the Wilcoxon test. The box represents 50% of the data. The black line inside the box is the median. The red line inside the box is the mean. Dotted line ends are maximum and minimum values. Circles are the atypical values. Confidence interval is 95%.

For tomato, the DQI was higher with the treatment with DM of *E. crassipes* (2.0%), so it was the most feasible and did not show significant differences with respect to the treatments with DM of *P. stratiotes* (0.5% and 1.0%). According to Rodríguez-Chow and Trujillo-Mendoza (2013), the soil used did not meet the P and K needs of tomatoes, so it can be inferred that the treatment with DM of *E. crassipes* (2.0%) and with DM of *P. stratiotes* (1.0%) covered these needs. However, factors such as the quality of irrigation water, the type of irrigation, and climate have played a role (López-Marín, 2017; Rodríguez-Chow & Trujillo-Mendoza, 2013).

DQI for pepper was higher in the treatment with DM of *P. stratiotes* (0.5%) that indicated that it was the best treatment and did not show significant differences with respect to the treatments with *P. stratiotes* DM (1.0%) and with DM of *E. crassipes* (2.0%). Indeed, this could have been the case, because although the soil contained most of the macronutrients necessary for the first 35 d of culture, according to the criteria of Álvarez & Pino (2018), they were not enough to cover the real needs of the species. The pepper is a vegetable with a high nutrient demand, which varies depending on the type of soil, the quality of irrigation water, and the climate (Álvarez & Pino, 2018). The treatment with DM of *E. crassipes* (4.0%) contained excessive macronutrients for peppers at the initial growth stage, which could have affected the quality of the seedlings.

The DQI values in peppers were in the range obtained by Álvarez-Romero (2022) in pepper seedbeds with red light and different substrates (between 0.01 and 0.03), but the values were below the values obtained in the seedbeds with blue light and different substrates (between 0.03 and 0.09). They were also within the range of values obtained by Silva *et al*. (2018), although these authors record maximum values between 0.02 and 0.03 with commercial substrates Plantmax, PlantHort II, and PlantHort III. Furthermore, they are lower than those reported by Hernandez-Huerta *et al*. (2023) in their study on the increase in the growth of pepper (between 0.018 and 0.040). In addition, our results were lower than those by Anjos *et al*. (2017) for the culture of pepper variety Morron with sunlight (0.06). The differences between the results of this study and the previous ones may be given by the variations of the environmental conditions, the type of substrate, and the variety used.

Conclusions

The best treatments for the initial growth of the tomato variety Amalia were those in which Ferralitic Red soil was used as a substrate, with DM of *P. stratiotes* at 1.0% and DM of *E. crassipes* at 2.0%. For the initial growth of pepper variety True Heart, the best treatments were those in which Ferralitic Red soil and DM of *P. stratiotes* at 0.5% or 1.0% and DM of *E. crassipes* at 2.0% were used as substrate. These results show that the invasive aquatic plants *P. stratiotes* and *E. crassipes*, far from having a negative impact on humans, can become an opportunity for sectors of economic importance, such as agriculture.

Acknowledgments

These results are part of the Territorial Project (PT: 121CA003-005) “Evaluation of the use and management of invasive aquatic plants *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms as an alternative for their use in urban agriculture in Ciego de Ávila” from the Center of Bioplants of Máximo Gómez Báez University in the province Ciego de Ávila (Cuba). The authors appreciate the collaboration of Vicente O. Rodríguez, Julia Martínez Rodríguez, José Ramón Laguna González, Lázaro A. Águila Armada, Yaisel Reina Rivero, Raúl D. Marchena Cervantes, and Liuba Peñate. The support of the Non-Governmental Organization Idea Wild is greatly acknowledged.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author’s contributions

LHF and YA designed the experiment and formulated the research goal and developed the methodology and research activity planning. LHF wrote the initial draft. RGZ, AJM and JCLF contributed to the data analysis. All authors reviewed the final version of the manuscript.

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Preliminary findings on biocontrol of bacterial wilt and canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis)* using *Trichoderma harzianum* after biofumigation

Hallazgos preliminares sobre el biocontrol del marchitamiento y cancro bacteriano del tomate (*Clavibacter michiganensis* subsp. *michiganensis*) utilizando *Trichoderma harzianum* luego de una biofumigación

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ABSTRACT

One of the most aggressive bacterial diseases in tomato crops is bacterial wilt and canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm). Chemical control is questioned due to its negative effects on health and the environment. Within integrated disease management, one alternative is biocontrol with *Trichoderma* species. Another technique is biofumigation, which releases volatile compounds into the soil that inhibit soilborne fungi and stimulate plant health. The aim of this study was to evaluate the potential of biofumigation and the use of *Trichoderma harzianum* for the control of bacterial wilt and tomato canker caused by Cmm *in vitro* and their effect on yields in a commercial tomato crop. The inhibition of phytopathogenic bacteria of the *in vitro* test and the number and weight of fruits per plant in a greenhouse were evaluated. The treatments were: tomato plants inoculated with Cmm with or without two strains of *T. harzianum,* alone and in combination with biofumigation. The *in vitro* test results showed, with both strains, no significant differences between the treatments, although the growth of Cmm was lower in the combination biofumigation and *T. harzianum*. One of the strains of *T. harzianum* (Th118) performed better than the other for yield (weight and number of fruits). However, the results do not show a synergistic effect between *T. harzianum* and biofumigation in the observed yield values.

Key words: biological control, phytopathogenic bacteria, tomato yield, microbial antagonists.

RESUMEN

Una de las enfermedades bacterianas más agresivas en el cultivo de tomate es el marchitamiento y cancro bacteriano ocasionado por *Clavibacter michiganensis* subsp. *michiganensis* (Cmm). Su control químico es cuestionado por sus efectos negativos en la salud y el ambiente. Dentro de un manejo integrado de enfermedades una alternativa es el biocontrol con especies de *Trichoderm*a. Otra técnica es la biofumigación que libera al suelo compuestos volátiles que inhiben fitopatógenos y favorecen la sanidad de las plantas. El objetivo del estudio fue evaluar el potencial de la biofumigación y el uso de *Trichoderma harzianum* para el control de la marchitez bacteriana y cancro del tomate causado por Cmm *in vitro* y observar el efecto sobre los rendimientos en un cultivo comercial. Se evaluó el número y peso de frutos por planta, donde los tratamientos fueron: plantas de tomates inoculadas con Cmm en presencia o ausencia de dos cepas de *T. harzianum* solas y en combinación con biofumigación. Los resultados de los ensayos *in vitro* mostraron que a pesar de que no hubo diferencias significativas entre los tratamientos, el crecimiento de Cmm fue menor en la combinación biofumigación y *T. harzianum*. Una de las cepas de *T. harzianum*, (Th118), tuvo mejor comportamiento que la otra, teniendo en cuenta el efecto sobre el rendimiento (peso y número de frutos). Por otro lado, los resultados no muestran un efecto sinérgico entre *T. harzianum* y la biofumigación en los valores de rendimiento observados.

Palabras clave: control biológico, bacterias fitopatógenas, rendimiento de tomate, antagonistas microbianos.

Introduction

Tomato wilt and bacterial canker is caused by *Clavibacter michiganensis* subsp. *michiganensis* (Davis *et al.,* 1984). It is present in practically all the producing areas of tomato worldwide (Leon *et al*., 2011; Osdaghi, 2015). This disease has caused large losses in tomato crops both in the field and in the greenhouse. The most relevant symptom is the wilting and death of plants, which causes large economic losses (Chalupowicz *et al*., 2016; Osdaghi, 2015; Rolleri & Romero, 2022). When the disease occurs, it can affect all plants in plots or greenhouses in a short time (EPPO,

Received for publication: May 27, 2024. Accepted for publication: July 15, 2024. Doi: 10.15446/agron.colomb.v42n2.114659

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2013; Kawaguchi *et al*., 2010). The disease is very difficult to control, and management practices have focused mainly on preventive measures and the use of chemical synthesis products (cuprics and antibiotics). However, these practices have not proven efficient. On the contrary, they not only increase production costs, but also cause harm to humans, animals and the environment. To reduce its incidence, rotation with non-susceptible crops is recommended. However, the effectiveness of this measure depends on the survival of the pathogens on the debris and/or crop supports or on its reintroduction to a new crop (Maeso *et al*., 2012; Malliarakis *et al*., 2023; Vega & Romero, 2015).

Management of diseases in horticulture has traditionally been carried out by soil fumigation, using toxic, volatile compounds (Martin, 2003). Alternative management methods for soil-borne plant pathogens are needed to maintain high agricultural production. In recent years, in the exploration for an ecofriendly disease control, efforts have been directed towards an enhanced understanding of the biological effects of natural products. Biofumigation by means of Brassicaceae green manure incorporation into soil is a promising, ecofriendly alternative to chemical fumigation by methyl bromide for the control of phytopathogens. This biological process is based on the release of glucosinolate-derived toxic compounds, facilitated by endogenous myrosinase (thioglucosidase EC 3.2.1.147) from Brassicaceae plant residues in the presence of water (Brown & Morra, 1997; Hanschen & Winkelman, 2020; Makane *et al.,* 2023; Mitidieri *et al*., 2015; Zhang *et al*., 2020). The beneficial effects observed may not always be related to the activity of glucosinolate-based hydrolysis compounds but may add to other mechanisms that improve plant health. These may play a complimentary or more dominant role in some disease suppression. This is probably due to the incorporation of large quantities of plant residues into the soil. Potentially, this improves soil structure, increases nutrient availability, increases water holding capacity, and stimulates antagonist microbial communities (Kirkegaard & Matthiensen, 2004; Rolleri *et al*., 2021). In addition, biofumigation favors the development of plants, making them more robust. Various authors (Daugovish *et al.,* 2009; Mitidieri *et al.,* 2015) attribute this to the contribution of mineral nutrients, such as nitrogen and phosphorus, and the increase in soil organic matter.

Trichoderma are free-living beneficial fungi commonly found in soil, useful for plant protection purposes in agriculture (Amerio *et al*., 2020; Guzman-Guzman *et al*., 2023). Commercial products based on certain *Trichoderma* isolates are currently utilized in the biocontrol of some soil borne and foliar pathogenic fungi (Sood *et al*., 2020). *Trichoderma* spp. are capable working together both in the plant rhizosphere and in the phyllosphere through several mechanisms, such as antagonism, competition for space and nutrients, mycoparasitism and the discharge of antibiotics and lytic enzymes, which directly inhibit phytopathogen growth (Amerio *et al*., 2020; Harman *et al.,* 2004). In this sense, numerous authors have studied biofumigation and *Trichoderma* spp. *in vitro* (Perniola *et al*., 2014) as well as their incorporation into the soil for management of phytopathogenic fungi (Berlanas *et al*., 2018; Makane *et al*., 2023; Morales-Rodriguez *et al*., 2018; Stocco *et al*., 2016).

This study aimed to explore the effect of biofumigation with *Eruca vesicaria* (L.) Cav. and the incorporation of two strains of *Trichoderma harzianum* on: 1) phytopathogenic bacteria present *in vitro* tests and 2) the control efficacy of bacterial canker and wilt on tomato plants grown under greenhouse conditions. In this regard, this research seeks to determine the synergistic effect of biofumigation and the application of *T. harzianum* on the manifestation of the disease.

Materials and methods

Fungal strains

Two strains of *T. harzianum* (Th118 and Th5cc) were used as antagonists. The Th118 strain was isolated from the tomato leaf phylloplane and previously tested in greenhouse trials. This strain reduced the incidence of the disease caused by *Botrytis cinerea* in tomato plants (Dal Bello *et al*., 2011). The Th5cc strain was isolated from the wheat phylloplane and was previously tested as an antagonist of *Zymoseptoria tritici* (Cordo *et al*., 2007). In addition, in a previous study, the Th5cc strain, when applied as a liquid formulation and as a coating on seeds, was the most effective in maintaining a high population of *T. harzianum* in soil, with a potential biocontrol effect (Stocco *et al.,* 2019). Both strains were molecularly identified following the technique described by Stocco *et al.* (2016) and were deposited in the database of the European Molecular Biology Laboratory (EMBL) under the accession numbers LN869400 (*T. harzianum* Th118) and LN869401 (Th5cc). These strains are also deposited in the fungal collection of the Centro de Investigaciones de Fitopatología (CIDEFI, UNLP, Argentina).

Bacterial strain

For this study, the strain of Cmm LPAb158 was used, which is deposited in the collection of microbial cultures of the Centro de Investigaciones de Fitopatología (CIDEFI, Argentina). It was identified by microbiological and molecular techniques using specific primers that amplify the intergenic region 16S-23S of rRNA (EPPO, 2013; Schaad *et al.,* 2001), as described by Rolleri (2015).

In vitro test

To evaluate the effect of biofumigation and the two strains of *Trichoderma harzianum* (Th5cc and Th118) on the pathogen, fresh plant tissue of arugula (*Eruca vesicaria*) was collected from the greenhouse experiments. The plants were uprooted at the 50% flowering stage and taken immediately to the laboratory in autoclaved polypropylene bags. The plants were washed with sterilized water, cut into small pieces, and then 5 g of the moistened plant tissue was placed at the bottom of a 9 cm Petri dish. For the preparation of the assay, a 4 cm long guideline was drawn on the bases of the Petri dishes where the bacteria were seeded with a bacteriological loop on Nutrient Agar. Then, Cmm was seeded 3 cm from the edge of the dish and 3 cm from the location where the antagonist was placed simultaneously. Five mm diameter discs of actively growing mycelium of *Trichoderma* strains were taken from the margins of 7-d-old cultures and transferred to Petri dishes, maintaining a distance of 4 cm between Cmm and *Trichoderma* (dual culture). One plug of each *Trichoderma* strain was seeded in each dual culture. The lid of the Petri dishes containing the pieces of arugula was replaced with the bottom of the Petri dishes with the fungal plug and bacteria. The pieces of arugula were not in contact with the bacteria or with *Trichoderma* sp. The plates were immediately sealed with parafilm and incubated in an inverted position at 27±2°C until the *Trichoderma* almost covered the medium surface. Petri dishes without biofumigation or *Trichoderma* were used as controls.

To evaluate the area of the Cmm colony, the growth in length was measured on the marked line of the bacterial colony and three perpendicular measurements of width were taken (at the center and 2 cm from it to the right and left). Two days after sowing, the three width measurements were averaged and multiplied by the growth length, thus obtaining the growth value of the bacteria (Peñalba, 2022). The treatments were: 1) Cmm, 2) Cmm + biofumigation, 3) Cmm + *T. harzianum* (Th5cc) or *T. harzianum* (Th118), 4) Cmm + *T. harzianum* (Th5cc) or *T. harzianum* (Th118).

To evaluate the growth of *T. harzianum*, the growth in length was measured 2 d after sowing.

Application of *Trichoderma harzianum* to tomato seedlings

The strains of *T. harzianum* were incorporated in liquid form to the substrate of tomato seedlings var. Elpida. For the incorporation, a suspension of spores of each strain of *T. harzianum* developed in PDA medium (potato dextrose agar at 2%) was prepared from a culture 8 d-old. The suspension was made by adding sterile water over the colonized Petri dish and scraping with a sterile ansa. Then it was adjusted to 1 x 10^8 spores ml^{-1} and Tween 20 (0.01%) was added. The fungal inoculum was applied only once, at the time of sowing, in the form of irrigation using 10 ml of suspension for each cell of the planting tray. Finally, tomato seeds were sown, one for each cell in the planting tray. The control treatment consisted of sowing tomato seeds on substrate without inoculum of *T. harzianum*. This methodology was used according to the results obtained by Rolleri *et al.* (2021), who tested two techniques for infesting the substrate with *Trichoderma* sp.

Greenhouse assays

The assays were carried out in a greenhouse (6 m x 20 m, with wooden masonry and 180 µm thick polyethylene) during September 2021 – January 2022 and September 2022 – January 2023. The greenhouse was located at the Chacra Experimental Gorina of the Ministry of Agrarian Development of the province of Buenos Aires, Argentina (34°54'56.4" S, 58°02'21.5" W) belonging to the Platense Horticultural Belt.

In the greenhouse, *E. vesicaria* seeds were sown at the rate of 10 g m^2 and after flowering the plants were cut and integrated into soil using a common rotary cultivator at a rate of 5 kg fresh biomass m^{-2}. After integration, the plot was sheltered with linear low-density polyethylene sheets and left for one month (biofumigation). At the time of tomato planting, the polyethylene sheet was removed and the soil was thoroughly mixed. In this greenhouse, the traditional management carried out by producers without the use of agrochemicals was followed. Biofumigation was carried out in half of the greenhouse, while the other half did not receive it. The tomato plants were transplanted at the state of three fully expanded leaves, at a distance of 0.35 m between plants and 0.60 m between rows. The treatments were the following: tomato seedlings inoculated with Cmm and biofumigation; tomato seedlings treated with *T. harzianum* (Th5cc), inoculated with Cmm and biofumigation; tomato seedlings treated with *T. harzianum* (Th118), inoculated with Cmm and biofumigation; tomato seedlings without

Trichoderma spp. inoculated with Cmm (control); seedlings treated with *T. harzianum* (Th5cc) and inoculated with Cmm, and tomato seedlings treated with *T. harzianum* (Th118) and inoculated with Cmm.

A single inoculation with the pathogenic bacteria was carried out at the time the first shoot was cut, when the plants had between 12 and 14 leaves. For this, the bacterial suspension was placed in the shoot wound. To prepare the bacterial suspension, the bacteria were streaked in Petri dishes, in Nutrient Agar (NA) medium, and incubated at 27°C (+/- 2°C) for 48 to 72 h. Subsequently, sterile distilled water was added, adjusting to a spectrophotometer reading of OD600 = 0.3 (~5.5 x 10^8 CFU ml^{-1}) diluted 1:10 to reach the final used concentration of 10^7 CFU ml^{-1}. The experimental design was a randomized block design with 6 treatments and 10 replicates.

The evaluation consisted of determining yield parameters, number and weight of fruits per plant. Three evaluations were carried out on a weekly basis.

Statistical analysis

To ensure reproducibility and reliability, the experiments were conducted twice. Data from the *in vitro* tests and the greenhouse trials were analyzed using analysis of variance (ANOVA) with the Infostat® program (Di Rienzo *et al.*, 2020). Treatment means for all variables were compared using the Tukey test at a significance level of 5% ($P \leq 0.05$). If the data did not meet the assumptions of normality, homoscedasticity and randomness, non-parametric statistics were applied using the Kruskal-Wallis test.

Results

In vitro test

The results of the *in vitro* assays showed that the diameter of the *Trichoderma* colonies (Th118 and Th5cc) did not present significant differences among the different treatments ($P \leq 0.05$) (Tab. 1).

According to the results, although significant differences were observed between treatments, the combination of Th5cc and biofumigation showed the lowest values in the growth of Cmm but did not differ significantly from the control (Cmm) (Fig. 1).

With respect to Th118, the bacterial growth was only slightly affected by biofumigation and the presence of *T. harzianum*. However, although no significant differences were observed between the treatments, the growth of Cmm was lower in the combination biofumigation and *T. harzianum*. These results can be observed in Figure 2.

TABLE 1. Growth (cm^2) of *Trichoderma harzianum* (Th118 and Th5cc) with and without biofumigation (Bio) in the presence or absence of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm).

Treatment	Mean	Standard deviation	Treatment	Mean	Standard deviation
Th118+Bio+Cmm	3.07	0.62	Th5cc+Bio+Cmm	5.57	0.53
Th118+Bio	4.64	1.20	Th5cc+Bio	5.57	0.53
Th118+ Cmm	3.39	1.26	Th5cc+ Cmm	5.05	0.27
Th118	3.37	1.00	Th5cc	5.09	0.35

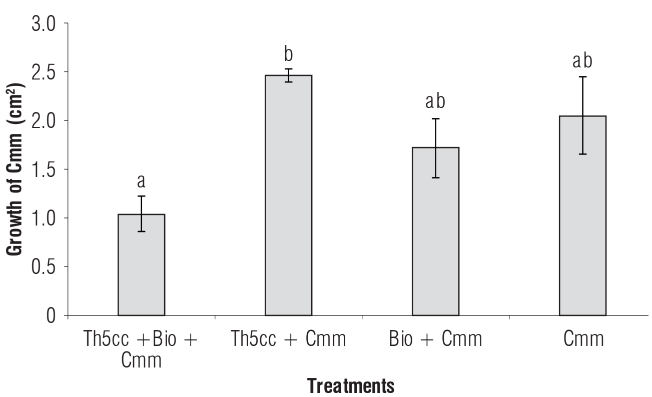

FIGURE 1. *In vitro* growth (cm^2) of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) with and without biofumigation (Bio) and in the presence or absence *Trichoderma harzianum* (Th5cc). Averages with the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). Vertical bars represent standard error (n=5).

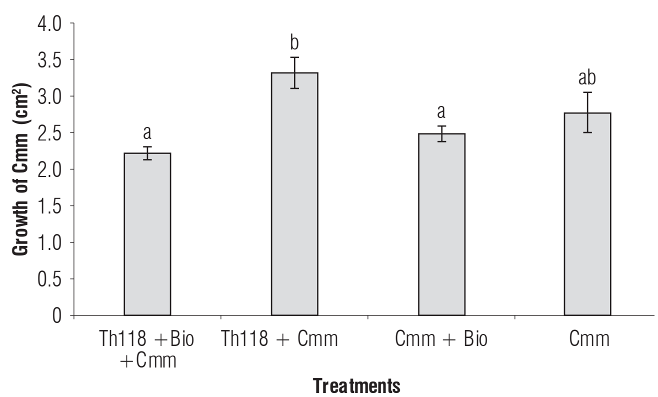

FIGURE 2. *In vitro* growth (cm^2) of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) with and without biofumigation (Bio) and in the presence or absence of *Trichoderma harzianum* (Th118). Averages with the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). Vertical bars represent standard error (n=5).

Greenhouse assays

Related to the assays in the greenhouse, in 2021-2022, the inoculated control with biofumigation presented the lowest yield in terms of the weight of the harvested fruits. The Th118 treatment produced the highest weight of tomato fruits, but it did not differ significantly from the control (Fig. 3).

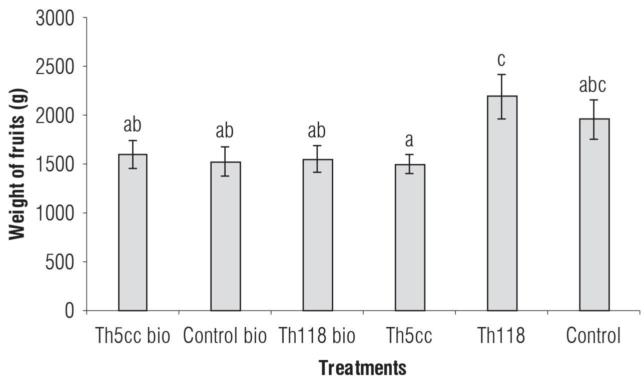

FIGURE 3. Effect of the treatments on the weight of tomato fruits during 2021-2022. Averages with the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). Th5cc bio: with *Trichoderma harzianum* 5cc and biofumigation; Th118 bio: with *T. harzianum* 118 and biofumigation; Control bio: with biofumigation without *T. harzianum*. The error bars correspond to standard error (n=27).

The effect of the treatments on the number of fruits is shown in Figure 4. The Th118 treatment had the greatest number of fruits, and it differed significantly from the control treatment with biofumigation. The same observation was made in relation to fruit weight.

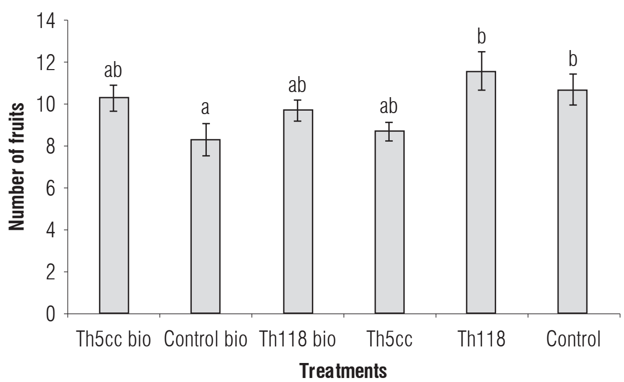

FIGURE 4. Effect of the treatments on the number of tomato fruits harvested during 2021-2022. Averages with the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). Th5cc bio: with *Trichoderma harzianum* 5cc and biofumigation; Th118 bio: with *T. harzianum* 118 and biofumigation; Control bio: with biofumigation without *T. harzianum*. The error bars correspond to standard error (n=27).

For tomato yield and weight of fruits, Th118 without biofumigation had the highest values and differed statistically from the treatments Th5cc with and without biofumigation and the treatment control without biofumigation (Fig. 5). For the numbers of fruits, the treatment Th118 without biofumigation had the highest value and differed statistically from the other *T. harzianum* strain, both with and without biofumigation (Fig. 6).

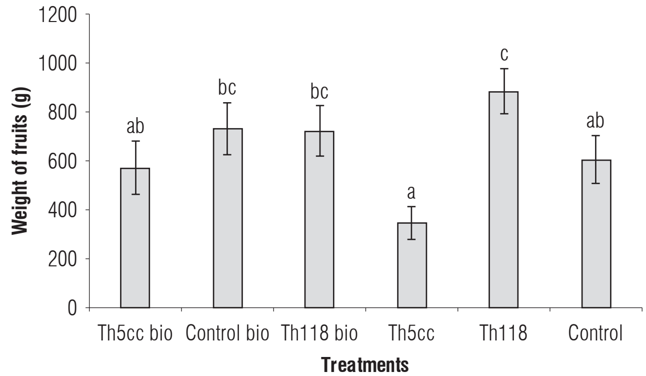

FIGURE 5. Effect of the treatments on the weight of tomato fruits during 2022-2023. Averages with the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). Th5cc bio: with *Trichoderma harzianum* 5cc and biofumigation; Th118 bio: with *T. harzianum* 118 and biofumigation; Control bio: with biofumigation without *T. harzianum*. The error bars correspond to standard error (n=27).

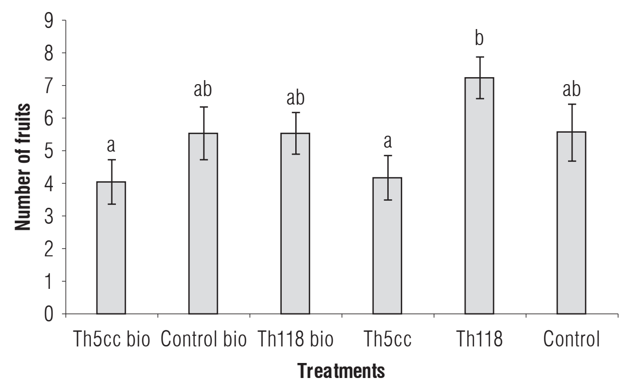

FIGURE 6. Effect of the treatments on the number of tomato fruits harvested during 2022-2023. Averages with the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). Th5cc bio: with *Trichoderma harzianum* 5cc and biofumigation; Th118 bio: with *T. harzianum* 118 and biofumigation; Control bio: with biofumigation without *T. harzianum*. The error bars correspond to standard error (n=27).

Discussion

In recent years, there has been increased interest in the biological control of plant pathogens, particularly phytopathogenic bacteria (Abo-Elyonsr *et al.*, 2019; Amerio *et al.*, 2020; Berlanas *et al.*, 2018; Sarandon & Flores, 2014; Zahir *et al.*, 2018). Within this context, and based on the results of the previous studies, the use of *T. harzianum* is a good alternative to control wilt and bacterial canker in tomato

(Rolleri *et al*., 2021). Additionally, the use of Cruciferous species for biofumigation, which can be carried out alone or combined with biocontrol microorganisms as control mechanisms for crop diseases, has gained significance (Dugassa *et al*., 2021; Mitidieri *et al*., 2015).

In this research, we studied the influence of the incorporation of *Eruca vesicaria* material in combination with *T. harzianum* biocontrol agent *in vitro* to control the pathogenic bacteria *Clavibacter michiganensis* subsp. *michiganensis* in tomato. In this assay, *Trichoderma* strains were not inhibited by the volatiles released by cruciferous species; similar results have been reported by Perniola *et al*. (2014). Kirkegaard and Matthiessen (2004) found that to stop the growth of certain pathogens, such as *Bipolaris* spp., *Sclerotinia* spp. or *Phytophthora* spp., low concentrations of isothiocyanates are necessary; however, to affect *Trichoderma* spp., high doses of these compounds are required. Furthermore, in our *in vitro* assay results, we observed that *T. harzianum* strains behaved differently. In this sense, the Th5cc and Th118 strains did not cause significant differences in the growth of the bacteria; similar results were observed with biofumigation. On the other hand, the Th118 strain, although it did not differ statistically from the control, caused a decrease in bacterial growth in combination with biofumigation, demonstrating a synergistic effect, as suggested by Perniola *et al*. (2014).

Additionally, the increase in microorganisms is undoubtedly due to the incorporation of cruciferous plant residues, which have an important role in the suppression of plant pathogens and improving plant health (Bakker *et al*., 2010; Bonanomi *et al*., 2010). In this sense, Mitidieri *et al*. (2015) mention that some fungi, such as *Trichoderma*, are tolerant to isothiocyanates. The management of bacterial diseases is difficult when epidemics develop during favorable weather. One of the ways to partially solve this problem may be the incorporation of Cruciferae residues in soil. Organic amendments improve soil contents of mineral nutrients, increase biodiversity, prevent degradation, and contribute to general suppressiveness through enhanced soil microbial biomass. Regarding the greenhouse results, one of the strains of *T. harzianum* (Th118) had a better performance than the other strain, considering the effect on the yield (with an average fruit weight between 900 and 2200 g). In this sense, *T. harzianum* Th118 strain exhibited a better performance than Th5cc, both in the in vitro and greenhouse assays. Rolleri *et al*. (2021) obtained similar results when they applied Th118 in the form of irrigation at the time of sowing in tomato plants from La Plata. This makes sense, considering that strain Th118 was isolated from the phylloplane of tomato plants (Dal Bello *et al*., 2011) and was better adapted to the agroecosystem studied. One concern about the use of *Trichoderma* spp. in greenhouses is the introduction of new species in the area, which is why native species are used (Dugassa *et al*., 2021; Guzman-Guzman *et al*., 2023; Zhang *et al*., 2020). And if, in addition to this effect, a biocontrol agent such as *T. harzianum* is incorporated, the effect on the development of the disease and the growth of the plants is enhanced.

Some authors, such as Galletti *et al*. (2008), found a synergistic effect of the two biological control methods carried out in soil under controlled conditions, applying separately and together *Trichoderma* spp. and biofumigation with seeds of *Brassica carinata*. However, in our study and according to Berlanas *et al*. (2018), we did not observe a synergistic effect between biofumigation and the incorporation of *T. harzianum* to the tray of tomato seedlings on the yield parameters (weight and number of the fruits). Regarding tomato yield and fruit weight, the Th118 treatment without biofumigation resulted in the highest values; the same treatment had the highest number of tomato fruits. This *Trichoderma* strain, applied as irrigation to the seedlings, could be a good alternative within an integrated disease management plan in tomato cultivation. In this sense, the proposed hypothesis could not be demonstrated, since the plants transplanted in a biofumigated soil did not present higher yield values when infested with *T. harzianum* and inoculated with Cmm.

Further research is required to analyze the effect of this integrated approach with different species of *Brassica* or non-*Brassica* genera on other phytopathogens and in field conditions to study the effect of biofumigation and *T. harzianum* on the incidence of bacterial wilt and canker of tomato.

Conclusions

This work highlighted the effect of *T. harzianum* (Th118) on the severity of bacterial canker and yield in tomato plants. We also demonstrated that no synergistic effect on yield was observed between Th118 and biofumigation. Further complementary studies are required to evaluate the integrated effect between biofumigation and other species of *Trichoderma*.

Acknowledgments

We express our gratitude towards the Estacion Experimental de Gorina (Argentina), where the assays were executed.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

MS, JR and CM designed the experiment, developed the methodology and analyzed the evaluated data. PM and JP carried out the experimentation in the greenhouse and collected data. MS performed the statistical analysis of the data.CM wrote the initial draft of the manuscrip. All authors reviewed the final version of the manuscript.

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Entomofauna associated with *Spodoptera frugiperda* in a maize agroecosystem in San José de las Lajas, Cuba

Entomofauna asociada a *Spodoptera frugiperda* en un agroecosistema de maíz en San José de las Lajas, Cuba

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ABSTRACT

The cultivation of maize (*Zea mays* L.) is of significant global economic importance as it is the third most widely grown cereal worldwide. Numerous harmful organisms affect this crop, impacting its yields. Among these, the "fall armyworm" *Spodoptera frugiperda* J. E. Smith, a corn pest, is the main culprit causing substantial economic losses because of its damage. Besides this pest, various beneficial insects are associated with the maize crops that play a crucial role in its control. The present research was conducted at the National Institute of Agricultural Sciences (INCA) in Cuba to list the insects associated with *S. frugiperda* in the maize agroecosystem with a 'MAIG-Diamante' cultivar. One hundred randomly selected plants were sampled in a zigzag pattern, and the inner whorl leaves and other parts of the plants were examined. Seven samplings were carried out weekly. Classification of species was done using taxonomic keys. In the agroecosystem, insects belonging to 7 orders, 22 families, and 28 species were found. These were categorized according to their habits as 15 phytophagous, 11 predators, 1 parasitoid, and 1 pollinator species. *Spodoptera frugiperda*, *Zelus longipes, Zelus* sp., *Nezara viridula, Peregrinus maidis, Oxymerus aculeatus*, an unspecified species of beetle, *Brachiacantha decora, Doru* sp., and *Apis mellifera* were very frequent. In contrast, the rest of the remaining species were somewhat frequent.

Key words: phytophage, beneficial insects, predators, parasitoids.

RESUMEN

El cultivo del maíz (*Zea mays* L.) tiene gran importancia económica a nivel mundial por ser el tercer cereal más extendido en todo el mundo. Sobre dicho cultivo inciden numerosos organismos nocivos, afectando su rendimiento, siendo *Spodoptera frugiperda* J. E. (Smith), la "palomilla del maíz", la principal plaga causante de grandes pérdidas económicas. Asociados a esta plaga existen diversos insectos benéficos que juegan un papel importante en su regulación. El presente trabajo se realizó en el Instituto Nacional de Ciencias Agrícolas (INCA) en Cuba, con el objetivo de catalogar la entomofauna asociada a *S. frugiperda* en un agroecosistema de maíz cultivar 'MAIG-Diamante'. Para el inventario se muestrearon 100 plantas seleccionadas al azar en forma de zig-zag y se revisaron los cogollos en su interior y otras partes de la planta. Se realizaron un total de 7 muestreos con una frecuencia semanal. La clasificación de las diferentes especies se realizó mediante diferentes claves taxonómicas. Se recolectaron insectos pertenecientes a 7 órdenes; 22 familias y 28 especies. Estos fueron ubicados según sus hábitos alimentarios en: 15 fitófagos, 11 depredadores, 1 parasitoide y 1 polinizador. Las especies *Spodoptera frugiperda, Zelus longipes, Zelus* sp., *Nezara viridula, Peregrinus maidis, Oxymerus aculeatus,* especie de escarabajo no determinada, *Brachiacantha decora*, *Doru* sp. y *Apis mellifera* resultaron ser muy frecuentes; mientras que el resto de las especies fueron frecuentes.

Palabras clave: fitófago, insectos benéficos, depredadores, parasitoides.

Introduction

Maize (*Zea mays* L.) is a crop of significant economic and nutritional importance and is considered one of the most cultivated cereals alongside rice (*Oryza sativa* L.) and wheat (*Triticum sativum* L.) (Chura *et al*., 2019). It is the most worldwide crop, cultivated in 170 countries, with over 200 million ha harvested in 2018 (Díaz *et al*., 2022). This crop serves as food for humans, livestock, poultry, and raw materials in various industries (Rodríguez-Soto *et al*., 2018).

In Cuba, maize holds a strong tradition as a crop. It is used for human and animal consumption, as an associated crop, living fence, and reservoir of insect predators, among other

Received for publication: June 13, 2024. Accepted for publication: August 30, 2024. Doi: 10.15446/agron.colomb.v42n2.115031

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roles (Gigato-Toledo *et al*., 2018). However, various harmful organisms affect this crop. During fall, armyworm *Spodoptera frugiperda* J. E. Smith is the most important pest that causes significant economic losses (MRI, 2019). Yield reductions of up to 0.8 t ha^{-1} of dry maize grain, equivalent to 40% of the production in Cuban conditions, can occur (Blanco & Leyva, 2009). The highest infestations occur during the vegetative stage when the larva feeds on the leaf tissues (Mirabal *et al*., 2018).

Associated with *S. frugiperda* are various beneficial insects that contribute to the natural regulation of its populations in crops. The conservation and management of these beneficial insects are paramount, as they provide valuable ecological services for farmers at no cost. They aid in the regulation of harmful organisms and reduce the use of pesticides that are detrimental to humans, animals, and the environment (DGSV-CNRF, 2021; García González *et al*., 2022).

In Cuba, maize is one of the most fundamental crops cultivated in spring. Many details are known about the insect fauna present in maize during the spring in different locations and associated with various varieties. However, data from this specific cultivar in a low-rainfall period are scarce. This research aimed to list the insects associated with *S. frugiperda* in maize crops of the 'MAIG-Diamante' (conventional) cultivar. We determined the relative percentage frequency of occurrence of each detected insect species on the Las Papas Farm of the National Institute of Agricultural Sciences (INCA). This data will contribute fundamental and essential knowledge for this crop's agroecological pest management approach.

Materials and methods

Experimental area

This research was conducted from October to January 2021-2022 in experimental areas of the National Institute of Agricultural Sciences (INCA) in San José de las Lajas, Mayabeque Province, Cuba, 3.5 km along the road to Tapaste. The geographic coordinates of the area are 22°59'40.79" N and 82°8'21.88" W at an altitude of 138 m a.s.l.

The agroecosystem where the experiment was conducted is characterized by a short duration of low rainfall from November to March (Gil-Reyes *et al*., 2020).

The monthly climatic variables recorded were precipitation (mm), average monthly temperature (°C), and relative air humidity (%), using the meteorological station number 78374 located 350 m away from the experimental area.

The average monthly temperatures during the research period ranged from 21.9°C to 28.3°C, corresponding to the cooler and drier months (November to April). Monthly precipitation varied from 90 mm in the drier period to 20.9 mm in the cooler period. Notably, the highest precipitation occurred in October and December, when precipitation was limited, necessitating artificial irrigation (sprinkling) (MINAG, 2013). Relative air humidity ranged from 77% to 93% during the experimental period.

The soil of the experimental area was a typical eutrophic leached Red Ferralitic soil, characterized by medium to high fertility (Hernández Jiménez *et al*., 2015). According to this author, the soil is deep with an acidic pH; it has a low percentage of organic matter and a high percentage of phosphorus and calcium. However, potassium and magnesium are deficient (Tab. 1). Soil analyses were carried out using the methodologies described by Paneque *et al*. (2010).

TABLE 1. Chemical characteristics of the soil where an inventory of the entomofauna associated with the maize cultivar 'MAIG-Diamante' was carried out.

Depth (cm)	pH (H_2O)	OM (%)	P (mg kg^{-1})	K^+	Ca^{2+}	Mg^{2+}
				(cmol$_c$ kg^{-1})		
0-20	6.4	2.11	234	0.52	9.93	1.80

OM: soil organic matter.

Experimental program

The maize cultivar 'MAIG-Diamante' was used and is characterized by having a medium growth cycle of 120 d to harvest maturity (MINAG, 2013). The crop reaches a height between 220 and 230 cm, with 12-16 thick and erect leaves. It is resistant to lodging, has a tassel and stigma, and its grains are yellow. Its potential yield is 5 t ha^{-1}.

The experimental area was 0.1 ha; the preceding crop was sweet potato (*Ipomoea batata* L.). Sowing of maize was carried out manually with a spacing arrangement of 0.90 m between rows and 0.30 m between plants. Nitrogen fertilization was applied at the time of planting at a rate of 50 kg ha^{-1} using urea, and potassium was applied at 100 kg ha^{-1} using potassium chloride.

Inventory of the insects associated with *S. frugiperda* in maize agroecosystem

To evaluate the entomofauna associated with *S. frugiperda* in maize crops, a detailed examination of the inner whorl leaves and other plant parts was conducted on 100 randomly selected plants in a zig-zag pattern using the simple systematic method. This was performed weekly, starting from 21 d after planting (from 14 to 56 d post-emergence), for 7 samplings.

Smaller insects (<3.0 mm) were collected using a handheld aspirator to gently collect insects. The insects were placed in labeled vials indicating the sampling site and date. In the case of highly mobile insects, an entomological net was used for collection at an angle of 90º in diagonal form.

Larger insects were stored in glass jars with 70% ethanol for preservation. All samples were taken to the Entomology Laboratory at the Faculty of Agronomy of the Agrarian University of Havana (UNAH). Species identification was done using a Novel stereoscopic microscope (PRC) with a magnification of 1.5-2. Taxonomic determination was performed using the keys of Cave (1993), Fernández (2002), Gordon (1985), and Nájera and Souza (2010).

Parasitoids were taken to the laboratory of the National Center for Agricultural Health (CENSA) for classification by specialized personnel using the guidelines proposed by Cave (1993). For each sampling, 20 larvae of *S. frugiperda* at different development stages were taken, placed separately in Petri dishes, and fed with leaves of *Sorghum halepense* (L.) Pers. until their life cycle was completed or until the emergence of parasitoids, which were placed in containers of ethanol at 70% for their identification.

Additionally, the samples were compared with specimens from the insect collection at the Entomology Laboratory of the Plant Health Department, Faculty of Agronomy of the Agrarian University of Havana.

Determination of the relative frequency index

The relative frequency index for each species was determined based on the data obtained from each weekly sample for each detected species. The following equation was used:

$$Rf = \left(\frac{Mi}{Mt}\right) * 100 \qquad (1)$$

where

Rf = relative frequency of species occurrence (%);

Mi = total number of samplings with the species i;

Mt = total number of samplings.

The assessment of relative frequency (Rf) values was carried out using the scale of Masson and Bryant (1974), which indicates that a species is considered very frequent if Rf > 30, frequent if 10 ≥ Rf ≤ 30, and rare if Rf < 10.

Results

Inventory of the insects associated with *S. frugiperda* in a maize agroecosystem

In the maize agroecosystem, various insect species were detected belonging to seven orders, 22 families, and 28 species, of which 25 were identified (Fig. 1). The orders with the highest number of species were Coleoptera, Hemiptera, and Hymenoptera, with 10, 8, and 4 species, respectively.

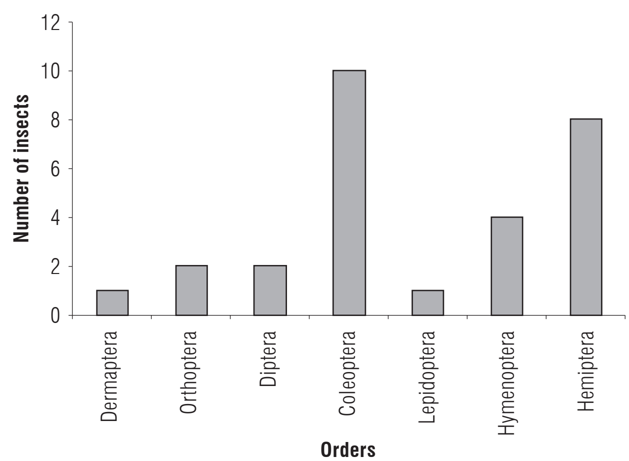

FIGURE 1. Number of species detected in each insect order in the maize cultivar 'MAIG-Diamante'.

List of phytophagous insects associated with maize crop

Table 2 lists the species of phytophagous insects distinguished from the 7 samples. Specifically, in this cultivar, 15 harmful species were identified. Among these, the primary species found and recognized in the literature as a pest capable of causing significant damage was *Spodoptera frugiperda*. Other insect species considered phytophagous for the crop were also recorded, including *Anasa andresii* Guér., *Nezara viridula* L., *Peregrinus maidis* Ashm., *Typophorus nigritus* Fabricius, *Diabrotica balteata* Le Conte, *Coptocycla guttata* Boheman, *Oxymerus aculeatus lebasi* Dup., *Cylas formicarius* Fabricius, *Atta insulares* Guér., *Conocephalus fasciatus* De Geer, *Gryllus assimilis* Fabricius, *Euxesta* sp., and two unidentified species from the order Coleoptera (Scarabaeidae and Curculionidae families).

TABLE 2. List of phytophagous insect species observed in the maize crop of the cultivar ‘MAIG-Diamante’.

Order	Family	Species
Lepidoptera	Noctuidae	*Spodoptera frugiperda* J. E. (Smith)
Hemiptera	Coreidae	*Anasa andresii* Guér.
	Pentatomidae	*Nezara viridula* L.
	Delphacidae	*Perigrinus maidis* Ashm.
Coleoptera	Chrysomelidae	*Typophorus nigritus* Fabricius
		Diabrotica balteata Le Conte
		Coptocycla guttata Boheman
	Cerambycidae	*Oxymerus aculeatus lebasi* Dup.
	Scarabaeidae	Undetermined species
	Curculionidae	Undetermined species
	Brentidae	*Cylas formicarius* Fabricius
Hymenoptera	Formicidae	*Atta insulares* Guér.
Orthoptera	Tettigonidae	*Conocephalus fasciatus* De Geer
	Gryllidae	*Gryllus assimilis* Fabricius
Diptera	Otitidae	*Euxesta* sp.

The species *P. maidis* belonging to the order Hemiptera and the family Delphacidae was found in the nymphal stage on the flag leaves and the plant’s tassel, 14 d after plant emergence (sampling 1). Concerning this delphacid, individuals were not detected forming colonies but rather were isolated on the leaves. In the last sample, one species of adult Diptera (*Euxesta* sp.) was detected on the maize crop.

List of beneficial insects associated with maize crop

Table 3 presents the beneficial insect species recorded in the maize crop during the research period.

TABLE 3. List of beneficial insect species associated with *Spodoptera frugiperda* in the maize agroecosystem.

Order	Family	Species
Predators		
Coleoptera	Coccinellidae	*Cycloneda sanguinea limbifer* Casey
		Brachiacantha decora Casey
		Brachiacantha sp.
Hymenoptera	Formicidae	*Pheidole* sp.
	Vespidae	*Polistes* sp.
Hemiptera	Reduviidae	*Zelus longipes* L.
		Zelus sp.
	Anthocoridae	*Orius insidiosus* Say
	Nabidae	Undetermined species
	Miridae	*Nesidiocoris tenuis* Reuter
Dermaptera	Forficulidae	*Doru* sp.
Diptera (Parasitoid)	Tachinidae	*Archytas marmoratus* Townsend
Hymenoptera (Pollinator)	Apidae	*Apis mellifera* L.

Thirteen species considered beneficial were identified as natural enemies of some harmful insects. Among these, 11 species exhibit predatory habits, while one is a parasitoid insect. Additionally, one species functions as a pollinator. These species spanned five orders and were represented in 10 families (Tab. 3).

The beneficial insects identified belonged to the orders Coleoptera, Hymenoptera, Hemiptera, Dermaptera, and Diptera, with Hemiptera having the highest number of species (5). In the study crop, the Coleoptera had three species from the family Coccinellidae: *Cycloneda sanguinea limbifer, Brachiacantha decora,* and *Brachiacantha* sp.

Only the parasitoid *Archytas marmoratus* in the adult stage was detected in field conditions. However, no parasitoid emerged when *S. frugiperda* larvae were placed in Petri dishes in the laboratory. Since the crop was established at a non-optimal time, the climatic conditions might not have been conducive to the development of parasitoid species.

Determination of the relative frequency index

Table 4 shows the relative frequency of the different species found in the studied crop. All the species detected in the research were determined as highly frequent and frequent.

TABLE 4. Relative frequency of entomofauna associated with the maize cultivar ‘MAIG-Diamante’.

Species	Relative frequency (%)
S. frugiperda	100.0 VF
A. andresii	14.28 F
Z. longipes	85.71 VF
Zelus sp.	71.42 VF
O. insidiosus	14.28 F
Undetermined species (Nabidae)	14.28 F
N. tenuis	14.28 F
N. viridula	42.85 VF
P. maidis	100.0 VF
T. nigritus	14.28 F
D. balteata	28.57 F
C. guttata	14.28 F
O. aculeatus	57.14 VF
Undetermined species (Scarabaeidae)	71.42 VF
Undetermined species (Curculionidae)	14.28 F
C. formicarius	14.28 F
C. sanguinea	14.28 F
B. decora	57.14 VF
Brachiacantha sp.	28.57 F

Continued

Species	Relative frequency (%)
A. insularis	14.28 F
Pheidole sp.	28.57 F
Polistes sp.	28.57 F
C. fasciatus	14.28 F
G. assimilis	14.28 F
Doru sp.	85.71 VF
A. marmoratus	28.57 F
A. mellifera	42.85 VF
Euxesta sp.	28.57 F

Abbreviations: VF – Very frequent; F – frequent; R – rare. * Very frequent if Rf >30, frequent if 10 ≥ Rf ≤ 30, and rare if Rf < 10.

The species *S. frugiperda, Z. longipes, Zelus* sp., *N. viridula, P. maidis, O. aculeatus*, and the undetermined beetle species *B. decora*, *Doru* sp., and *A. mellifera* were very frequent. The remaining species were frequent in the crop.

Discussion

According to Blanco Valdes (2016), research focused on establishing coexistent relationships between associated insects and crops is a relatively underexplored topic despite its significant importance for safeguarding economic crops. These authors mention that the diversity of colors and scents attracts entomofauna, benefiting economic crops.

The insect orders with the most detected species associated with maize crops were Coleoptera, Hemiptera, and Hymenoptera. These results align with those obtained by Mirabal *et al.* (2018). When assessing the insects associated with different maize agroecosystems across various San José de las Lajas farms, those authors found that these orders exhibited the highest number of species. The authors pointed out that the orders Coleoptera, Hemiptera, and Hymenoptera showcased the greatest diversity of families and species in maize crops. Merino (2016) reports the orders Coleoptera, Hemiptera, Diptera, Orthoptera, Hymenoptera, and Lepidoptera as the most representative in maize diagnostics, while Blanco and Leyva (2009) identify 7 orders, including Coleoptera, Dermaptera, Diptera, Hymenoptera, Lepidoptera, Orthoptera, and Hemiptera.

Spodoptera frugiperda larvae were found in all the samples taken. Simón and Golik (2018) suggest that the larvae are active day and night, feeding on tender tissues, leaves, and shoots. By the IV instar, they measure 11 to 15 mm and enter the plant's tassel, making their control difficult due to their lack of exposure. So, application or control measures should be conducted up to the third instar of the larvae (Fernández, 2002). Merino (2016) states that damage is inflicted during the initial days of crop development by devouring the plant close to the ground, defoliating it wholly or partially, and sometimes even leading to its death. In the later stages, these insects feed on the tassel and ear's rolled tender leaves, occasionally consuming the grains.

Varón *et al.* (2022) mention that *P. maidis* females primarily lay their eggs on the veins of the flag leaves. Many individuals can also be found in the tassel and areas where water accumulates. The highest incidence of this delphacid occurred from October to March, coinciding with data obtained by Padrón Padrón *et al.* (2008). The fact that *P. maidis* individuals were found isolated rather than in colonies on the leaves could be attributed to the planting season and various natural enemies, notably ladybirds and predatory bugs. This aspect agrees with results obtained by Mirabal *et al.* (2018), who report these natural enemies as regulators of *P. maidis* populations and other phytophagous insects in the crop.

Other hemipterans found were *N. viridula* and *A. andresii* (Hemiptera: Heteroptera). Simón and Golik (2018) point out that the damage caused by stink bugs and squash bugs is inflicted by the adult insects and nymphs from the fourth to fifth instar. In late plantings, more significant damage is typically observed.

In the order Coleoptera, the species *T. nigritus, C. guttata*, and *C. formicarius*, belonging to Chrysomelidae and Brentidae, were detected. These species are phytophagous pests of sweet potato crops, and their presence in the maize crop might be attributed to the preceding crop, which in this case was sweet potato.

Mirabal *et al.* (2018) suggest that the larvae of chrysomelid beetles *D. balteata* and *Cerotoma ruficornis* (Coleoptera: Chrysomelidae) feed on maize plant roots, affecting leaf development. The adults consume foliage and stamens, resulting in semi-empty cobs and reduced productivity.

Adult specimens of a longhorn beetle, morphologically matching the description of *O. aculeatus*, were also captured. This beetle is known to cause damage to plant species. In ordinary language, insects from this family are called borers, longhorn beetles, or sawyers, among other names. In studies conducted by Martins *et al.* (2011), specimens of *O. aculeatus* were found in maize crops at the Federal University of Viçosa, Minas Gerais state, Brazil, during the flowering period; these caused damage to the plant's reproductive parts.

Atta insularis (Hymenoptera: Formicidae) (leaf-cutting ants) is another species found in the crop. These are the primary herbivores in the Neotropics and are responsible for significant defoliation (Molina-Ochoa *et al*., 2004).

Conocephalus fasciatus and *G. assimilis* of the order Orthoptera are insects that impacted this experimental maize crop. However, their population values were low, possibly attributed to the timing of the research (October-January), a period of low rainfall. In this context, Huerta *et al*. (2014) indicate that these species' nymph and adult stages cause severe damage, consuming almost half of their body weight in green forage in a single day. Generally, they invade crops from July to September.

Adult insects of the order Diptera observed in the crop corresponded to those described for the genus *Euxesta* (Diptera: Otitidae), formerly considered secondary pests that cause severe damage to maize (Martos, 1983). The larvae of this dipteran species start by damaging the silks, leading to the emptying of the grains, especially the apical ones. However, the damage can extend to the entire ear. The activity of these larvae also serves as a gateway for saprophytic microorganisms, resulting in product loss and preventing its direct consumption (Rojas Borrel *et al*., 2017).

In studies conducted by Camacho-Báez *et al*. (2012), these flies are sometimes associated with grain rot where some type of pathogen is present; Rojas Borrel *et al*. (2017) found that the damage caused by the fall armyworm *S. frugiperda* can attract these flies.

Camacho-Báez *et al*. (2012), in their study of natural enemies of maize silk flies in Mexico, noted that the pirate bug *O. insidiosus* preys on larvae of these dipterans and is considered promising for use as a biological control agent. It has also been observed that the pirate bug feeds on lepidopteran eggs. Considering this element is of utmost importance given the very scarce number of these flies captured, with only three specimens found. Thus, *O. insidiosus* could have contributed to the natural control of their populations. In this regard, Blanco and Leyva (2009) stated that *O. insidiosus* feeds on silk fly eggs during the autumn-winter agricultural cycle.

Tchao *et al*. (2022) report in a study conducted in maize that the main predator insects belong to four orders and one suborder within Hemiptera, which are Hymenoptera, Hemiptera, Coleoptera, Dermaptera, and the suborder Heteroptera. These results agree with the findings of the present research.

Among the species identified in the studied agroecosystem, assassin bugs, *Zelus* sp. and *Z. longipes* of the Reduviidae family, were found. These are generalist predators that contribute to the natural control of phytophagous insects. These two species stand out as being highly prevalent on cultivated plants. Some prey items for these predators include larvae of lepidopterans, phytophagous mites, and aphids (Ordáz-Silva *et al*., 2014). Cuesta (2011), in a study conducted in Cienfuegos Province, Cuba, report that *Z. longipes* and *Coleomegilla cubensis* Casey play a significant role within maize crops as predators of *S. frugiperda*.

The species from the Nabidae family (unidentified) and the bug *Nesidiocoris tenuis* from the Miridae family detected in the experiment are considered predators by various authors. Species from the Nabidae family prey on and consume aphids, thrips, mites, whiteflies, and small lepidopteran caterpillars in their early stages, among other insects (Romero Sueldo *et al*., 2014).

In the studied agroecosystem, three species from the family Coccinellidae were detected within the order Coleoptera: *C. sanguinea limbifer*, *B. decora*, and *Brachiacantha* sp. This finding is consistent with the results obtained by Mirabal *et al*. (2018). In a national survey conducted in Cuba, Milán Vargas *et al*. (2008) report the presence of 14 genera and 22 species of ladybugs (coccinellids), which are considered widespread biocontrol agents for eggs and early larval instars of insects, especially Hemiptera. Moreover, Gordon (1985) highlights Coccinellidae as the second most represented family of entomophagous insects associated with the corn earworm with 11 species. Carabidae and Reduviidae were the leading families, with 16 species each, collectively accounting for 76.1% of the total biocontrol agents.

Another group of insects in the study were earwigs in the order Dermaptera. These insects are primarily predators of lepidopteran larvae (Jaraleño *et al*., 2020). In maize fields, both nymphs and adult earwigs feed on eggs and larvae of *S. frugiperda*, making them one of the most effective natural controls for *S. frugiperda*. Earwigs can also prey on other insects associated with maize crops, such as *Diatraea saccharalis* Fabricius and *Rhopalosiphum maidis* Fitch, which can serve as alternative prey if the corn earworm population decreases due to the action of these predators or pesticide application (Romero Sueldo *et al*., 2014).

During our research, two species from the order Hymenoptera were detected, one from the family Formicidae, *Pheidole* sp., and another from the family Vespidae, *Polistes* sp. These Hymenoptera species are considered another group of predators in agricultural systems, and their role is crucial in regulating harmful organisms (Molina-Ochoa *et al*., 2004). An adult of *A. marmoratus*, a tachinid fly (Diptera: Tachinidae) associated with the fall armyworm, was also found. In this regard, Rojas Borrel *et al*. (2017) researched the natural enemies of *S. frugiperda* in maize crops in Ciego de Ávila. They found 9 species of parasitoids from the orders Hymenoptera and Diptera, among which they also included *A. marmoratus*. Molina-Ochoa *et al*. (2004) emphasize *A. marmoratus* as a significant parasitoid for the corn earworm.

Another species found in our research was the honeybee (*A. mellifera*). These hymenopterans are the most important pollinators, especially the domestic honeybee (*A. mellifera*) (Mendoza Betancourt *et al*., 2021). Genaro and Loriga (2018) state that *M. beecheii* and *A. mellifera* are the only two species of social bees living on the island, where they are raised and managed by humans for their products and services in agricultural crop pollination.

Various Cuban and international researchers and authors have reported all the species recorded who have studied pests and beneficial organisms related to maize and bean crops (Mendoza Betancourt *et al*., 2021).

All the species detected in the maize crop were very frequent or frequent, which agrees with the results obtained by Mirabal *et al*. (2018). This element in our population dynamics study plays a significant role in managing harmful and beneficial organisms, as it provides information about their incidence in different crop phenological stages. This information is essential for making decisions regarding their management.

In the case of *S. frugiperda*, the main maize pest, the insect was found to be very frequent, similar to the beneficial species that naturally control it, such *as Doru* sp. This predator is recognized as a highly efficient predator of the corn earworm. These findings correspond to Romero Sueldo *et al*. (2023), who highlight this dermapteran as frequent in untreated maize fields.

Two species from the *Zelus* genus were widespread, consistent with Álvarez Hernández *et al*. (2004). In their study on natural enemies of the lepidopteran *Heliothis virescens* F. in tobacco cultivation across four locations in Villa Clara, they report *Z. longipes* as very frequent.

Conclusion

Several phytophagous insects were detected in the agroecosystem of maize cultivar ‘MAIG-Diamante’. Experimental crops and beneficial species contributed to the natural regulation of their populations in agricultural systems. Hence, their conservation and proper management are essential for establishing an agroecological management program for *Spodoptera frugiperda* in maize cultivar ‘MAIG-Diamante’.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author’s contributions

YBV, NC, and JVGP designed the experiments. TVM carried out the field research, YBV, JVGP, NC, and ALP conceptualization, research, original draft, visualization, writing, and editing. FAR and OECR contributed to the data analysis. All authors have read and approved the final version of the manuscript.

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Soil quality index using the CASH methodology and Rainforest Alliance Scorecard in the coffee zone of the Caldas department, Colombia

Índice de calidad del suelo utilizando la metodología CASH y Rainforest Alliance Scorecard en la zona cafetera del departamento de Caldas, Colombia

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ABSTRACT

The Comprehensive Assessment of Soil Health (CASH) methodology provides a broad approach to evaluate soil health, helping farmers and researchers to identify management practices that can enhance soil health and increase agricultural sustainability in coffee crops. When integrated with regenerative agriculture strategies such as cover cropping, intercropping, agroforestry, and crop rotation, these practices can effectively enhance soil health and productivity. The aim of this research was to develop a soil health index using the CASH approach adjusted to the conditions of the coffee zone in the Caldas department (Colombia) and to adapt the index of the Rainforest Alliance Scorecard guidelines. A database containing 3,332 soil analyses from different coffee zones in the department was used, carried out between 2005 and 2021. The index obtained with historical data shows that 66% of the properties were classified as Bronze, 18% Silver, and 16% Gold, whereas the validation data showed that 49% of the properties were classified as Bronze, 21% Silver, and 30% Gold. The most important variables identified in soil health were organic matter content, organic carbon stock, CO_2 equivalent, pH, bulk density, and nutrient content related to N, P, K, Ca, and Mg. CASH can be employed to evaluate the soil health status in the field using quantitative indicators linked to the Rainforest Scorecard and is a useful tool to promote regenerative practices in soil adjusted to Caldas coffee zones.

Key words: regenerative agriculture, base line, soil indicator.

RESUMEN

La metodología de la Evaluación Integral de la Salud del Suelo (CASH, por sus siglas en inglés) proporciona un enfoque amplio para evaluar la salud del suelo. Puede ayudar a los agricultores e investigadores a identificar prácticas de manejo que puedan mejorar la salud del suelo y aumentar la sostenibilidad agrícola en los cultivos de café. Cuando se integran con estrategias de agricultura regenerativa como los cultivos de cobertura, cultivos intercalados, la agrosilvicultura y la rotación de cultivos, estas prácticas pueden mejorar eficazmente la salud y la productividad del suelo. El objetivo de esta investigación fue desarrollar un índice de salud del suelo utilizando el enfoque CASH ajustado a las condiciones de la zona cafetera en el departamento de Caldas (Colombia) y adaptar el índice de las directrices del Rainforest Alliance Scorecard. Se utilizó una base de datos que contenía 3332 análisis de suelos de diferentes zonas cafeteras del departamento, realizados entre los años 2005 y 2021. El índice obtenido con datos históricos muestra que el 66% de las propiedades fueron clasificadas como Bronce, 18% Plata y 16% Oro, mientras que los datos de validación mostraron que el 49% de las propiedades fueron clasificadas como Bronce, 21% Plata y 30% Oro. Las variables más importantes identificadas en la salud del suelo fueron el contenido de materia orgánica, el stock de carbono orgánico, el CO_2 equivalente, el pH, la densidad aparente y el contenido de nutrientes relacionados con N, P, K, Ca y Mg. CASH se puede emplear con éxito para evaluar el estado de salud del suelo utilizando indicadores cuantitativos vinculados al Rainforest Scorecard; y se identifica como una herramienta útil para promover prácticas regenerativas en el suelo ajustadas a las zonas cafeteras de Caldas.

Palabras clave: agricultura regenerativa, línea base, indicador del suelo.

Introduction

In coffee-producing regions of Colombia, the Green Revolution (GR) brought significant changes to coffee production, including the introduction of high-yielding coffee varieties, synthetic fertilizers, and pesticides (Osorio-García *et al*., 2020). While these changes have brought benefits, such as increased coffee yields and incomes for farmers, they have

Received for publication: June 2, 2024. Accepted for publication: August 22, 2024.

Doi: 10.15446/agron.colomb.v42n2.114840

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also had significant impacts on soil health and environmental sustainability (Breitler *et al*., 2022).

The impact of the GR on soil health has been documented, highlighting the changes in soil properties and nutrient cycling associated with the implementation of technologies in coffee production. Manson *et al*. (2022) found that soil pH decreased significantly in coffee farms that used synthetic fertilizers, while organic matter and nutrient availability also decreased over time.

There is growing recognition of the need for more sustainable approaches to coffee production that prioritize soil health and environmental sustainability. Regenerative agriculture (RegAg) (Al-Kaisi & Lal, 2020), for instance, offers a promising approach to enhance soil health, productivity, and environmental sustainability in coffee.

RegAg strategies, such as cover cropping, intercropping, agroforestry, and crop rotation, aim to restore soil organic matter, reduce soil erosion, and enhance nutrient cycling while improving soil structure and promoting beneficial microorganisms (O'Donoghue *et al*., 2022).

RegAg can effectively enhance soil health and environmental sustainability in coffee-producing regions of Colombia. Rekik *et al*. (2020) found that cover cropping and intercropping can effectively reduce soil erosion and enhance soil health in coffee-producing regions in Colombia.

Santos *et al*. (2021) evaluated the soil health of coffee crops in Brazil. Their study compared conventional and organic management practices and found that the organic coffee farm had significantly higher soil health indicators such as microbial biomass, total carbon, and permanganate oxidizable carbon. The study suggests that organic management practices can enhance soil health in coffee crops.

The CASH methodology of a Comprehensive Approach to evaluate Soil Health provides a holistic approach by integrating physical, chemical, and biological indicators, offering a comprehensive view of soil functionality to help farmers and researchers identify management practices that can enhance soil health and increase agricultural sustainability in different crops (Fine *et al*., 2017; Moebius-Clune *et al*., 2017).

Rekik *et al*. (2018) conducted a study in Cauca, Colombia, to develop specific scoring functions to serve as benchmarks for soil health (SH) in coffee production. A total of 3,332 soil samples were collected from coffee farms in six municipalities, analyzing 13 SH indicators including wet aggregate stability, available water capacity, respiration rate, pH, and contents of active carbon, organic matter, protein, and various nutrients (P, K, Mg, Mn, Fe, and Zn). A scoring function was developed for each indicator using the cumulative normal distribution. The results showed that active carbon was the best predictor of soil health.

The Caldas region coffee production is primarily done by small-scale farmers, most of whom own less than 5 ha of area. According to FNC (2021), smallholder coffee farms in the Caldas region have 32,144 farmers and 40,227 farms in 59,282 ha. The intensive use of soils in the department of Caldas due to coffee cultivation has significantly impacted soil health over time. Continuous monocropping and the use of agrochemicals in coffee production have led to soil degradation, including the loss of organic matter, reduced biodiversity, soil compaction, and nutrient imbalances. These practices disrupt the natural processes that maintain healthy, productive soils, ultimately reducing soil resilience, fertility, and long-term sustainability.

Developing soil health indicators using the Comprehensive Assessment of Soil Health (CASH) methodology is essential for evaluating the current state of these soils. Utilizing historical soil analysis data from Caldas allows for the creation of a local Soil Health Index, which is critical for understanding long-term trends in soil degradation and identifying areas for improvement.

This study aimed to provide a tool for interpreting soil analyses, which corresponds to the CASH indices, adjusted to local conditions, to help make decisions that contribute to keeping the soils of the Caldas coffee region in good condition and to adapt the index of the Rainforest Alliance Scorecard guidelines.

Materials and methods

Study area

The study covered seven distinct zones within Caldas, which are critical for coffee production. Each zone presented a variety of soil types, primarily belonging to the Andisol and Inceptisol soil orders, known for their volcanic origin, high organic matter content, and good structure. These soils are conducive to coffee cultivation but present differences in nutrient retention, pH buffering, and susceptibility to erosion. The specific characteristics of each zone are described in Table 1.

TABLE 1. Representative zones of coffee production in the Caldas department.

Zone	Municipality	Number of samples	Modal unit of soils (Gómez *et al.*, 1991)
Aguadas	Aguadas	500	Typic Melanudands
Alto	Marmato, Riosucio, Supía	1,115	Andic Eutrudepts
Anserma	Anserma, Risaralda	157	Typic Melanudands, Typic Hapludands
Bajo Occidente	Belalcazar, San Jose, Viterbo	55	Typic Hapludands, Typic Eutropepts
Centro	Chinchiná, Manizales, Neira, Palestina, Villamaria	976	Typic Melanudands
Norte	Aranzazu, Filadelfia, La Merced, Pacora, Salamina	221	Typic Eutropepts, Typic Melanudands
Oriente	Florencia, Manzanares, Marquetalia, Marulanda, Pensilvania, Samaná	308	Typic Melanudands, Typic Troporthents

Elaboration based on internal data from Fundación Local Partners and Ecotopos cafeteros (Gómez *et al.,* 1991).

Soil inventory and analysis

A database was consolidated with 3,332 soil analyses obtained from seven different zones representing 25 municipalities in the department, located between 1,000 to 2,100 m a.s.l. and specifically conducted for coffee crops collected between 2005 and 2021. The samples were collected from 0 to 15 cm depth and composited.

The following soil variables were measured according to Carrillo (1985): biological: organic matter by Walkley and Black (%), % organic carbon (calculated), and equivalent CO_2 stock (t ha^{-1} $year^{-1}$) (calculated) using the IPCC parameter (Publications - IPCC-TFI, 2023); chemical: pH (potentiometric), nitrogen (calculated) (Sadeghian, 2010), phosphorus (mg kg^{-1}) by Bray II, potassium, calcium, magnesium (Ammonium acetate 1 N / Atomic Absorption Spectrophotometer (AAS)) and aluminum (cmol(+) kg^{-1}) by KCl 1 N / Volumetric); boron and sulfur (Monobasic calcium phosphate / Turbidimetric), and minor elements Fe, Mn, Zn, Cu (mg kg^{-1}) by EDTA extraction and ammonium acetate / AAS; the effective cation exchange capacity (ECEC) is calculated as the sum of the milliequivalents of the exchangeable bases (Ca, Mg, K, Na) plus exchangeable acidity (often measured separately as Al^{3+} and H^+). ECEC is expressed in cmol(+) kg^{-1} soil and cation saturation of Ca, Mg, K, and Al (%) (calculated)); physical: sand, clay, silt (%) by Bouyoucos-Hydrometer / Gravimetric, bulk density (kg m^{-3}) using a cylinder of known volume, and water aggregate stability (WAS %) by Wet sieving method.

Statistical analysis

Descriptive statistical analysis for each variable, including minimum, maximum, mean, variance (n-1), standard deviation (n-1), and variation coefficient were used. Analysis of variance was conducted for seven regional subsets for all soil parameters. Means separation was computed using the LSD test. Pearson correlation coefficients for physical, biological, and chemical indicators were calculated.

CASH methodology

This article follows the analytical pathway developed by Fine *et al.* (2017) and described in the Manual Comprehensive Assessment of Soil Health: The Cornell Framework (Moebius-Clune *et al.*, 2017). Scoring functions were used to interpret measured physical, chemical, and biological indicator values for CASH. Means and standard deviations for each indicator were calculated and used to determine the scoring function as a cumulative normal distribution (CND) using the following function:

$$\mathrm{Fx(x)} = \int_{-\infty}^{x} \mathrm{N}(z; \mu, \sigma^2)\mathrm{d}z \quad (1)$$

where $N(z; \mu, \sigma^2)$ is the normal (Gaussian) probability density function evaluated at z. It is defined by: μ: The mean of the distribution, representing the central value around which the data is distributed; σ^2: The variance of the distribution, representing the spread or dispersion of the data. The square root of the variance, σ, is the standard deviation.

CND represents the probability of a normally distributed random variable being less than or equal to x, e is Euler's number, and t is the standard normal random variable. The CND provides a score on a scale from 0 to 100 (Fine *et al.*, 2017).

CASH - Alignment with sustainability standards of Rainforest Scorecard

The indices were constructed for Bronze, Silver, and Gold levels of the Rainforest Alliance scorecard (2022) using CND values to set thresholds for rating soil health indicators: 1) scores between 0 and 20 are considered very low; 2) scores between 20 and 40 are considered low; 3) scores between 40 and 60 are considered medium (below 60 are all Bronze level); 4) scores between 60 and 80 are considered high (Silver level); 5) scores between 80 and 100 are considered very high (Gold level).

Multivariate statistics (Principal Component Analysis - PCA) were evaluated using the database, including only samples that had measured values for all indicators (n = 640). The first two PCs were visualized in two-dimensional space. The PCA was used to obtain a minimum data set (MDS) described by Mukherjee and Lal (2014). The ranges defined by the index from 0 to 100 were as follows: Bronze: $0 \leq I < 60$, Silver: $60 \leq I < 80$, and Gold: $80 \leq I \leq 100$.

Validation of index

The final index model to measure the soil health index-based CASH – Soil Quality Index (SQI) was applied in farms using the following equation:

$$n = \frac{N * Z_\alpha^2 * p * q}{d^2 * (N-1) + Z_\alpha^2 * p * q} \quad (2)$$

where N = total population (9200 farms), Z_α= 1.96 (95%), p = expected proportion (5% = 0.05), q = 1 – p (1-0.05 = 0.95), d = precision (5% = 0.05).

The chi-square test was used to test for a significant association between CASH and the VALIDATION dataset (frequency table), with the following null hypothesis H_0: There is no association between soil properties and CASH and VALIDATION. All statistics were computed using XLSTAT 2014.5.03 (Addinsoft, New York, USA).

Results

Datasets represent the maximal variability of soil in Caldas department

The diverse array of samples, encompassing varied locations and highlighting the influence of soil-forming factors such as parent material, topography, climate, biological activity, and time, altitudes, time of sampling, sample handling, land use, and land management practices, resulted in a broad spectrum of values (Tab. 2). Each variable presented a considerable standard deviation in comparison to the means, including soil pH, which was influenced by the farmer's practices. The soil analysis revealed significant variability in key physical and chemical properties of soil across the study region, impacting soil health and fertility. The high variability in soil properties, such as aluminum saturation, nutrient availability, and organic matter content, indicates the need for localized soil management practices.

The analysis of variance revealed highly significant differences among the coffee subregions for all soil parameters. The LSD test demonstrated differences among zones for each indicator (Tab. 3), thus highlighting the pronounced soil variability in the coffee-growing regions and the imperative to develop locally focused soil quality indicators.

For aggregate stability, the value of 4.25 suggests that the soil has a moderate level of structural stability. Stable aggregates are vital for resisting erosion and maintaining soil porosity (Lince-Salazar *et al.,* 2020). This value indicates that the region is performing better than zones such as the "Centro" and "Norte" but lags behind "Oriente", which has a superior score of 6.11.

For soil organic matter (OM) content and organic carbon (%), with an organic matter content of 8.80% and organic carbon at 5.10%, "Bajo occidente" shows relatively high fertility. High organic matter supports microbial activity and nutrient cycling, essential for coffee production (González-Osorio *et al.,* 2008). The organic carbon levels also reflect carbon sequestration, an increasingly important metric for soil health (Lal, 2020). This performance is comparable to other regions but still slightly behind "Oriente" (13.11% OM and 7.59% organic carbon).

For soil nutrient content (N, P, K, Ca, Mg), the region demonstrates moderate nitrogen (N) content at 0.73%, phosphorus (P) at 5.89 mg kg^{-1}, potassium (K) at 0.31 cmol kg^{-1}, and calcium (Ca) at 5.07 cmol kg^{-1}. These levels are essential for coffee plants, supporting vegetative growth and coffee bean formation. While the nitrogen levels are at the lower end, phosphorus content is relatively high compared to regions like "Norte" (23.26 mg kg^{-1}). Calcium and magnesium contents in soil were well-balanced, promoting soil pH stability and reducing aluminum toxicity (Sadeghian, 2008).

For effective cation exchange capacity (ECEC), the cation exchange capacity in "Bajo occidente" is 12.68 cmol kg^{-1}, which is among the highest in the region, indicating a strong ability to retain and supply essential nutrients like calcium, magnesium, and potassium. This is a critical factor for long-term soil fertility, especially in tropical regions.

For cation saturation levels (Ca, Mg, K, Al), the region demonstrates a calcium saturation of 47.95%, magnesium saturation of 16.62%, and potassium saturation of 4.02%, suggesting a well-balanced nutrient profile (Sadeghian, 2008). The aluminum saturation, which can be detrimental to root growth, is relatively low at 9.55%, indicating reduced aluminum toxicity, which is crucial for crop performance. This value is significantly better than in zones like "Centro" (13.44% aluminum saturation).

TABLE 2. Descriptive statistics of CASH indicators for all soil samples (total n= 3332) in Caldas coffee zone.

Soil property	Units	Min	Max	Mean	Standard deviation (n-1)	Variation coefficient (%)
Bulk density	(kg m^3)	0.73	1.77	1.01	0.20	20
WAS		0.44	12.30	4.27	1.78	42
Sand		2.00	85.90	37.82	16.22	43
Clay	%	1.50	95.00	35.89	17.55	49
Silt		2.40	67.00	26.91	9.34	35
Organic matter (OM)		0.00	27.40	8.84	4.12	47
Organic carbon (OC)		0.00	15.87	5.12	2.39	47
Organic carbon stock	(t ha^{-1})	0.00	2.39	0.90	0.26	29
eq CO_2	(t ha^{-1} $year^{-1}$)	0.00	8.99	3.38	0.99	29
pH		3.50	7.70	4.97	0.43	09
Al	cmolc kg^{-1}	0.00	13.07	1.48	1.36	92
N	mg kg^{-1}	0.00	1.35	0.38	0.17	45
P	mg kg^{-1}	0.00	303.00	19.79	34.26	173
K		0.00	8.11	0.37	0.38	103
Ca	cmolc kg^{-1}	0.00	28.28	4.20	3.73	89
Mg		0.03	12.76	1.44	1.50	104
Na		0.00	0.58	0.04	0.05	144
Fe		2.34	3171.00	252.00	188.77	75
Mn		0.00	300.00	38.20	31.98	84
Zn	mg kg^{-1}	0.00	82.90	5.44	6.37	117
Cu		0.02	118.60	5.48	5.73	105
S		0.00	212.60	25.26	20.20	80
B		0.00	2.99	0.32	0.20	62
ECEC	cmolc kg^{-1}	0.16	35.84	7.51	5.09	67.7
Ca		0.00	95.25	49.96	19.47	39
Mg		0.00	78.81	16.48	8.72	53
K	% saturation	0.00	100.00	6.52	6.01	92
Al		0.00	94.44	26.49	22.47	84
Na		0.00	23.80	0.52	0.95	180

WAS – water aggregate stability.

The high variability in soil properties across the coffee-growing regions can be attributed to the diverse range of soil types, influenced by volcanic parent material, steep topography, and differential agronomic management practices. Soil acidity and aluminum toxicity are significant constraints in many areas, requiring site-specific interventions such as liming and organic matter amendments. Variability in nutrient availability highlights the need for tailored fertilization strategies to optimize coffee production.

TABLE 3. ANOVA results represented by the p level for subregion group effect. Different letters indicate statistically significant differences at $P = 0.05$ for LSD comparisons between the zones.

Soil property		F-Value (Pr >F***)	AG	B.Oc	AL	N	AN	C	Or
Bulk density		41.52	1.01 ^{B}	1.00 ^{B}	1.02 ^{B}	1.09 ^{C}	1.03 ^{B}	1.02 ^{B}	0.84 ^{A}
WAS		64.90	4.37 ^{C}	4.25 ^{BC}	4.20 ^{BC}	3.83 ^{A}	4.01 ^{AB}	3.86 ^{A}	6.11 ^{D}
Sand		242.86	41.37 ^{E}	39.43 ^{ED}	31.28 ^{B}	38.86 ^{D}	41.62 ^{E}	37.17 ^{C}	56.57 ^{F}
Clay		420.76	32.33 ^{D}	24.25 ^{B}	45.93 ^{F}	30.15 ^{C}	27.12 ^{B}	34.73 ^{E}	16.61 ^{A}
Silt		97.33	26.70 ^{C}	36.32 ^{F}	23.89 ^{B}	31.18 ^{E}	31.39 ^{E}	28.66 ^{D}	26.95 ^{C}
OM		64.90	9.08 ^{C}	8.80 ^{BC}	8.68 ^{BC}	7.84 ^{A}	8.24 ^{AB}	7.91 ^{A}	13.11 ^{D}
% OC		64.90	5.26 ^{C}	5.10 ^{BC}	5.03 ^{BC}	4.54 ^{A}	4.77 ^{AB}	4.58 ^{A}	7.59 ^{D}
OC stock		57.38	0.91 ^{C}	0.89 ^{BC}	0.88 ^{BC}	0.82 ^{A}	0.86 ^{AB}	0.85 ^{A}	1.16 ^{D}
eq CO_2		57.38	3.43 ^{C}	3.36 ^{BC}	3.32 ^{BC}	3.10 ^{A}	3.22 ^{AB}	3.20 ^{A}	4.36 ^{D}
pH		30.52	5.07 ^{D}	5.00 ^{CD}	5.05 ^{CD}	5.08 ^{D}	5.04 ^{CD}	4.86 ^{B}	4.80 ^{A}
Al		192.21	0.88 ^{AB}	0.73 ^{AB}	2.48 ^{D}	0.71 ^{A}	0.85 ^{AB}	1.08 ^{C}	1.01 ^{BC}
N		131.24	0.39 ^{BC}	0.41 ^{C}	0.36 ^{B}	0.35 ^{AB}	0.38 ^{BC}	0.33 ^{A}	0.63 ^{D}
P		69.71	21.63 ^{CD}	5.89 ^{A}	7.35 ^{A}	23.26 ^{D}	16.24 ^{BC}	36.75 ^{E}	12.05 ^{AB}
K		9.51	0.41 ^{BC}	0.31 ^{AB}	0.34 ^{B}	0.40 ^{BC}	0.43 ^{C}	0.41 ^{BC}	0.24 ^{A}
Ca		87.51	5.03 ^{C}	5.07 ^{CD}	5.45 ^{D}	5.72 ^{D}	4.77 ^{C}	2.69 ^{B}	1.48 ^{A}
Mg		91.64	1.98 ^{D}	2.01 ^{D}	1.92 ^{D}	1.85 ^{D}	1.56 ^{D}	0.79 ^{B}	0.39 ^{A}
ECEC		118.14	8.96 ^{C}	12.68 ^{E}	10.60 ^{D}	11.48 ^{DE}	11.99 ^{E}	5.15 ^{B}	3.18 ^{A}
Saturation	Ca	60.09	54.97 ^{C}	47.95 ^{B}	47.50 ^{B}	54.86 ^{C}	49.96 ^{B}	58.65 ^{D}	36.07 ^{A}
	Mg	37.50	20.71 ^{D}	16.62 ^{BC}	16.86 ^{BC}	17.13 ^{BC}	15.48 ^{B}	17.53 ^{C}	10.73 ^{A}
	K	189.11	5.56 ^{B}	4.02 ^{AB}	3.68 ^{A}	4.89 ^{B}	5.89 ^{B}	13.44 ^{D}	8.87 ^{C}
	Al	76.71	15.68 ^{A}	9.55 ^{A}	29.58 ^{B}	10.53 ^{A}	11.79 ^{A}	50.26 ^{D}	41.84 ^{C}
	Na	204.43	0.08 ^{A}	0.48 ^{C}	1.06 ^{D}	0.27 ^{B}	0.25 ^{B}	0.07 ^{A}	0.46 ^{C}

AG = Aguadas; B.Oc = Bajo occidente; AL = Alto; N = Norte; AN = Anserma; C = Centro; Or = Oriente. WAS – water aggregate stability; OM – organic matter; OC – organic carbon; ECEC - effective cation exchange capacity. Pr>F *** indicate significant differences (ANOVA). Averages with the same letter indicate non-significant differences (LSD test).

Correlation analysis between soil health indicators

A correlation matrix (Tab. 4) was created by computing Pearson correlation coefficients for each pair of soil health indicators, including percent sand, silt, and clay. Strong positive correlations were particularly observed among biological indicators, such as percentage of soil organic matter (OM) with % organic carbon (0.99), OM with carbon stock (0.99), and OM with eq CO_2 (0.99), as well as negative correlations between bulk density and OM (-0.91) and carbon stock (-0.92).

Negative correlations ($r \geq 0.2$-0.4) were found between silt and Ca, Mg, ECEC, Fe, Mn, Sand, and Clay with OM, % Organic carbon, and eq CO_2, while Mg showed a negative correlation with OM, % Organic carbon, and eq CO_2.

The correlations between biological, chemical, and physical soil properties provide valuable insights into the key factors that influence soil health. Nitrogen content and organic matter emerge as critical biological indicators, while pH, ECEC, and bulk density serve as important chemical and physical indicators, respectively.

Scoring functions for soil quality index

The best indicators of soil health are described below. The indicators include WAS, OM, organic carbon, eq CO_2, pH, N, P, K, Ca, Mg, ECEC, and saturation of Ca, Mg, and K. These indicators are scored using a "more is better" function, where increasing measured values result in higher scores.

The overall soil health index is calculated as the unweighted mean of individual indicator scores, with nutrients combined into a single metric. The scoring functions for the Caldas department include regional soil health statistics, such as the CND function, mean, and standard deviation, as shown in Table 5.

TABLE 4. Pearson correlation coefficients for CASH indicators for all textural groups (total n = 3,332). Values in bold have absolute correlation coefficients greater than 0.4; ns indicates not statistically significant at $P = 0.05$.

Variable	N	OM	OC %	Bulk density	OC stock	eq CO_2	K	Ca	Mg	Al	P	Sand	Clay	Silt	ECEC
pH	- 0.14	- 0.11	- 0.11	0.12	- 0.12	- 0.12	0.05	0.49	0.40	- 0.37	- 0.13	- 0.18	0.19	- 0.04	0.35
N	1.00	0.95	0.95	- 0.86	0.94	0.94	- 0.05	- 0.30	- 0.33	- 0.05	- 0.09	0.42	- 0.41	0.02	- 0.23
Organic matter		1.00	1.00	- 0.91	0.99	0.99	- 0.04	- 0.31	- 0.32	- 0.02	- 0.09	0.20	- 0.20	0.02	- 0.20
Organic carbon (OC) %			1.00	- 0.91	0.99	0.99	- 0.04	- 0.31	- 0.32	- 0.02	- 0.09	0.20	- 0.20	0.02	- 0.20
Bulk density				1.00	- 0.92	- 0.92	0.03	0.34	0.36	0.03	0.06	- 0.12	0.14	- 0.05	0.23
OC stock					1.00	1.00	- 0.04	- 0.31	- 0.33	- 0.02	- 0.08	0.18	- 0.19	0.03	- 0.21
eq CO_2						1.00	- 0.04	- 0.31	- 0.33	- 0.02	- 0.08	0.18	- 0.19	0.03	- 0.21
K							1.00	0.23	0.13	- 0.04	0.22	0.03	- 0.08	0.08	0.21
Ca								1.00	0.72	- 0.10	0.04	- 0.15	0.18	- 0.06	0.72
Mg									1.00	- 0.04	- 0.07	- 0.21	0.26	- 0.11	0.62
Al										1.00	- 0.05	- 0.23	0.36	- 0.26	0.06
P											1.00	0.11	- 0.18	0.13	- 0.04
Sand												1.00	- 0.83	- 0.18	- 0.38
Clay													1.00	- 0.37	0.17
Silt														1.00	0.36

Values in bold have absolute correlation coefficients that are statistically significant at $P = 0.05$.

TABLE 5. Scoring functions for physical, biological, and chemical CASH indicators and relevant soil processes (n= 3,332 soil analyses). Scoring functions are based on the dataset's cumulative normal distribution (CND) indicated by CND (mean; StdDev).

Type	Soil health indicator (Type of scoring)	CASH scoring function
Physical	Bulk density (LB)	S = 100* 1-CND (1.01; 0.20)
	WAS (MB)	S = 100* CND (4.27; 1.78)
	% Sand (MB)	S = 100* CND (37.82;11.22)
	% Clay (LB)	S = 100*1- CND (35.89; 17.55)
	% Silt (MB)	S = 100* CND (26.91; 9.34)
Biological	% OM (MB)	S = 100* CND (8.84; 4.12)
	OC stock (MB)	S = 100* CND (0.90; 0.26)
	eq CO_2 (MB)	S = 100* CND (3.38; 0.99)
Chemical	pH (MB)	S = 100* CND (4.97; 0.43)
	Al (LB)	S = 100* 1-CND (1.48; 1.36)
	N (MB)	S = 100* CND (0.38; 0.17)
	P (MB)	S = 100* CND (19.79; 34.26)
	K (MB)	S = 100* CND (0.37; 0.38)
	Ca (MB)	S = 100* CND (4.20; 3.73)
	Mg (MB)	Se = 100* CND (1.44; 1.50)
	ECEC (MB)	S = 100* CND (8.25; 6.65)
	Saturation Ca (MB)	S = 100* CND (51.36; 20.14)
	Saturation Mg (MB)	S = 100* CND (17.02; 8.98)
	Saturation K (MB)	S = 100* CND (7.44; 7.68)
	Saturation Al (LB)	S = 100* 1-CND (32.24; 38.51)

Abbreviations: S – score. Type of scoring: MB – more is better; LB – less is better. WAS – water aggregate stability; OM – organic matter; OC – organic carbon; ECEC – effective cation exchange capacity.

Principal component analysis

The analysis revealed that six PCs accounted for 83.4% of the total variability in the raw dataset, with eigenvalues greater than one and a cumulative fraction of total variance of at least 70% (Fig. 1).

PC1 explained 37% of the variance, with twelve variables having high positive loadings (N > WAS > eq CO_2 > bulk density > Mn > Ca > Mg). PC2 had high positive loadings for Sand, Al, and Na, and high negative loadings for Ca saturation, representing 14% of the total variance. The minimum data set (MDS) identified by PCA includes twenty soil variables that are bulk density, WAS, Sand, Clay, Silt, OM, eq CO_2, pH, Al, N, P, K, Ca, Mg, ECEC, saturation of Ca, Mg, K, Al.

Table 6 displays various indicators of soil quality in coffee-growing zones in Caldas. These indicators are categorized into physical, biological, and chemical, each evaluated according to three quality levels: Bronze, Silver, and Gold. The percentages shown correspond to how often the soil properties fall into each of these categories, both for the general database (n = 3332) and for the validation set (n = 400).

Physical properties: For bulk density, in the general database, 53% of the samples fall into the Bronze category, with lower percentages for Silver (24%) and Gold (23%). In the validation set, the Gold category is more frequent (43%), followed by Bronze (38%) and Silver (18%).

WAS (Water aggregate stability): Most soils fall into the Bronze category (66%), with smaller proportions in Silver (17%) and Gold (17%). The validation set shows a similar trend, but with a higher percentage of soils in Gold (35%).

Sand, clay, and silt content: The texture of the soil varied significantly. For sand, the general database shows 79% in Bronze, while the validation set shows a higher proportion in Gold (54%). For clay, the Gold category is predominant in both datasets, with 56% for the general set and 67% for validation. Silt is predominantly found in the Bronze category in the general database (82%), but in the validation set, it is more evenly distributed between Bronze (34%) and Silver (45%).

Biological soil properties: For organic matter (OM), most soils fall into the Bronze category (66% for the general set and 45% for validation), with smaller proportions in Silver and Gold.

Organic carbon stock and eqCO_2: These indicators followed a similar pattern, with the general database having more Bronze-rated soils (~65%), while the validation set shows a strong presence of soils in the Gold category (85%).

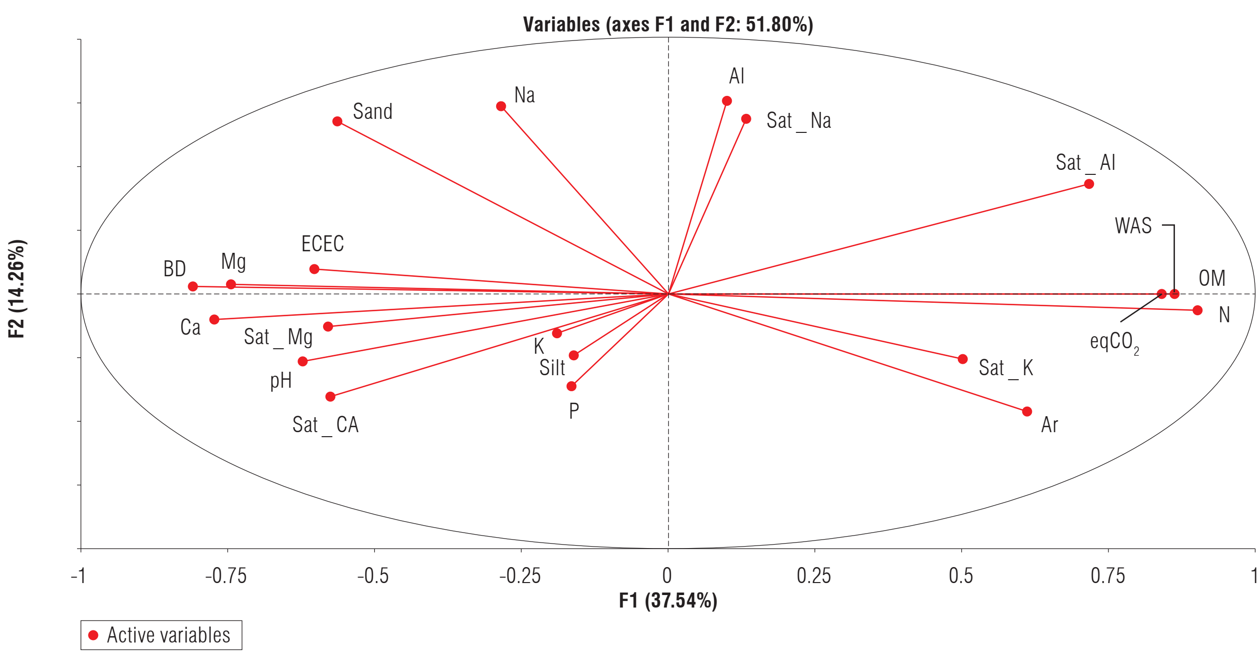

FIGURE 1. Loading plot from the principal components analysis (PCA) performed on CASH indicators presented in Table 5 (n = 640). WAS – water aggregate stability; OM – organic matter; BD – bulk density; ECEC – effective cation exchange capacity.

Chemical properties: For pH, in the general database, 59% of soils fall into Bronze category, with fewer in Silver (23%) and Gold (18%). The validation set shows a shift towards more soils in the Bronze category (62%) compared to the other two categories.

Nutrient content (N, P, K, Ca, Mg): The distribution varies across the Bronze, Silver, and Gold categories. For example, Nitrogen (N) is mainly present in Bronze in both datasets, while Phosphorus (P) is also largely concentrated in Bronze, particularly in the validation set (88%). Potassium (K) and Calcium (Ca) are similarly skewed toward Bronze.

Effective cation exchange capacity (ECEC): The general set shows a predominant Bronze category (69%), similar to that of the validation set (79%).

Saturation levels (Ca, Mg, K, Al): Most soils fall in the Bronze category for saturation of Ca, Mg, and K. Interestingly, for saturation of Al, the general set shows a unique split with a higher percentage of Silver (53%) compared to Bronze (47%), while the validation set has a higher proportion of Silver (61%).

For all indicators, the general database categorizes 66% of soils as Bronze, 18% as Silver, and 16% as Gold. In the validation set, there is a higher distribution of Bronze (49%) and Gold (30%), with fewer soils in Silver (21%).

Table 6 reveals that the majority of soils in the coffee-growing regions of Caldas fall into the Bronze category, especially for chemical properties like pH, ECEC, and nutrient content. The validation set slightly favors Gold in soil properties like bulk density and organic carbon stock, indicating that a subset of soils may be more suitable for high-quality coffee cultivation. However, soil physical properties like texture (*e.g.*, sand, clay, and silt) and biological properties like organic matter are more variable, suggesting that soil management practices could be targeted to improve specific properties in the different regions.

TABLE 6. CASH indicator values for general database (n = 3,332) and validation (n = 400) soil analysis in Caldas department of the Colombian coffee growing zone.

Type of soil properties	Soil property	CASH %			Validation %			Chi^2 (*P*-value)
		Bronze	Silver	Gold	Bronze	Silver	Gold	
Physical	Bulk density	53	24	23	38	18	43	9.38 ***
	WAS	66	17	17	45	20	35	10.44 ***
	Sand	79	8	13	23	23	54	63.09 ***
	Clay	33	11	56	15	18	67	9.42 ***
	Silt	82	10	8	34	45	21	47.96 ***
Biological	Organic matter	66	17	17	45	20	35	10.44 8 ***
	Organic carbon stock	65	20	15	8	6	85	101.04 ***
	eq CO_2	65	20	15	8	6	85	101.04 ***
Chemical	pH	59	23	18	62	22	15	0.36 ns
	Al	51	28	21	26	38	36	13.57 ***
	N	69	14	17	38	12	50	25.38 ***
	P	82	7	10	88	4	8	1.24 ns
	K	77	14	9	74	10	16	2.68 ns
	Ca	67	17	16	72	12	16	1.04 ns
	Mg	72	12	16	79	7	14	1.77 ns
	ECEC	69	15	16	79	11	10	2.67 ns
	Saturation Ca	53	26	21	50	24	26	0 .69 ns
	Saturation Mg	64	18	17	79	10	11	5 .14 ns
	Saturation K	74	11	15	72	18	10	2 .71 ns
	Saturation Al	47	53	0	39	61	0	1.30 ns
Grand total		66	18	16	49	21	30	7.00 **

*** $P < 0.01$; ** $P = 0.01 – 0.05$; ns indicates not statistically significant at $P = 0.05$. WAS – water aggregate stability. ECEC – effective cation exchange capacity.

For the chemical variables, the Chi^2 results show that only two variables, Al and N contents, are significantly associated with the validation and CASH results, with Chi^2 values of 13.58 and 25.39, respectively (both $P<0.01$).

Discussion

Caldas is a region in Colombia known for its high-quality coffee production (Araque-Salazar & Duque, 2019), which relies heavily on the health and fertility of Andosols and Inceptisols in the area. The management of these soils is essential for the sustainability of coffee production as well as for the preservation of the natural resources of the region. The "Bajo occidente" zone performs well in terms of nutrient availability, organic matter content, and cation exchange capacity. While not the best in every metric, this zone maintains a balance that supports coffee cultivation. Zones like "Oriente" may have higher organic matter and carbon content, but "Bajo occidente" benefits from lower bulk density, better nutrient retention (ECEC), and lower aluminum saturation, which are critical soil health indicators (Rekik *et al*., 2018).

Soil health of Caldas is particularly important for coffee production because it directly affects the quality and yield of coffee crops (Tobasura Acuña *et al*., 2015). Coffee plants require specific soil conditions to grow and produce high-quality beans, including optimal pH, organic matter content, and nutrient availability. Andosols have a high capacity to retain nutrients and water, making them ideal for coffee production (Rekik *et al*., 2019). However, erosion, overuse of agrochemicals, and deforestation can lead to a decrease in soil health, which can ultimately affect the quality and yield of coffee crops (Lal, 2015).

According to a study on soil health assessment for coffee farms on Andosols in Colombia, carbon and organic matter contents are the best predictors of overall soil health and can be used in a simplified test (Rekik *et al*., 2018). The study found that the organic matter contents, organic carbon stock, and CO_2 equivalent stock sequestered in the soil showed the highest values in the defined Gold range.

One of the most critical chemical properties of soil is pH, which affects nutrient availability and soil microbial activity. Coffee crops require acid and moderately acid soils with a pH range of 5.0-6.5 for optimal growth and yield (Manson *et al*., 2022). Soil pH can be managed through the application of lime, which raises the pH, or sulfur, which lowers it. However, excessive use of these chemicals can lead to soil degradation and negatively impact soil health (Krishnan *et al*., 2020).

Lower bulk densities are generally associated with better root penetration and water infiltration (Chalise *et al*., 2019). Organic matter (OM) and organic carbon also reflect carbon sequestration, an increasingly important metric for soil health (Rainford *et al*., 2021). Nutrient content of N, P, K, Ca, Mg should also be well-balanced, promoting soil pH stability and reducing aluminum toxicity (Martins *et al*., 2015).

ECEC is a critical factor for long-term soil fertility, especially in tropical regions (Domingues *et al*., 2020). In our case, we found evidence of soil degradation, particularly in the depletion of nutrient contents and soil acidification, which adversely affects the plant's response in terms of the quality of coffee beans. This calls for changes in the practices in the coffee crops to promote the recovery of soil health under regenerative agriculture practices.

This is the first evidence of using the Rainforest Scorecard (Rainforest Alliance, 2022) to promote regenerative practices and linked to soil health indicators under the CASH methodology, adjusted to tropical zones. The Scorecard evaluates the chemical, physical, and biological characteristics of soils (Mukherjee, 2014), making it an important tool for identifying areas in need of improvement and encouraging best management practices by taking the list of regenerative practices to a quantitative level and applying it to the development of soil quality and health indicators.

Conclusions

The findings of this study were used to develop novel CASH scoring functions that were substantiated by a comprehensive analysis of a diverse dataset. The results indicate that it may be appropriate to create region-specific scoring functions once regional soil health data analyses are more complete.

The results suggest that several soil properties are good predictors of CASH, while others are not. These findings could have important implications for soil management and the Soil Quality Index and soil health.

The CASH approach can be successfully employed to evaluate soil health status in soils managed differently in the field, with the possibility of region-specific parameterization for soils in Caldas that are used to cultivate coffee crops.

The implementation of the Scorecard represents a significant step forward in rainforest scorecard efforts and highlights the importance of adopting regenerative practices to promote soil health and sustainability. Further research is needed to better understand the complex interactions between soil health, management practices, and coffee bean quality in the Caldas department.

Acknowledgments

The authors wish to thank Cafexport SARL and the Local Partners Foundation for funding this study.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

AMVH contributed to the conceptualization, data collection, analysis and writing of the manuscript; MAAR and JDR organized the data, collected data, analyzed and interpreted the results; EOS and JCAS helped to conceptualize and interpret the results and revised the manuscript. All authors approved the final version of the manuscript.

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Agroclimatic modeling of the water requirement of the oil palm (*Elaeis guineensis* Jacq.) crop in the Cesar department, Colombia

Modelación agroclimática del requerimiento hídrico del cultivo de palma de aceite (*Elaeis guineensis* Jacq.) en el departamento del Cesar, Colombia

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ABSTRACT

The limited availability of water in the Cesar department (Colombia), along with the high-water consumption associated with the lack of knowledge of water requirements and the low efficiency of irrigation systems in the oil palm (*Elaeis guineensis* Jacq.) crop, has led to an inefficient use of water resources and affected the crop productivity. The main objective of this research was to determine the water demand of oil palm cultivated in areas with homogeneous agroclimatic conditions in the Cesar department. For this purpose, climatic information of the department was obtained from meteorological stations and climatic satellite data. The water demand was also determined by obtaining the crop coefficient (Kc) for plants aged 10 and 15 years, using the water balance methodology. The Kc obtained was 0.91 and 0.84, respectively. As a result of the zoning, 10 agroclimatic zones were identified in the department, of which 8 were found to be suitable for oil palm cultivation. In these zones, water requirements are expected to be homogeneous due to similar soil and climate characteristics. This information was used to estimate the regional water balance, allowing farmers to plan water management and optimize the use of available resources.

Key words: water balance, water use efficiency, cluster, climatic variables, soil water capacity.

RESUMEN

La limitada disponibilidad de agua en el departamento del Cesar (Colombia) y los elevados consumos de agua asociados al desconocimiento del requerimiento hídrico sumado a sistemas de riego de baja eficiencia en el cultivo de la palma de aceite (*Elaeis guineensis* Jacq.), han incidido en un uso ineficiente del recurso hídrico y en la afectación de la productividad del cultivo. El objetivo principal de este trabajo fue determinar la demanda hídrica del cultivo de la palma de aceite en zonas con condiciones agroclimáticas homogéneas en el departamento del Cesar. Para esto se obtuvo la información climática del departamento mediante estaciones meteorológicas y datos climáticos satelitales. También se determinó la demanda hídrica de este cultivo mediante la obtención del coeficiente del cultivó (Kc) en plantas de dos edades, 10 y 15 años, mediante el uso de la metodología de balance hídrico, obteniéndose un Kc de 0,91 y 0,84 respectivamente. Como resultado de la zonificación, se identificaron 10 zonas agroclimáticas en el departamento, de las cuales 8 resultaron ser aptas para el cultivo de la palma de aceite. En estas zonas, se espera que los requerimientos hídricos sean homogéneos debido a las características similares de suelo y clima. Con esta información se hizo la estimación del balance hídrico regional, permitiendo a los agricultores planificar la gestión del agua, optimizando el uso de los recursos disponibles.

Palabras clave: balance hídrico, eficiencia del uso de agua, clúster, variables climáticas, capacidad hídrica del suelo.

Introduction

Oil palm production is of great importance for the Cesar department, as it contributes 17% of the national agricultural GDP and ranks fourth in the country's agricultural exports (Murcia, 2023). The department currently has 77,869 ha planted in 23 of the 25 municipalities (Fedepalma, 2021). This crop requires certain climatic conditions to achieve its proper development and production. In the Cesar department, the accumulated annual precipitation is estimated between 1250 and 1750 mm per year (IDEAM, 2022), which is insufficient to meet the crop water requirements. Woittiez *et al.* (2017) determined that oil palm cultivation in Southeast Asia requires 1900 to 3500 mm of water annually, due to a transpiration rate of 4.0 to 6.5 mm d^{-1} in the rainy season and 1.0 to 2.5 mm d^{-1} in the dry season, with an average evapotranspiration of 6 mm of water per day under normal conditions.

Received for publication: May 15, 2024. Accepted for publication: August 9, 2024. Doi: 10.15446/agron.colomb.v42n2.114042

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In adult oil palm, Lascano and Munévar (2000) found values of crop coefficient (Kc) in a range between 0.75 and 1.2 daily, with water requirement for a crop in northern Colombia ranging between 350 and 500 L per palm per day. Henson (1995), in Malaysia, where ETo varies between 6 and 7 mm d^{-1}, reported Kc between 0.8 and 1.2. Dufrene *et al*. (1992), in Ivory Coast, estimated Kc for the area in the rainy season between 0.69 and 0.72. Arshad (2014), in studies conducted in the Malay Peninsula, obtained evapotranspiration of oil palm crop in a range between 1583 and 2003 mm $year^{-1}$. The crop coefficient (Kc) estimated in this study was 1.0 (Arshad, 2014). Water requirements for the study area remain unknown since the Kc varies depending on the climatic conditions where the crop is grown.

In recent years, there has been a reduction in the availability of water sources in northern Colombia, aggravated by the low efficiency of water use in oil palm crops due to poorly performing irrigation systems (Alvarez *et al*., 2007). The lack of knowledge about climatic conditions and natural resources, such as water and soil, also contributes to inadequate water management planning for most crops (Ongley, 1997). Therefore, it is essential to carry out the characterization of these resources through zoning methodologies. According to the Instituto Geográfico Agustin Codazzi (IGAC, 2014), zoning is defined as the identification of homogeneous clusters that allow the delineation of similar agroclimatic regions (Zhou *et al*., 2009). These regions become essential tools for the planning of spatial projections in agriculture, thus facilitating a more effective and sustainable management of irrigation in crops (Molina *et al*., 2022).

Most of the currently published climate or ecological research work has been conducted by using assessments of experts and qualitative analysis of on-site data (Zhou *et al*., 2009). These traditional methods of manual regional delineation are laborious and time-consuming processes. Therefore, in this work, we applied a methodology designed to generate hierarchical structures (clusters), enabling the correlation of agroclimatic variables according to their geographic location (Morales *et al*., 2006). This methodology was used because the traditional methods of climatic classification do not meet the proposed needs, providing insufficient detail and relying on predefined climatic groups that fail to reflect variations under the conditions of the Cesar department (Terán *et al*., 1998).

The most relevant climate classification studies in Colombia are those provided by the IGAC, which integrate the Caldas-Lang methodological proposal (IGAC, 2018). This climate classification is applied to the American tropics and is based on temperature values with respect to their elevation variability, which indicates the thermal floors and is complemented with precipitation (Castañeda Tiria, 2014). The Cesar department is composed of three morphostructural units: La Sierra Nevada de Santa Marta, la Serranía de los Motilones (or del Perijá), and the sedimentation basin of the Magdalena and Cesar (IGAC, 2017). This means that the Caldas-Lang climate classification does not show significant variations for the department since the valley of the basin of the Magdalena and Cesar rivers occupy the highest portion of the area of Cesar. This landscape is located in the basins of the Cesar and Magdalena rivers at altitudes ranging from 150 to 400 m a.s.l. (Gobernación del Cesar, 2020). This area is one of the hottest zones in the country, with averages above 28°C and minimum temperature changes (IDEAM, 2023); according to the Caldas-Lang methodology, it is a single climatic zone comprised by the valleys found in the basins of the Cesar and Magdalena rivers.

Cluster analysis is used in various areas such as biology, medicine, engineering, and social sciences to identify patterns and relationships between data and thus make decisions (Plazas Niño, 2021). Considering the above, this work sought to produce a zoning that accounts for the temporal-spatial variation of the climatic elements, which were classified through grouping techniques of the agroclimatological variables based on their nature and affinity (statistical and mathematical methods of grouping) (Cardona Arévalo, 2019). In Colombia, the zoning of climatic elements by cluster analysis methods has been implemented mainly due to the country's location on the equatorial strip, which causes climatic variation over short distances, influenced by topography, vegetation and other conditions (Terán *et al*., 1998).

The purpose of this zoning design is to contribute to the efficient use of water resources in the Cesar department, through the determination of agroclimatic parameters that can help optimize available resources and the rational distribution of natural resources (Zhou *et al*., 2009) such as, in the present case, the water resource for the oil palm crop.

Materials and methods

This study was conducted for the department of Cesar, which is located between the coordinates 7°40'38" and 10°52'17" N and between 72°53'06" and 74°07'47" W. It has an area of 22,905 km^2 (Gobernación del Cesar, 2020). The department has a predominantly flat topography below

400 m a.s.l., highlighting the mountain systems that make up the Sierra Nevada de Santamarta and the Serranía del Perijá, with an average temperature of 28°C and rainfall ranging between 1000 and 1500 mm per year. In the center of the department, and especially on the eastern fringe, precipitation volumes increase to values close to 2000 mm (CORPOCESAR, 2023).

The research was conducted in two phases: first, a strategy was developed to delimit agroclimatic homogeneous zones using GIS combined with multivariable analysis for the entire Cesar department and the municipality of Codazzi. This was done following the research trials established in two commercial oil palm plantations for the determination of the crop's water requirements at different ages. After that, the evapotranspiration values were determined for the crop of two ages: 10 and 15 years.

To formulate the zoning, climatic information (precipitation, air temperature, relative air humidity, solar radiation, and wind speed), topography of the Cesar department, and water retention capacity of soil were used. The climatic information was obtained from the network of meteorological stations of the Instituto de Meteorología y Estudios Ambientales (IDEAM), with information from 72 meteorological stations: 52 pluviometric (PM) and 20 from the categories of main climatic (MC), ordinary climatic (OC), and agroclimatological (AC). To complement the climatic information, satellite climatic systems (Copernicus ERA-5) were used. The climatic information was for a stable period of 31 years, following the indications of the World Meteorological Organization (WMO, 2018). The selected stations underwent quality control, including determination of missing data, checking and cleaning of atypical data, filling of missing data, consistency and homogeneity tests to validate the data obtained, in order to determine whether the data were inaccurate, incomplete or incompatible (WMO, 2017).

With the climatic data of the Cesar department, the reference evapotranspiration (ETo) and the precipitation in both annual accumulated and monthly accumulated multiannual terms were obtained. For this, the first step was to calculate ETo, estimated using the FAO Penman-Monteith method, which determines ETo using climatic parameters such as net solar radiation, average air temperature, wind speed, and relative air humidity. This method was used as it is considered one of the most accurate for the estimation of ETo in any location evaluated and because it is widely used in agriculture and hydrology (Allen *et al*., 2006). ETo was calculated daily at the point locations of the weather stations employed.

In relation to the water retention capacity of soils in the Cesar department, a generalized soil study of the department was used (IGAC, 2017), in which the characteristics of the edaphic mosaic and its relationships with the geomorphologic and geologic variety of the soil are found. The results of the soil physical analysis of the points analyzed by the IGAC were used with a total of 55 locations, where the soil water storage was determined up to 60 cm depth considered to be the effective depth for the oil palm crop (Fedepalma, 2001). The amount of rapidly usable water (available water holding capacity, AWHC) was obtained, which refers to the amount of water available for plants to absorb and is determined as the difference between the field water capacity (FC) and permanent wilting point (PWP) to the depth in the soil profile (z) (Eq. 1).

$$AWHC = \frac{(FC - PWP)}{100} \times z \tag{1}$$

With the climatic information (precipitation, ETo), the topographic profile of the department, and the water storage capacity, we proceeded to spatially represent it through a Geographic Information System (GIS), using the Inverse Distance Weighted (IDW) model to perform the interpolation. In this model, the sampling points are weighted during interpolation in such a way that the influence of a point in relation to others decreases with the distance from the unknown point to be created (Villatoro *et al*., 2008).

This research sought to develop a zoning map according to the data shown by Torres Bernal (2024) and following procedures similar to those used by Molina Moral *et al*. (2022) and Teran *et al*. (1998), among others, which determine agroclimatic homogeneous zones. For this, the georeferenced data from the 18402 established points and the monthly values of these variables at these points were used. The zoning was performed through clustering algorithms using unsupervised machine learning to create models that do not require training data, with the purpose of grouping data automatically into different categories or clusters based on their similarity (Jolly, 2018). This process was carried out by applying different clustering methodologies. To evaluate the most appropriate methodology for zoning, four different clustering algorithms were employed. Initially, the k-means algorithm was used, based on Euclidean distance (Kong *et al*., 2021). The second methodology was similar to the first one, but based on the distance of the data to the centroids of the created clusters. The third algorithm was based on the creation of hierarchical clusters; at this point, the Ward model was implemented (Gallardo San Salvador & Vera Vera, 2004). Finally, the Gaussian mixture model

(GMM) algorithm was used. This probabilistic model assumes that the data are generated from a mixture of several Gaussian distributions (Avila & Hauck, 2017).

The best methodology was determined using an expert analysis that allowed choosing which of the zonings was more in accordance with reality, considering that the climatology of Cesar department is regulated or influenced by several circulation patterns associated with the topography and hydrographic systems of the region.

The water requirement was determined using the method of water balance for the two ages of the crop, using a randomized block treatment model for the two experimental units (10-year-old and 15-year-old palms (*Elaeis guineensis* Jacq.)). For each age, five treatments were applied: Treatment T1 (50 Liters-Palm-Day (LPD), Treatment T2 (150 Liters-Palm-Day (LPD), Treatment T3 (300 Liters-Palm-Day (LPD), Treatment T4 (450 Liters-Palm-Day (LPD), and Treatment T5 (600 Liters-Palm-Day (LPD)). These treatments were applied with different irrigation flow rates. Each treatment had four replicates, and each replicate consisted of sixteen palms. A statistical analysis (ANOVA) was performed between replicates and treatments to determine significant differences between replicates and treatments.

Crop evapotranspiration was obtained using the water balance method. This method consists of evaluating the water flows into and out of the crop within a given period of time. Water inflows are mainly due to irrigation (I) and precipitation (P), while outflows are due to runoff (RO), deep percolation (Dp), and evapotranspiration (ETc) (Ordoñes, 2011).

$$ETc - (P + I) - RO - Dp = \pm \Delta W \tag{2}$$

Using this methodology, the ETc was obtained by measuring the daily moisture content of the soil. This was determined with the use of a FDR Diviner 2000 moisture sensor (Vienna Scientific Instruments GmbH), which provided moisture content values on a volumetric basis down to 100 cm depth. With the ETc values obtained, the crop coefficient (Kc) was calculated by relating it to the reference evapotranspiration (ETo):

$$ET_c = Kc \times ET_0 \tag{3}$$

Results

Analysis of climatic variables

Precipitation

The climatic variables analyzed included multi-year monthly averages of evapotranspiration and precipitation for the period 1991 to 2021. From these data, multi-year monthly distributions of ETo and precipitation were generated. Figure 1 shows the monthly variation of precipitation in the analyzed period, which exhibits a bimodal trend. The precipitation peaks are observed in May and October with values above 190 mm, while the driest month is January with precipitation below 60 mm, although in June and July the decrease in precipitation is less pronounced than in December and January.

In multiannual terms, precipitation shows significant variation, which is largely influenced by the El Niño and

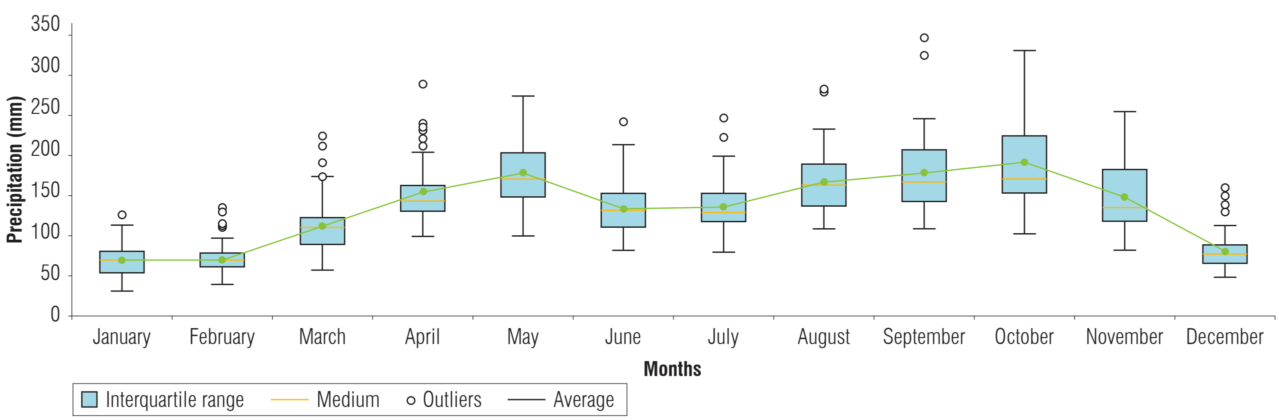

FIGURE 1. Boxplot of the monthly behavior of precipitation (P) in the Cesar department.

La Niña (ENSO) phenomena. These natural phenomena, characterized by fluctuations in ocean temperatures in the central and eastern region of the equatorial Pacific, have a substantial impact on climatic conditions in various parts of the planet (WMO, 2023). In the case of the Cesar department, the average annual accumulated precipitation was 1574 mm, with a maximum annual accumulated precipitation of 2440 mm in 2010 and a minimum value of 1154 mm in 2015. Notably, these years coincide with the extreme phenomena of El Niño and La Niña. Figure 1 represents the behavior of precipitation in wet and dry years obtained using the quintile methodology. The years 1995, 1996, 1998, 1999, 1999, 2008, 2010, 2011, and 2017 are considered wet years, with maximum precipitation values above 1730 mm per year and an average precipitation of 1942 mm per year.

Reference evapotranspiration (ETo)

The average ETo value for the entire department was 5.61 mm d^{-1} for the period analyzed (31 years). The data series has an average annual accumulated value of 2046 mm $year^{-1}$, with a minimum in 2012 of 1822 mm $year^{-1}$ and a maximum reported in 2015 of 2196 mm $year^{-1}$. Regarding the multiannual monthly accumulated ETo, analysis, the series has a monthly average of 170 mm/month, and an inverse bimodal trend to that observed for precipitation is evident throughout the year, with March being the month with the highest evapotranspiration, with a value of 185 mm, and November the month with the lowest ETo, with a value of 156 mm (Fig. 2).

Spatial distribution of precipitation and ETo in the Cesar department

The spatial analysis for the Cesar department shows a differentiated behavior in the climatic variables analyzed (Fig. 3). The southern part of the department, including the municipalities of San Alberto and San Martín, shows the highest precipitation and the lowest ETo. For the ETo variable, another area with low values is observed in the foothills of the Sierra Nevada de Santa Marta. On the other hand, the center-north of the department, including the Cesar River sedimentation basin, shows the lowest precipitation and the highest ETo.

Climatic water balance

The climatic water balance was obtained by using the maps of cumulative P and ETo, annual and monthly multiannual for the Cesar department. At the annual level, the values obtained vary in a range from 400 mm to a minimum peak value of -900 mm. Negative values indicate maximum water deficit events, which indicate that the annual precipitation level is not able to compensate for the losses caused by evapotranspiration. Positive values are recorded in the south of the department, mainly in the municipalities of San Alberto and San Martín. With regard to monthly behavior (Fig. 4), generally for the department, it is observed that May, September and October exhibit a positive water balance, which is concentrated mainly in the southern and central areas of the department, with a maximum value of around 25 mm in October; however, in October, some areas in the north of the department reported a negative

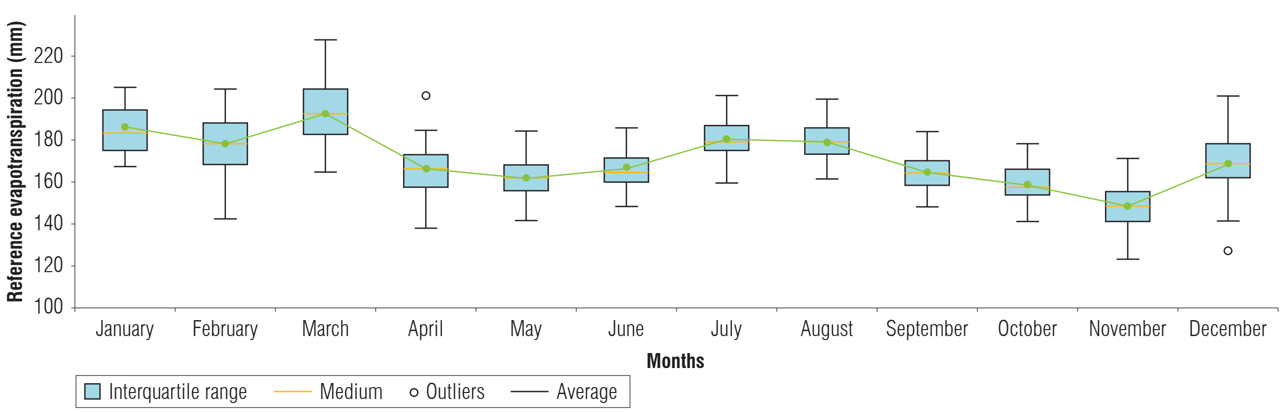

FIGURE 2. Boxplot of the monthly behavior of the reference evapotranspiration (Eto) in the Cesar department.

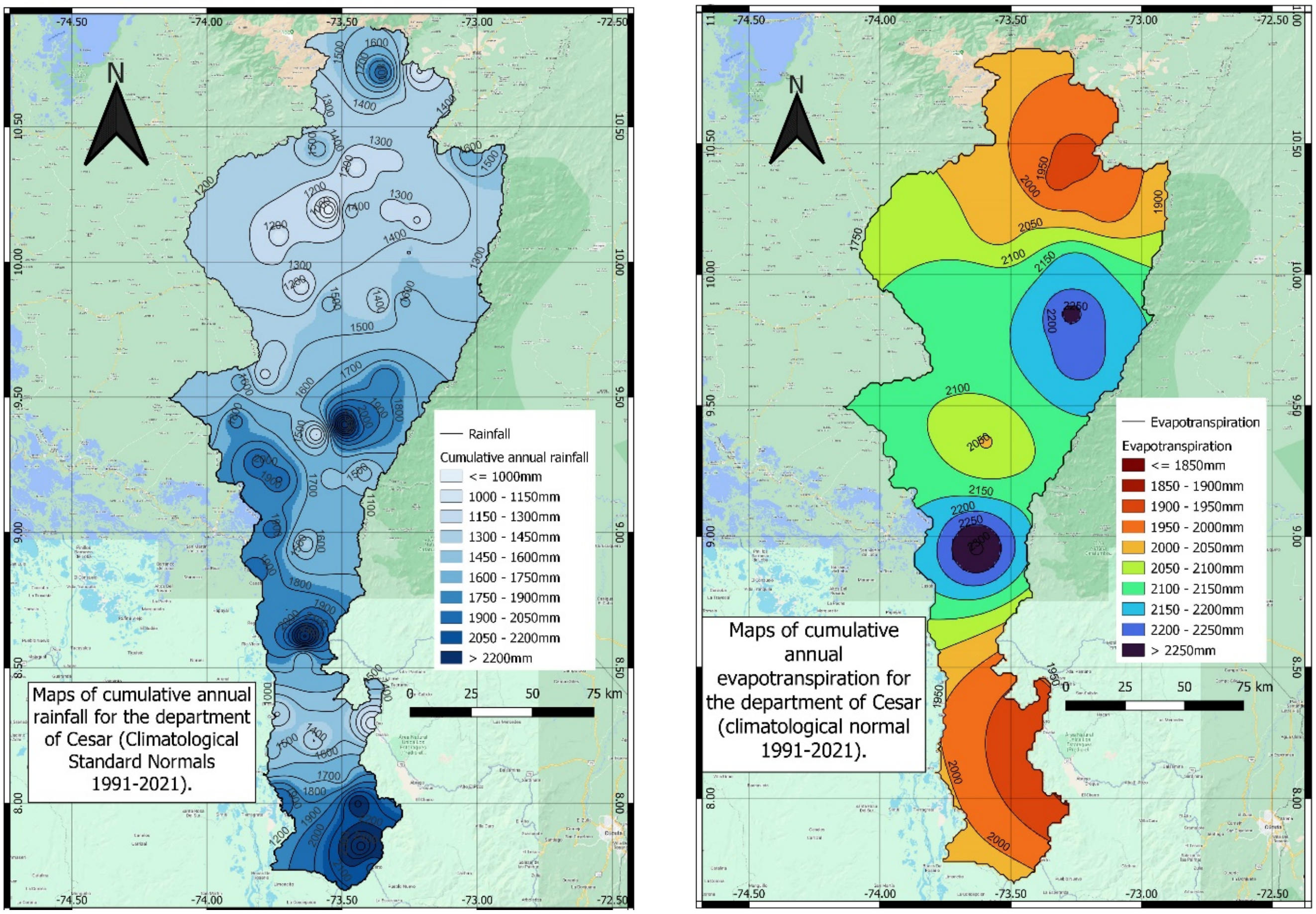

FIGURE 3. Maps of cumulative annual rainfall and cumulative annual evapotranspiration for the Cesar department.

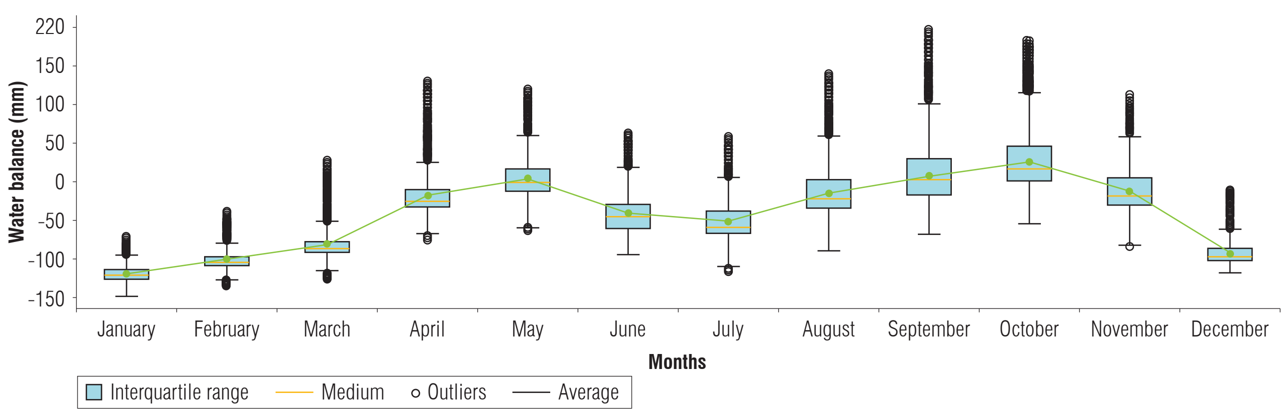

FIGURE 4. Average behavior of the monthly climatic water balance in the Cesar department.

water balance. In the rest of the year, mainly in January, February, March, and December, negative water balances were recorded throughout the department. January had the highest accumulated water deficit of -121 mm, which was more critical in the regions located in the basin of river Cesar, particularly in the municipalities of Copey, Bosconia, Valledupar, La Paz, San Diego, and Agustín Codazzi.

Agroclimatic zoning of the Cesar department

Based on the information of the accumulated annual and accumulated monthly multiannual data of the climatic water balance, soil storage capacity and topography, the four zoning models were carried out. Using expert analysis (Galicia Alarcón *et al.*, 2017), the zoning was determined using the k-means Euclidean distances methodology as the best approximation, thus determining the homogeneous zones in the Cesar department.

As a result of this zoning, 10 different agroclimatic zones were determined (Fig. 5). Zones 1 to 8 correspond to areas with altitudes below 640 m a.s.l., where oil palm cultivation can be developed. Zone 9 corresponds to areas with altitudes between 640 and 1950 m a.s.l., and Zone 10 corresponds to areas with altitudes between 1950 and 4897 m a.s.l. These zones were excluded from the analysis because they are part of the Sierra Nevada de Santa Marta and the Serranía de los Motilones or del Perijá, and are areas that are not suitable for oil palm cultivation due to their climatic and altitudinal conditions.

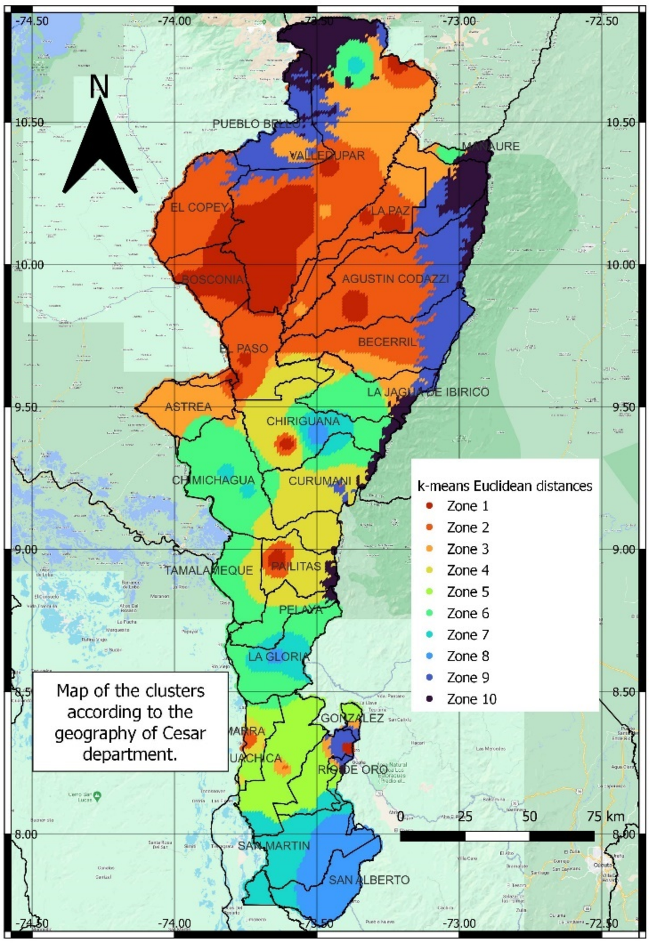

FIGURE 5. Spatial representation of the clusters according to the geography of the Cesar department.

In Table 1, the climatic parameters of the different zones are summarized, including the area covered by the crop in each zone. It is worth noting the climatic variability found in the regions where oil palm is grown, with significant differences between the areas sampled. Annual accumulated rainfall shows a difference of 906 mm between the zone with the lowest rainfall (Zone 1) and the zone with the highest (Zone 8). As for evapotranspiration, minor differences of 253 mm were observed between the zone with the highest ETo (Zone 2) and the zone with the lowest ETo (Zone 5), which shows that the extreme values do not necessarily correspond to the zones with the highest or lowest water deficit.

The area under oil palm crop across the different zones reveals that Zone 2 has the largest cultivated area in the department, occupying 23.8% of the planted area. Zone 8 is the smallest identified zone, with 4.88% of the total area of the department and it is the only zone that, in annual terms, does not show a water deficit. On the contrary, Zone 8 has a positive water balance of 235 mm and is also characterized as the second zone with more hectares of oil palm planted, with 17.4% of the oil palm planted in this department.

Water demand of the oil palm crops

Two ANOVA tests were performed on the results obtained (Tabs. 2 and 3). The first analyzed the similarity of the

TABLE 1. Summary of the agroclimatic indicators for the zones suitable for oil palm cultivation in the Cesar department.

Zone	Water deficit (mm)	P accum (mm)	ETo accum (mm)	ETo (mm)	Mean T (°C)	TMax (°C)	TMin (°C)	Altitude (m a.s.l.)	Area (km^2)	Planted area (ha)
1	-821.5	1271	2098.1	5.8	28.9	34.5	23.2	138.6	2096.3	4380
2	-711.9	1394.1	2110.6	6	28.6	34.4	22.8	237.3	5439.9	27112
3	-561.9	1472.9	2036.4	5.5	28.1	34.1	22.7	215.9	2605.4	4362
4	-515.2	1695.8	2144.8	6.1	27.6	32.9	22.3	185.7	2453.6	8231
5	-404	1539.8	1957.9	5.4	24	31.9	20.6	92.5	1292.1	4458
6	-313.5	1782.3	2107.5	5.8	27.4	33.3	22.2	159.1	3378.5	10850
7	-78.5	1890.5	2020	5.5	25.6	30.2	19.4	106.3	1461.4	12435
8	235.4	2177.8	1968.9	5.4	24.5	29.9	18.8	325.1	959.8	15089

P – Precipitation, ETo – Evapotranspiration, T – temperature, accum – accumulated.

different replicates according to the treatments, with the null hypothesis (Ho) indicating that the replicates of the same treatment are equal. The results of the test did not reject the hypothesis, since it showed a significant level of more than 5% (Tab. 2). This procedure was performed for the soil depth up to 60 cm. The second ANOVA test, with the null hypothesis (Ho) suggesting that the treatments have no significant differences, rejected this hypothesis, showing a significance level of less than 5% (Tab. 3).

According to Table 4, ETc values ranging from 1.5 to 8.58 mm d^{-1} were observed for the 10- and 15-year-old palms. Treatment T1 (50 Liters-Palm-Day (LPD)) had the lowest ETc values, with an average of 3.31 mm d^{-1} during the period analyzed, while treatment T4 (450 LPD) had the highest ETc values, with an average of 4.21 mm d^{-1} during the period sampled. A similar trend was observed in the 15-year-old palms, where treatment T1 (50 LPD) showed the lowest ETc with 3.29 mm d^{-1}, while the experimental units of treatment T4 (450 LPD) had average ETc values equal to 4.07 mm d^{-1}.

With the ETc obtained (Tab. 4) and the ETo calculated using the Penman-Monteith equation as found in FAO guide No. 56 (Allen *et al*., 2006), the crop coefficient (Kc) was determined. The results were presented as a monthly average (Tab. 3), with an average Kc value of 0.91 for the 10-year-old palms and 0.84 for the 15-year-old palms, showing variable

TABLE 2. Results of variance ANOVA test analyzing the similarity of the different replicates according to treatments.

	Origin of variations	Sum of squares	Degrees of freedom	Mean squares	F	Significance level	Critical value for F
Treatment 1 (50 LPD)	Between replicates	3.677	3	1.226	2.162	0.098	2.699
	Within replicates	54.43	96	0.567			
Treatment 2 (150 LPD)	Between replicates	2.056	3	0.685	1.809	0.151	2.699
	Within replicates	36.36	96	0.379			
Treatment 3 (300 LPD)	Between replicates	0.223	3	0.074	0.22	0.882	2.699
	Within replicates	32.453	96	0.338			
Treatment 4 (450 LPD)	Between replicates	1.241	3	0.414	1.113	0.348	2.699
	Within replicates	35.669	96	0.372			
Treatment 5 (600 LPD)	Between replicates	0.825	3	0.275	0.637	0.593	2.699
	Within replicates	41.469	96	0.432			

LPD – Litters-Plant-Day.

TABLE 3. Results of variance ANOVA test analyzing the similarity of the different treatments.

Origin of variations	Sum of squares	Degrees of freedom	Mean squares	F	Significance level	Critical value for F
Between treatments	397318.020	4	99329.505	251.277	4.62E-162	2.378
Within treatments	543139.076	1374	395.297			

TABLE 4. Monthly crop evapotranspiration of the different treatments in the two experimental units of oil palm measured between September 2022 and September 2023 in the Cesar department (treatment T1 50 LPD, treatment T2 150 LPD, treatment T3 300 LPD, treatment T4 450 LPD, and treatment T5 600 LPD).

Months		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Mean
Crop evapotranspiration (mm) 10-year-old palms	**T1**	2.7	3.3	5.2	6.7	6.3	3.8	2.1	2.1	2.3	2.6	2.3	2.4	2.6	3.3
	T2	3.6	4.5	3.6	7.3	6.1	3.0	3.3	3.0	3.5	2.4	2.9	3.2	2.9	3.7
	T3	3.2	4.6	7.0	6.7	3.9	5.0	3.4	3.0	2.6	2.5	2.6	2.4	2.6	3.7
	T4	2.9	4.2	5.3	6.0	6.3	5.0	5.8	3.4	3.2	2.5	3.4	3.2	3.8	4.2
	T5	4.6	4.4	4.9	8.6	5.4	4.4	3.8	2.9	2.9	2.5	2.1	3.2	3.0	4.1
Crop evapotranspiration (mm) 15-year-old palms	**T1**	3.5	5.6	5.1	6.2	4.4	3.4	2.7	2.5	3.3	2.2	1.9	2.1	2.1	3.3
	T2	3.5	4.0	3.5	7.2	5.5	3.9	2.6	2.6	2.8	2.0	1.8	2.1	1.8	3.2
	T3	4.6	5.5	4.4	2.0	5.7	3.5	3.8	2.5	3.1	2.8	3.4	3.1	2.7	3.8
	T4	2.8	3.1	3.3	7.8	5.8	5.8	4.3	4.5	3.4	3.2	3.6	3.4	3.8	4.1
	T5	3.4	1.5	7.4	5.1	4.3	3.4	2.8	4.1	3.1	2.4	4.1	3.1	2.9	3.5

behavior throughout the year, characterized by increasing values in the dry months (December, January, February, and March). In these months, a maximum Kc was identified in December for both plantations, with values of 1.52 for 10-year-old palms with daily irrigation and 1.27 for 15-year-old palms. In contrast, in the less dry months, a lower Kc was observed, with average values of 0.75 and 0.71 in 10- and 15-year-old palms, respectively (Tab. 5).

Water balances according to the agroclimatic zone

Finally, the monthly behavior of the climatic variables was analyzed. This was done by presenting the water balance of the zones monthly, in accordance with the general characteristics of the study and the different parameters obtained. This approach will support more effective planning of the agronomic operations of the crop according to the agroclimatic conditions identified in each zone.

In Figure 6, the monthly behavior of climatic variables in the different zones is presented together with their corresponding water balance. The previously mentioned pattern is confirmed, where Zone 1 exhibits the greatest water deficit, maintaining a negative water balance during all months of the year. In contrast, Zone 8, although it does not present a water deficit in annual terms with respect to the climatological norm, shows a deficit in January and February with an average of 63 mm in these months. The other zones show variable monthly behavior of water deficit influenced by climatic conditions.

Discussion

The zoning was carried out using a cluster analysis methodology, allowing for an unbiased selection of zones, corresponding to both annual and monthly multiannual climatic behavior. The delimitation of zones depends on the selection of defined parameters and the appropriate weight for each parameter. These methods can be replicated for other regions as well as for different regionalization themes at various scales. The cartographic procedures are quantitative and automated; therefore, the resulting maps have less uncertainty, and the biases of human judgment are reduced (Morales *et al*., 2006). This zoning approach provides valuable knowledge that can be used to create a more specific approach to farming regions, making it possible to objectively identify areas with similar characteristics, without the need for predefined values, as is common in other zoning strategies.

This zoning for the department seeks to contribute to the development of strategies to meet the water demand of the crop according to its location. Among these strategies is the implementation of efficient irrigation systems to optimally meet crop water requirements. Additionally, the implementation of efficient irrigation systems will not only contribute to ensure water supply for crops but also maximize agricultural productivity and minimize the negative impact on local water resources (Sanchez Arzapalo & Acosta Sanchez, 2023).

TABLE 5. Average crop coefficient Kc in the different treatments for 10- and 15-year-old palms between September 2022 and September 2023 in Cesar department (treatment T1 50 LPD, treatment T2 150 LPD, treatment T3 300 LPD, treatment T4 450 LPD, and treatment T5 600 LPD).

Months		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Mean
Crop Kc 10-year-old palm	T1	0.63	0.7	1.07	1.43	1.25	0.93	0.71	0.53	0.54	0.68	0.5	0.53	0.6	0.78
	T2	0.84	0.9	0.99	1.59	1.29	1.02	1.01	0.76	0.71	0.63	0.7	0.71	0.79	0.92
	T3	0.8	0.9	1.43	1.45	1.09	0.98	1.19	0.77	0.66	0.64	0.7	0.71	0.86	0.94
	T4	0.81	0.9	1.09	1.29	1.32	1.21	1.18	0.86	0.75	0.65	0.8	0.71	0.84	0.96
	T5	0.92	0.9	1.01	1.85	1.04	1.17	1.02	0.75	0.64	0.66	0.7	0.69	0.97	0.94
Mean		0.8	0.9	1.12	1.52	1.2	1.06	1.02	0.73	0.66	0.65	0.7	0.67	0.81	0.91
Crop Kc 15-year-old palm	T1	0.67	1.2	1.14	1.41	0.96	0.72	0.49	0.6	0.78	0.59	0.5	0.54	0.52	0.77
	T2	0.67	0.8	0.98	1.58	1.21	0.97	0.65	0.65	0.65	0.53	0.4	0.52	0.45	0.78
	T3	0.9	1.1	0.98	0.45	1.26	0.77	0.61	0.6	0.72	0.74	0.8	0.78	0.68	0.8
	T4	0.53	0.6	0.72	1.78	1.28	1.29	0.98	1.0	0.81	0.86	0.8	0.87	0.87	0.96
	T5	0.65	0.3	2.04	1.14	0.95	0.8	0.71	0.93	0.74	0.63	1.0	0.77	0.73	0.87
Mean		0.68	0.8	1.17	1.27	1.13	0.91	0.69	0.76	0.74	0.67	0.7	0.7	0.65	0.84

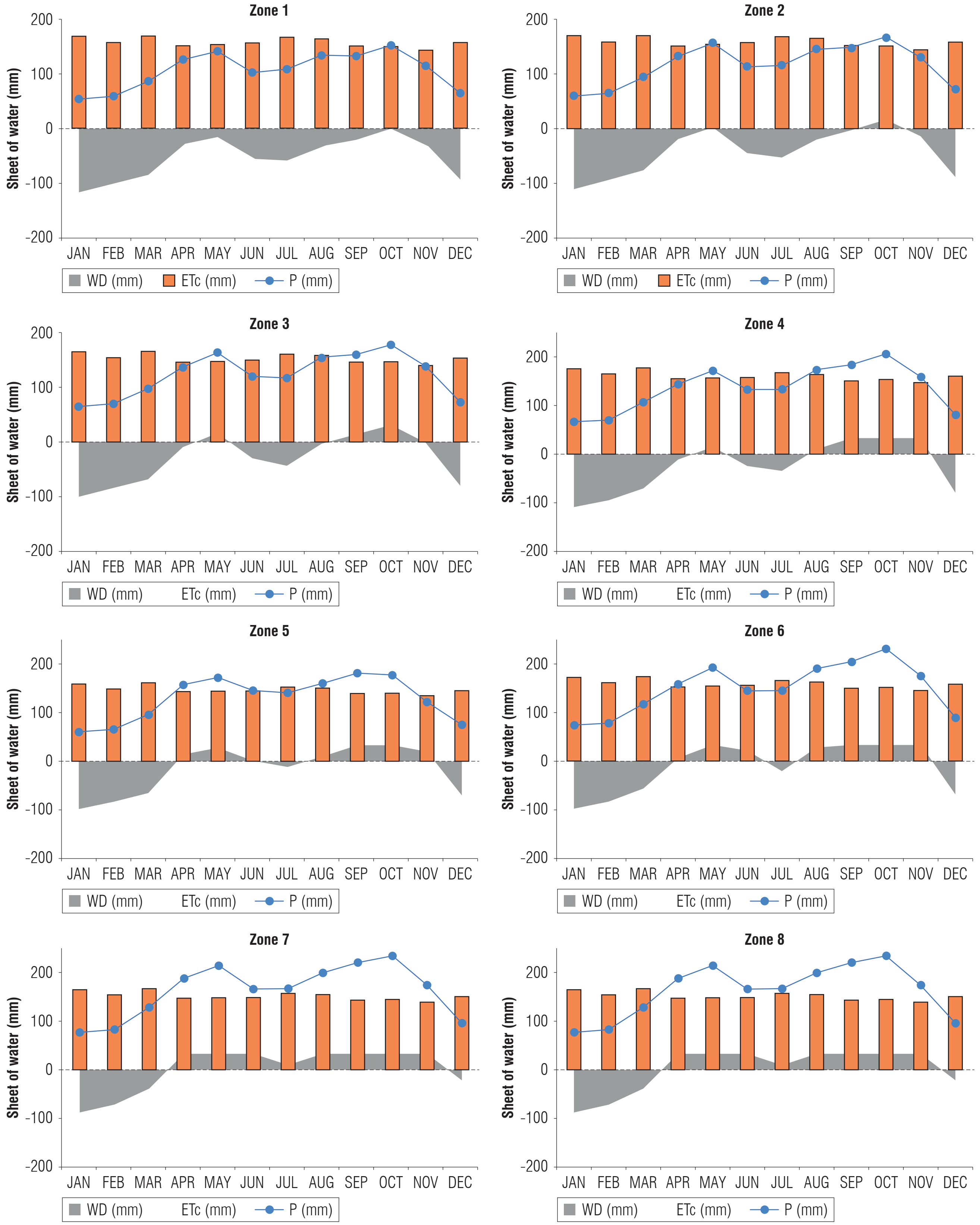

FIGURE 6. Behavior of water deficit (WD), evapotranspiration (ETc), and precipitation (P) in the Cesar department.

The determination of the crop coefficient (Kc) through the WD methodology can provide valuable information on water consumption by oil palms in these areas. However, as noted by Allen *et al*. (2011), this methodology has a potential source of error in the determination of ETc. This uncertainty is caused by the deep percolation of the measured area, as well as the rising capillary movements, making these errors difficult to determine. To reduce the error associated with the established methodology, Nachabe (1998) used the theory of drainage and redistribution of water in the soil, stating that the water flow occurs when the soil moisture is above FC, while below FC, this flow could be 0.05 mm d^{-1} or less. This is considered negligible. Therefore, the use of the accumulated film of water up to 60 cm (palm root depth) was proposed to determine water consumption by the plants, with measurements at 60 and 70 cm to determine percolation losses.

The statistical analysis showed that treatment T5 (600 LPD), which was subjected to excess water, did not show a relevant benefit compared to treatment T4 (450 LPD), which was subjected to a lower excess water. This evaluation was based on the response variables, including the number of arrows (the arrows on the oil palm refer to leaves in their early stages, unopened, which are indicators of water deficit or excess in the crop (Fedepalma, 2021)). This lack of benefit is explained by the fact that crop water consumption depends on climatic conditions, plant type, soil conditions, and phenological stage. Crops have a maximum water absorption capacity (Marin *et al*., 2019); if more water is applied than the plant can use, it will simply be wasted by percolation. However, excess water can be detrimental to crops, causing phytosanitary affectations, such as root rot, which could result in decreases in production (Cobo Romero, 2016).

Meanwhile, treatments T1 (50 LPD) and T2 (150 LPD), which experienced water deficiency, had a lower Kc value compared to the other treatments. This can be explained by the defense mechanism of plants in a situation of water deficit, where closure of stomata prevents the escape of water vapor. However, this leads to a lower CO_2 input, which reduces the photosynthetic rate (Moreno, 2009). The reduction of the photosynthetic rate alters yield since the plants use energy to obtain water, significantly impacting production.

These findings highlight the importance of not relying on an average Kc for a given plant age, as this approximation may not accurately reflect crop water requirements at different times of the year. The relationship between the reference evapotranspiration (ETo) and crop coefficient (Kc) was evaluated using daily data, showing that at higher ETo values, Kc tends to be lower. This phenomenon has been corroborated by previous studies (Gonçalves *et al*., 2023; Marin *et al*., 2019; Marin *et al*., 2020), who reached similar conclusions. Their research indicates that the use of an average Kc can result in an overestimation or underestimation of irrigation requirements, especially in crops exposed to high evaporation conditions in soils with different drainage levels.

Assuming the average Kc, the evaluation of water consumption in the different zones determined suitable for the crop was carried out. In all these zones, the implementation of irrigation systems is essential to maintain crop productivity. However, irrigation strategies must be adapted to the specific conditions of each zone. In areas with a greater water deficit (Zones 1 and 2), it is necessary to implement highly efficient irrigation systems that minimize the use of water resources. In addition, it is crucial to consider water storage methodologies for use during the months with the greatest deficit (Jasso Ibarra *et al*., 2007). In contrast, in zones with a lower deficit (Zones 7 and 8), although irrigation systems are still necessary, less efficient systems can be used due to favorable climatic conditions; furthermore, the installation of drainage systems has to be considered.

It is important to point out that the analysis carried out in this research contemplated both wet and dry years in the Cesar department, which reinforces the need for irrigation systems in oil palm cultivation.

Agroclimatic zoning, such as the one presented in this study, has a series of highly relevant applications. First, it plays a fundamental role in the formulation of research projects, allowing the definition of homogeneous zones for the precise location of experiments. This facilitates the extrapolation of results to similar areas or regions with similar characteristics, thus promoting technology transfer. In addition, agroclimatic zoning plays a crucial role in the efficient management of water resources by defining zones according to their topographic characteristics, soil and climatic conditions, and by providing a valuable tool for optimizing agricultural production. This implies making informed decisions based on the conditions of each zone. To summarize, agroclimatic zoning is proposed as an essential resource in agricultural planning and management as well as in the research and development of projects aimed at the agricultural sector.

Conclusions

From this research, it was possible to achieve the zoning of the water demand of the oil palm crop (*Elaeis guineensis* Jacq.) in the Cesar department. This methodology allowed for a spatial-temporal analysis of the results obtained, providing an integral and detailed view of water consumption by differentiating agroclimatically homogeneous areas. The development of this methodology for agroclimatic modeling in the Cesar department is not only applicable at the local level but also lays the groundwork for its replication at the national level in similar agricultural contexts.

The crop coefficients (Kc) obtained for 10- and 15-year-old palms, with average values of 0.91 and 0.84, respectively, highlight the need to adapt water management strategies precisely throughout the crop cycle. The evaluation of Kc in different treatments shows that variations in moisture content generated substantial changes in Kc values. In this regard, decision-making in agricultural water management should incorporate the variability of soil moisture content to ensure accurate and efficient irrigation application, especially in areas with higher water deficits.

This methodological approach lays the groundwork for agroclimatic research and establishes a valuable precedent for future studies in the field. It contributes significantly to the understanding and efficient management of water resources in comparable agricultural environments, providing a robust framework for addressing challenges related to water consumption in oil palm cultivation and, potentially, in other agricultural crops under similar conditions.

Acknowledgments

This work was developed within the framework of the project entitled "Development of an integrated agronomic management system for palm cultivation as a response to the effects of climate variability in the Cesar department" carried out by Cenipalma with the support of the Sistema General de Regalías of the Cesar department (SGR), which aimed to identify strategies for the crop management and mitigate the impacts of climate variability. One of the results of the project was the Master's thesis entitled "Zoning of the water demand of the oil palm (*Elaeis guineensis* Jacq.) crop under the agroclimatic conditions of the department of Cesar". We thank the Oil Palm Research Center (Cenipalma) and the Sistema General de Regalías of the Cesar department (SGR) for the funding.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

FLT, TED, and CAG designed the experiments, FLT and GSL carried out the field experiments, FLT and CAG contributed to the data analysis, FLT, TED, and CAG wrote the manuscript, NAA, TED, and GSL supervised the experiments. All authors reviewed the final version of the manuscript.

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Azospirillum brasilense and jasmonic acid as mitigators of water stress in creole corn plants

Azospirillum brasilense y ácido jasmónico como atenuadores del estrés hídrico en plantas de maíz criollo

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ABSTRACT

The aim of this research was to evaluate the action of *Azospirillum brasilense* and jasmonic acid as mitigators of water stress caused by various irrigation levels in Creole corn crops. The experiment was conducted in a greenhouse using a completely randomized factorial design consisting of 2 levels of *A. brasilense* (absence and presence) x 3 doses of jasmonic acid x 3 irrigation depths determined by three evapotranspiration (ET0) multipliers (0.6, 0.8, and 1.0 of the evapotranspiration measured in the crop area). The design included 4 replicates, totaling 72 experimental units. Evaluations were carried out at the R6 physiological maturity stage for number of tassels, tassel branch number, number of leaves on the corn cob, and number of ears per plant. Analysis of variance showed significant difference among the variables studied. The irrigation depth of 0.8 of the evapotranspiration showed significant interactions between *A. brasilense* and jasmonic acid in reducing water stress in creole corn plants. The results for the irrigation depth 0.6 of the evapotranspiration, with the use of mitigators, showed smaller reductions in stress (11.91% reduction compared to 0.8 of the ET0). The treatment of 10 μmol L^{-1} jasmonic acid was the most favorable for reducing stress and consequently increasing the productivity of creole corn plants, coming closest to the estimated optimal dosage.

Key words: production, resistance, signaling, water stress, growth regulator.

RESUMEN

El objetivo de esta investigación fue evaluar la acción de *Azospirillum brasilense* y ácido jasmónico como atenuadores del estrés hídrico provocado por diferentes niveles de riego en el cultivo de maíz criollo. El experimento se llevó a cabo en un invernadero, el diseño experimental fue completamente al azar en un esquema factorial con dos niveles de *A. brasilense* (ausencia y presencia) x 3 dosis de ácido jasmónico x 3 profundidades de riego determinadas por tres coeficientes multiplicadores de la evapotranspiración (ET0) (0,6, 0,8 y 1,0 de la evapotranspiración medida en el área del cultivo), con 4 repeticiones, para un total de 72 unidades experimentales. Las variables estudiadas fueron: número de panículas, número de ramas de la panoja, número de hojas en la mazorca de maíz y número de mazorcas por planta. Se realizó un análisis de varianza, en el cual se demostró que hubo diferencias significativas entre los tratamientos para las variables estudiadas. La lámina de riego de 0,8 de la evapotranspiración mostró interacciones significativas entre *A. brasilense* y ácido jasmónico en la reducción del estrés hídrico en plantas de maíz criollo. Los resultados para la lámina de riego de 0,6 de la evapotranspiración, con el uso de atenuadores, mostraron reducciones del estrés (reducción del 11,91% respecto al 0,8 de la ET0). El tratamiento de 10 μmol L^{-1} de ácido jasmónico fue el que más se acercó a las dosis estimadas y resultó ser el más favorable para la reducción del estrés y consecuente aumento de la productividad de las plantas de maíz criollo.

Palabras clave: producción, resistencia, señalización, estrés hídrico, regulador de crecimiento.

Introduction

Corn (*Zea mays* L.) stands out internationally as one of the most relevant agricultural products and is indispensable in animal feed as it is the main source of energy. It is also used in the production of renewable fuel. In 2024, Brazil will be the second largest corn producer in the world, behind the United States. Production is estimated at 115.72 million t, including the three harvests, which is 12.3% or 16.17 million t below that produced in 2022/23 (Companhia Nacional de Abastecimento – Conab, 2024).

Received for publication: May 9, 2024. Accepted for publication: August 15, 2024. Doi: 10.15446/agron.colomb.v42n2.100956

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Oliveira *et al*. (2021) pointed out that creole corn can be an alternative to hybrid cultivars, which are dependent on chemical inputs and more advanced technologies. Another peculiar characteristic of native plants, resulting from genetic factors, is their resistance to biotic and abiotic stresses (Modesto *et al*., 2021).

The cultural role of Creole maize is of great relevance, as well as its economic influence in certain countries around the world, specifically those in America, especially Latin America, where the first maize cultivars originated (Jasper & Swiech, 2019). Generally, native plantations are inferior in terms of productivity when compared to commercial ones; however, they are essential for establishing a source of genetic variability (Araújo *et al*., 2018).

Creole varieties tend to be resistant to environmental changes and phytopathologies, as they adapt better to unfavorable circumstances, such as high temperatures and pest attacks (Eicholz *et al*., 2018). One of the most effective techniques to conserve Creole varieties is the use of local seed banks (Mateus *et al*., 2020). An indication of the superiority of Creole varieties over commercial ones is their productivity, whether in grains or in volume of straw for feeding dairy cattle (Sah *et al*., 2020).

According to Pandit *et al*. (2018), the choice of corn cultivation period takes into account the rainfall patterns in different Brazilian regions to obtain better results in productivity. Sah *et al*. (2020) reported that water management techniques are essential to increase crop production and, above all, to improve the efficient use of water, and should focus on irrigated agriculture planning.

The use of Plant Growth-Promoting Bacteria (PGPB) can favor the cultivation of corn (Silva *et al*., 2022). According to Schaefer *et al*. (2019), PGPB act as a defense mechanism against adverse factors such as water deficit. With the use of *Azospirillum brasilense*, the synthesis of plant hormones that signal stress such as jasmonic acid and abscisic acid, as well as growth promoters, has been observed, especially for the increase of the root system, which provides greater water absorption (Fukami *et al*., 2018).

Jasmonic acid is a phytohormone that plays a role in the growth, development, and response to various stress states in plants (Wang *et al*., 2020). According to Kerbauy (2019), some studies have suggested that jasmonic acid may be associated with the expression of resistance genes and involved in the signaling response to biotic and abiotic stress conditions.

Oliveira *et al*. (2020) report that irrigation of plantations is not always carried out to fully meet the crop's water needs, and management is guided by economic objectives rather than by maximum physiological productivity. It is essential to study the action of mitigators of low water conditions in the soil, which can also provide greater plant performance and enhance the productivity of Creole corn.

According to Crisóstomo *et al*. (2018), there is a lack of information on Creole corn cultivars in Brazil, considering the limited availability of production technology and distinctions of size and arrangement of leaves in the plants, making further studies regarding the population management of Creole corn plants indispensable. Given the above, the aim of this study was to evaluate the action of *Azospirillum brasilense* and jasmonic acid as mitigators of water stress caused by various irrigation levels in Creole corn crops.

Materials and methods

Description of the experimental area

The experiment was conducted from November 2020 to February 2021 in a greenhouse belonging to the Integrated Management of Weeds of the Amazon (Mipdam) group, located at the Institute of Agrarian Sciences (ICA) of the Federal Rural University of the Amazon (UFRA), in the municipality of Belém (PA). This site is geographically located at 48°30'16" W and 1°27'21" S, at an average altitude of 10 m a.s.l. and temperature and average relative humidity of 36.5°C and 66% RH, respectively, measured with a digital thermo-hygrometer model K29-5070H (Kasvi®).

Experimental design

The experimental design was completely randomized in a factorial scheme consisting of 2 levels of *A. brasilense* (absence and presence) x 3 irrigation depths determined by three evapotranspiration multiplier coefficients (0.6, 0.8, and 1.0 of the evapotranspiration measured in the crop area) (Deaquiz *et al*., 2014) x 3 doses of jasmonic acid (Sigma Chemical Co., USA) (0, 10, and 20 μmol L^{-1}), with 4 replicates, totaling 72 experimental units. Five seeds were sown per pot, with a capacity of 11.7 L, containing a dystrophic yellow Latosol soil substrate, which was sent for analysis (Tab. 1) after being previously sieved in a 2 mm sieve. The first thinning was performed at 7 d, leaving only two plants per pot, and the second was performed at 14 d, leaving one plant per pot (which served as each of the experimental units). Nitrogen fertilization with urea was carried out at planting and covering was done as recommended by the liming and fertilization recommendation book for the State of Pará (Brasil *et al*., 2020).

TABLE 1. Results of the soil chemical analysis.

pH		TOC	OM	P	N	K	Ca	Mg	Al (KCl)	H+Al	SB
$CaCl_2$	Buffer solution (SMP)	g dm^{-3}		mg dm^{-3}	mg L^{-1}	mmolc dm^{-3}					
5.9	6.5	40.0	94.0	87.0	2688.0	2.9	31.0	19.0	0.0	25.0	65.1

TOC: Total organic carbon, OM: soil organic matter, P: phosphorus, N: nitrogen, K: potassium, Ca: calcium, Mg: magnesium, Al: aluminum, H: hydrogen, SB: sum of bases, SMP: Shoemaker-MacLean-Pratt buffer.

The content of each mineral nutrient was determined in the Soil laboratory of the Brazilian Institute of Analysis (IBRA) according to the methods described by IAC (2001) and EMBRAPA (2009).

Creole corn seeds of the variety BRS 4157 Sol-da-morning Nitroflint, provided by the Popular Peasant Movement (MCP) of the municipality of Igarapé-açú were used. The seeds underwent asepsis with 1% sodium hypochlorite for 3 min and then were washed with distilled water and placed on paper towels to dry (Cicero & Silva, 2003).

Subsequently, the corn seeds were inoculated (Fig. 1) with *A. brasilense,* strains AbV5 and AbV6, in a peat-based vehicle, about 2 h before sowing, applying a 10% glucose solution to moisten the seeds and then the inoculant (50 g of inoculant/10 kg of seeds), covering all the seeds. The seeds were left in the shade and in an airy place for drying and adherence of the inoculant, according to the manufacturer's instructions (Ministério da Agricultura, Pecuária e Abastecimento, 2009).

FIGURE 1. Seed inoculation.

All treatments were irrigated daily for 7 d until total evapotranspiration (1.0) was restored for seedling establishment (Ministério da Agricultura, Pecuária e Abastecimento, 2009). From that point on, no irrigation was carried out in the treatments with 0.6, and 0.8 of the evapotranspiration. Only the treatment with 1.0 of the evapotranspiration was irrigated every day. Once all treatments reached their respective percentages, daily irrigation was done according to the treatments.

The application of jasmonic acid (Sigma Chemical Co., USA) was carried out in three treatments – control (without application), 10 µmol L^{-1}, and 20 µmol L^{-1} – via foliar spray with a manual sprayer (Fig. 2B) using 5 ml per plant, applied adaxially and abaxially to all leaves. The solution was diluted in distilled water and Tween 20 (0.05%) to enhance adhesion to the leaves (Lopes *et al*., 2009). The applications took place at 20 and 40 d after sowing.

Biometric variables

When the plants reached the R6 stage, between 90 and 105 d after sowing, which characterizes physiological maturity, the following evaluations were performed: number of tassels (NT): counting all tassels per plant, according to the methodology described by Vital *et al*. (2015); tassel branch number (TBN); number of leaves on the corn cob (NLC): counting all leaves with on the corn cob, according to the methodology described by Vital *et al*. (2015); and number of ears of corn (NE): counting all the ears , according to the methodology described by Martins *et al.* (2016).

Statistical analysis

The experimental data were submitted to the Shapiro-Wilk and Levene tests at 5% of significance to verify the normality and homoscedasticity of the data, respectively. Subsequently, analysis of variance was carried out, in which the developments that proved to be significant were evaluated. The effects of jasmonic acid doses on the presence and absence of *A. brasilense* at different irrigation depths were studied by polynomial regression analysis, observing the results of the F test (P<0.05) from the analysis of variance with the Sisvar statistical software (Ferreira, 2019).

Results and discussion

The number of tassels (NT) did not show adjustment to the linear or quadratic models (Tab. 2), indicating that the plants maintained a pattern in this variable that did not change according to the treatments used. At 58 d after sowing more than 90% of the plants already had tassels, normally following their phenological stage. Modesto *et al*. (2021) observed the phenological stages in corn plants, noting that about 90% of the plants had tassels at 60 d after sowing.

For Singh *et al*. (2021), Creole varieties, as they are genotypes with a broad genetic base, are able to respond better to abiotic and biotic stresses, which may present productive potential to match or exceed the production of hybrid cultivars, thus making them an alternative for sustainable production, reducing production costs with inputs and minimizing the use of technological packages.

Jasmonic acid (JA) is a phytohormone that acts as a plant growth regulator and plays a role in the growth and development of plants under environmental stress. Higher levels of jasmonates accumulate in actively growing tissues, such as hypocotyls, flowers, and pods (Yang *et al*., 2019). JA also regulates such processes of plant development and growth as fruit ripening and maturation and production of viable pollen, seed germination, and development of anthers and pollen grains (Ruan *et al*., 2019). JA can regulate various aspects of plant development, including root growth, stamen development, flowering, and leaf senescence (Wang *et al*., 2020).

It can be seen from Table 3 that there was an adjustment to the quadratic model in tassel branch number (TBN) in the irrigation conditions of 0.6, and 0.8 of the ET0 with the presence of *A. brasilense*, obtaining the maximum technical efficiency (Ymte) at 0.8 of evapotranspiration (ET0), with an increase of 11.91% compared to 0.6 of the ET0. In the absence of *A. brasilense*, an adjustment to the increasing linear model is observed, indicating that the higher the dose of jasmonic acid, the higher the TBN. In the condition of 1.0 of the ET0 and absence of *A. brasilense*, there was an adjustment to the decreasing linear model, showing that the JA doses had a negative effect on these treatments. In the presence of *A. brasilense,* the TBN did not present adjustments to the quadratic and linear model, showing that the results were similar despite the doses of JA. JA can stimulate flowering and raise tolerance to low water conditions (Kerbauy, 2019). According to El Sabagh *et al*. (2018), minimizing the effects of water deficit is one of the benefits of inoculation with *A. brasilense* in forages.

Bacteria of the genus *Azospirillum* are free-living microorganisms capable of proliferating in the roots of plants, promoting benefits such as stimulating root growth. This results in different effects including an increase in the absorption surface of the roots, which leads to an increase in the volume of the exploited soil, an increase in water

TABLE 2. Number of tassels (NT) of creole corn plants subjected to three irrigation depths with *A. brasilense* and jasmonic acid as mitigators.

ET0 (irrigation)	*A. brasilense* (Absence/Presence)	JA (µmol L^{-1})			Equation	R^2	JA
		0	10	20			
0.6	Absence	1.00	1.00	1.00	ns	-	-
	Presence	1.00	1.00	1.00	ns	-	-
0.8	Absence	1.00	1.00	1.00	ns	-	-
	Presence	1.00	1.00	1.00	ns	-	-
1.0	Absence	1.00	1.00	1.00	ns	-	-
	Presence	1.00	1.00	1.00	ns	-	-

ET0 – evapotranspiration. The irrigation depths were determined by three evapotranspiration multiplier coefficients. ns: not significant according to the F test (P<0.05), R^2 – determination coefficient, JA – jasmonic acid.

TABLE 3. Tassel branch number (TBN) of creole corn plants cultivated at three irrigation depths with *A. brasilense* and jasmonic acid as mitigators.

ET0 (irrigation)	*A. brasilense* (Absence/Presence)	JA (µmol L^{-1})			Equation	R^2	JA
		0	10	20			
0.6	Absence	7.00	7.50	7.75	$Y=7.04+0.037X$	0.96	-
	Presence	8.50	9.00	5.75	$Y=8.50+0.24X-0.019X^2$	0.99	6.33
0.8	Absence	8.75	9.75	11.25	$Y=8.67+0.12X$	0.98	-
	Presence	9.25	10.50	9.50	$Y=9.25+0.24X-0.011X^2$	0.99	10.56
1.0	Absence	10.75	9.75	9.25	$Y=10.67-0.075X$	0.96	-
	Presence	11.50	10.50	10.50	ns	-	-

ET0 – evapotranspiration. The irrigation depths were determined by three evapotranspiration multiplier coefficients. ns: not significant according to the F test (P<0.05), R^2 – determination coefficient, JA – jasmonic acid.

absorption and mineral nutrient acquisition, and greater tolerance to stress. These factors contribute to plants with more vigor and productivity (Fukami *et al*., 2018). Inoculation makes it possible, in some cases, to obtain good yields or gains in crop growth (Leite *et al*., 2018; Modesto *et al*., 2021).

Inoculation of corn with diazotrophic bacteria reduces the need for nitrogen fertilization and mitigates environmental contamination risks due to the bacteria's biological nitrogen-fixation capacity. *A. brasilense* and nitrogen fertilization resulted in increased corn biomass, production and yield in an integrated crop-livestock system (Shaefer *et al*., 2019).

From Table 4, it can be seen that NLS did not show adjustment to the linear or quadratic models, indicating that there was no maximum technical efficiency in this variable. Despite the lack of differences in NLS, stigmas can be observed 58 d after sowing, reaching more than 90% of the plants at 63 d after sowing, matching the period of the usual phenological stage of the culture. Sah *et al.* (2020), when evaluating the impact of water deficit stress in maize, observed that the interval from cultivation to flowering of maize plants increased from 80 to 120 d, on average, after the sowing of different maize genotypes in India, which resulted in losses of 30 to 60% in grain yield.

In the variable of number of ears of corn (NE) (Tab. 5), an adjustment to the decreasing linear model was observed under the condition of irrigation of 0.8 of the ET0 and in the presence of A. *brasilense,* indicating that the doses of jasmonic acid promoted an inverse effect for these circumstances. The other treatments showed no adjustment to the linear or quadratic models, suggesting a consistent pattern of NE in each plant subjected to the aforementioned treatments.

According to Modesto *et al.* (2021), jasmonic acid provokes a signal transduction network that leads to a cascade of events responsible for the physiological adaptation to the state of abiotic stress. The inoculant A. *brasilense* mitigated the deleterious effects caused by drought and promoted better growth of the root system, enhancing the tolerance of maize plants to water deficit (Marques *et al*., 2021).

Conclusions

The irrigation depth of 0.8 of the evapotranspiration showed the most favorable interactions between

TABLE 4. Number of leaves on the corn cob (NLC) of creole maize plants submitted to three irrigation depths with *A. brasilense* and jasmonic acid as mitigators.

ET0 (irrigation)	*A. brasilense* (Absence/Presence)	JA (μmol L^{-1})			Equation	R^2	JA
		0	10	20			
0.6	Absence	1.25	1.25	1.75	ns	-	-
	Presence	1.25	1.50	1.25	ns	-	-
0.8	Absence	1.25	1.00	1.00	ns	-	-
	Presence	1.00	1.00	1.00	ns	-	-
1.0	Absence	1.00	1.00	1.00	ns	-	-
	Presence	1.50	1.00	1.00	ns	-	-

ET0 – evapotranspiration. The irrigation depths were determined by three evapotranspiration multiplier coefficients. ns: not significant according to the F test (P<0.05), R^2 – determination coefficient, JA – jasmonic acid.

TABLE 5. Number of ears per plant (NE) of creole corn plants subjected to three irrigation depths with *A. brasilense* and jasmonic acid as mitigators.

ET0 (irrigation)	*A. brasilense* (Absence/Presence)	JA (μmol L^{-1})			Equation	R^2	JA
		0	10	20			
0.6	Absence	0.75	1.00	0.75	ns	-	-
	Presence	0.75	0.75	1.00	ns	-	-
0.8	Absence	1.00	0.75	1.00	ns	-	-
	Presence	1.25	1.00	0.75	Y=1.25-0.025X	0.99	-
1.0	Absence	0.25	0.75	0.75	ns	-	-
	Presence	1.50	0.75	0.75	ns	-	-

ET0 – evapotranspiration. The irrigation depths were determined by three evapotranspiration multiplier coefficients. ns: not significant according to the F test (P<0.05), R^2 – determination coefficient, JA – jasmonic acid.

Azospirillum brasilense and jasmonic acid in reducing water stress in creole corn plants. The results for the irrigation depth of 0.6 of the evapotranspiration, with the use of mitigators, showed smaller reductions in stress (11.91% reduction in relation to 0.8 of the ET0). The treatment with 10 µmol L^{-1} of jasmonic acid was the closest to the estimated dosages that proved to be the most favorable for reducing stress, consequently increasing the productivity of creole corn plants.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

EFLS: conceptualization, methodology, validation, formal analysis, research, and writing – original draft; KBST: designed the experiment, analyzed the data, wrote, and edited the manuscript; GGTNM: resources, writing, and review & editing; ACS: validation, resources, writing, and review & editing; SCSO: validation, writing – review & editing; BFG: conceptualization, resources, writing – review & editing, supervision; PAS: validation, writing – review & editing; JTO: validation, writing – review & editing; GMRP: validation, writing – review & editing; CFON: validation, analysis and interpretation of data, writing the draft of the manuscript. All authors have read and approved the final version of the manuscript.

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Relationship between spectral response and manganese concentrations for assessment of the nutrient status in rose crop

Relación entre la respuesta espectral y las concentraciones de manganeso para evaluar el estado nutricional en el cultivo de rosa

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ABSTRACT

The present research was conducted on a Freedom rose (*Rosa* sp.) variety grown in greenhouses in the municipality of Tocancipá, Cundinamarca department (Colombia), to assess the relationship between reflectance and manganese content in leaves. A randomized complete block design was implemented, including five treatments with different manganese doses (0%, 25%, 50%, 75%, and 100% of the commercial dose, which is 2 mg L^{-1}), each with five replicates. Samplings at five phenological stages were carried out, with 10 plants analyzed per treatment for each sampling, totaling 50 plants per sampling. Spectral responses were taken from the adaxial surface of the leaves using a FieldSpec® 4 spectroradiometer, covering a wavelength range from 350 to 2500 nm. As the concentration of manganese in the leaves decreased, the reflectance values increased, showing an inverse relationship between these two parameters. The increase in reflectance values was particularly pronounced in the spectral regions between 560 nm and 840 nm. Among the vegetation indices evaluated, GNDVI, DATT4, DATT2, and D1 stood out; DATT4 and GNDVI showed the most promising results. DATT4 exhibited correlations greater than 0.6 during the "palmiche" (induction of the floral primordium) and "rice" (flower bud less than 4 mm in diameter) phenological stages, while GNDVI presented correlations of 0.64 in the "chickpea" (peduncle with an average length of 4 cm) phenological stage and 0.52 in the "scratch color" (the color of the petals could be observed) phenological stages.

Key words: crop nutrition, reflectance spectra, spectral indices, spectroradiometer, simple linear regression.

RESUMEN

La presente investigación se realizó en rosa (*Rosa* sp.), variedad Freedom, cultivada bajo invernaderos en el municipio de Tocancipá, departamento de Cundinamarca (Colombia), para evaluar la relación entre la reflectancia y el contenido de manganeso en las hojas. Se implementó un diseño experimental de bloques completos al azar, que incluyó cinco tratamientos con diferentes dosis de manganeso (0%, 25%, 50%, 75% y 100% de la dosis comercial, que es de 2 mg L^{-1}), cada uno con cinco repeticiones. Se realizaron muestreos en cinco etapas fenológicas, analizándose 10 plantas por tratamiento para cada muestreo, totalizando 50 plantas por muestreo. Las respuestas espectrales se tomaron de la superficie adaxial de las hojas utilizando el espectrorradiómetro FieldSpec® 4, cubriendo un rango de longitud de onda de 350 nm a 2500 nm. A medida que disminuía la concentración de manganeso en las hojas, los valores de reflectancia aumentaban, mostrando una relación inversa entre estos dos parámetros. El aumento de los valores de reflectancia se observó particularmente en las regiones espectrales entre 560 nm y 840 nm. Entre los índices de vegetación evaluados destacaron GNDVI, DATT4, DATT2 y D1; DATT4 y GNDVI mostraron los resultados más prometedores. DATT4 exhibió correlaciones superiores a 0,6 durante las etapas fenológicas de "palmiche" (inducción del primordio floral) y "arroz" (botón floral de menos de 4 mm de diámetro), mientras que GNDVI presentó correlaciones de 0,64 en el estado fenológico "garbanzo" (pedúnculo con una longitud media de 4 cm) y de 0,52 en los estados fenológicos "color de rayado" (el color de los pétalos podía observarse).

Palabras clave: nutrición de cultivos, espectro de reflectancia, índices espectrales, espectroradiómetro, regresión lineal simple.

Introduction

The flower industry arrived in Colombia in the 1960s through an alliance between foreign investors and local entrepreneurs. Gradually, it consolidated, becoming one of the main sectors in the agricultural economy and a strong export product in Colombia, competing with major flower producers worldwide. Colombia is now the leading flower exporter in Latin America and the second in the world, surpassed only by the Netherlands (ICA, 2024).

The increase in flower exports, improvement of sector competitiveness, and the demands of international markets, such as consistency in stem quality, size of flower buds, uniformity in the cutting point, and intensity of color, have elevated quality standards. To meet these parameters, an

Received for publication: July 23, 2024. Accepted for publication: August 29, 2024. Doi: 10.15446/agron.colomb.v42n2.110294

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integrated crop management approach is essential. This includes an appropriate technological package which supplies nutrients in quantities required by the crops to obtain high-quality flowers (Ruppenthal & Castro, 2005).

Mineral nutrient supply is indispensable for obtaining commercially valuable flowers. Nutrients are categorized as macronutrients and micronutrients, depending on their concentrations in leaf tissue, with macronutrients present in higher concentrations. Despite their lower concentrations, micronutrients such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), boron (B), chlorine (Cl), and molybdenum (Mo) are equally crucial for the growth and development of crops and for producing flowers that meet the market quality standards (Rashed *et al*., 2019).

One of the nutrients present in low concentrations in rose plants is Manganese (Mn), which is essential for floral development and differentiation. However, it is prone to losses, such as through leaching, making it important to incorporate Mn into the soil or substrate at the right time and correct amounts (González Hurtado *et al*., 2007). If not managed appropriately, deficiency of this nutrient can lead to nutritional problems.

Manganese is crucial for plant growth; its deficiency affects the oxygen-evolving complex of photosystem II (PSII). This leads to detrimental effects on the photosynthetic apparatus, oxidative stress and reduced water-use efficiency (Schmidt *et al*., 2016). This is because the Mn-cluster that splits water molecules is anchored to PSII, and its deficiency reduces this binding, leading to the disintegration of PSII complexes. Consequently, there is a marked reduction in the D1 protein and intrinsic PsbP and PsbQ proteins, affecting the PSII core due to oxidative damage and leading to reduced carbon assimilation and lower plant yield (Schmidt *et al*., 2016).

Distinctive foliar symptoms of manganese deficiency become unmistakable when the growth rate is significantly restricted. These symptoms include diffuse interveinal chlorosis in fully expanded leaves. Severe necrotic spots may also form. Symptoms often first appear in middle leaves, unlike magnesium deficiency symptoms that appear in older leaves. In roses specifically, deficiency of Mn halts cellular growth in the leaf tissues between the veins, leading to cell death overtime and the appearance of brown spots (Humphries *et al*., 2006).

The diagnosis of the nutritional status of the rose crop is of fundamental importance as it influences decision-making regarding the formulation of exogenous fertilizer applications. These applications can be made via foliar spraying, but mainly through fertigation systems. The formulations, interactions, and relationships between soil/substrate, plant, and water, along with the timing of these applications, are factors that determine the quality and productivity indicators of the crop. These indicators are closely related to the sustainability and viability of the crop, since adequate supplementation of micronutrients substantially increases crop productivity. For this reason, it is essential to maintain plant nutrition in optimal balance, which requires the timely diagnosis of the nutritional status of the crop (Hariyadi *et al*., 2019).

In flower crops, as well as in other crops, nutrient analyses are traditionally performed in the laboratory using methods that are destructive and demanding in terms of time and labor, both for sample collection and processing. This leads to delays in the nutrient diagnosis of the crop. Hence, there is an interest in methods such as the use of sensors estimating certain parameters of the plants, such as the nutrient content in leaves. In this regard, the measurement of crop reflectance is a non-destructive, rapid, and integrative alternative for characterizing the nutrient status. Schmidt *et al*. (2016) emphasize that timely and efficient diagnosis and remediation of Mn deficiency in plants is a significant challenge in plant production systems.

In this regard, various studies have established relationships between spectral information and Mn content in plants, such as in cotton seed flour, where near-infrared spectroscopy (NIRS) and chemometrics were employed (Yu *et al*., 2019). These authors carried out the spectral corrections using the standard normal variable along with the first derivative, as well as applying Monte Carlo methods (MCUVE) and the successive projections algorithm to extract informative variables from complete NIR spectra (Yu *et al*., 2019). As a result, optimal models were developed to predict Mn content in cotton seeds, with a root mean square error of prediction of 1.99, a coefficient of determination R^2 of 0.94, and a residual predictive deviation of 4.37 (Yu *et al*., 2019).

Researchers assessed mineral element contents in common beans using spectral reflectance techniques, as these are affected in plants under abiotic stress such as salinity and drought (Boshkovski *et al*., 2020). Multivariate regression identified a relationship between reflectance in specific regions of the spectrum with foliar concentration of phosphorus (P) and the Normalized Difference Vegetation Index (NDVI), indicating significant correlation with foliar concentrations of B, Fe, K, Mn, P, and Zn. Furthermore, these authors developed customized spectral indices that show significantly high correlation with B, Fe, K, Mg (magnesium), Mn, Na (sodium), P, Zn, and N (nitrogen) (Boshkovski *et al*., 2020).

Other researchers studied the nutrient content of oil palm leaves, using spectral reflectance data to determine suitable wavelengths for predicting the levels of the most important leaf nutrients: N, P, K, Ca, Mg, B, Cu, and Zn (Santoso *et al*., 2019). These authors have built prediction models through stepwise regression followed by principal component regression. The resulting models showed strong positive correlations with foliar contents of N ($R^2 = 0.53$) and Ca ($R^2 = 0.50$). The contents of P, K, Mg, B, Cu, and Zn exhibited moderately positive correlations, with R^2 values ranging from 0.33 to 0.49 (Santoso *et al*., 2019).

Based on the above, this research was conducted to evaluate the relationship between spectral reflectance and foliar manganese content compared to chemical analysis of foliar tissue to diagnose the nutrient status of the rose crop.

Materials and methods

Experimental design

The research was conducted on a Freedom rose (*Rosa* sp.) variety grown under greenhouse conditions in the municipality of Tocancipá, Cundinamarca department (Colombia) at an altitude of 2605 m a.s.l. with coordinates 4°58'40.1" N, 73°59'06.6" W. The experimental field covered an approximate area of 176 m^2. In a randomized complete block design (RCBD), five doses of manganese applied through fertigation were evaluated in plants grown in a coconut fiber substrate. Five treatments with five replicates were carried out, consisting of Mn doses relative to the recommended dose of 2 mg L^{-1} with the product MF ACTIVA Mn 12% EDTA, as shown in Table 1, ensuring pH of the fertilizer solution between 5.3 and 5.8 and an electrical conductivity between 1.5 and 1.8 dS m^{-1}. These nutrients were supplied through drip fertigation based on the amount of water used by the crop, which was 120 L per standard 32 m bench, corresponding to 4 pulses of 17 L per plot per day, i.e., 4.25 L per plot per pulse, distributed throughout the day during all days of the experiment.

Each of the 25 plots had an area of 1.35 m^2 (0.3 m x 4.5 m) and contained a total of 60 rose plants. Five plots were arranged per each of the five hydroponic benches, avoiding the first and last plots to minimize edge effects. Each bench received the five treatments through a drip irrigation system.

TABLE 1. Nutrient composition of solutions (mg L^{-1}) used in the experiment.

	T1 (Mn 0%)	T2 (Mn 25%)	T3 (Mn 50%)	T4 (Mn 75%)	T5 (Mn 100%)
N	160	160	160	160	160
P	10	10	10	10	10
K	180	180	180	180	180
Ca	100	100	100	100	100
Mg	40	40	40	40	40
S	14	14	14	14	14
Mn	0	0.5	1	1.5	2
Zn	0.7	0.7	0.7	0.7	0.7
Cu	1.2	1.2	1.2	1.2	1.2
Fe	2	2	2	2	2
B	0.2	0.2	0.2	0.2	0.2
Mo	0.09	0.09	0.09	0.09	0.09

T – treatment.

Irrigation system

To apply each treatment to its corresponding plot, each nutrient solution was prepared in a separate tank connected to a half-horsepower centrifugal pump (0.5 hp), resulting in five pumps. Each pump was connected to a system of pipes and drippers that worked independently and distributed the nutrient solutions differentially to each plot.

The irrigation was remotely activated through the implementation of smart power outlets connected to a Wi-Fi network via a router located at the top of the greenhouse. The irrigation system was controlled from a mobile device with an Android operating system, using a downloaded app that allowed for the programming of the irrigation schedule.

Leaf chemical analysis

The chemical analysis consisted of determining the contents of Mn in rose leaves. The analysis was conducted using the AGRILAB laboratory, and the Mn content was analyzed using Microwave-Assisted Extraction methodology with the use of HNO_3 and H_2O_2 and read through Inductively Coupled Plasma Optical Emission Spectroscopy (Ghosh *et al*., 2013).

Measurement of spectral responses in the laboratory

To measure spectral responses, the FieldSpec® 4 spectroradiometer (Malvern Panalytical Ltd, UK (ASD, 2012)) was utilized in the Geomatics Laboratory, Faculty of Agricultural Sciences of the Universidad Nacional de Colombia (Bogotá campus). The reflectance was measured using the leaf clip of the spectroradiometer, specifically in the fourth and fifth fully developed leaves (with five or more leaflets), using the floral bud as a reference and counting the leaf number from the top down towards the base of the stem. The spectroradiometer recorded readings between 350 and 2500 nm with spectral resolutions of 1.4 nm from 350 to 1050 nm and 2 nm from 1050 to 2500 nm. This device operates by measuring light at different wavelengths, converting photons into electrons, and recording a signal over a defined integration time, typically 17 ms, which can increase to 34 ms under low-light conditions (ASD, 2012). The spectral reflectance of each leaf was measured using the leaf clip with the plant probe, which utilizes an internal broad-spectrum halogen light source. Prior to reflectance measurement, the spectroradiometer was standardized using a white reference standard (Spectralon, Labsphere Inc., Sutton, NH, USA).

The leaves used for measuring spectral responses were obtained in different phenological stages of plant development. The following sequential phenological stages were assessed, which were named according to Valencia *et al.* (2018): "palmiche" (induction of the floral primordium) followed by "rice" (flower bud less than 4 mm in diameter), "chickpea" (peduncle with an average length of 4 cm and approximately thirteen pairs of leaves), "scratch color" (the color of the petals could be observed due to the advance in flower development), and "straight sepals". In each phenological stage, a total of 50 leaflets were evaluated per treatment. The leaves were counted from the top downwards (from the youngest leaves to the mature ones), and a destructive sampling method was employed by removing the 4th and 5th leaves.

We ensured that the leaves were in good condition (without pest and disease damage), free from visual traces of agrochemicals, and kept hydrated during transportation to the laboratory. Once the leaves were collected in the field, they were stored in a styrofoam cooler with a refrigerant gel and transported within 5 h after recollection to the Geomatics Laboratory of the Universidad Nacional de Colombia for spectral response measurements. Each leaf underwent five readings of spectral responses corresponding to the five individual leaflets.

Data analysis

The evaluated spectral indices are presented in Table 2, where "R" represents Reflectance and "D" represents the First Derivative of reflectance. An analysis using a box plot was conducted to identify outliers. Shapiro-Wilk test was employed to assess the normality of the data, while the Bartlett test was used to evaluate the equality of variance across different populations. If the statistical

TABLE 2. Vegetation spectral indices evaluated in rose plants. Adapted from Lehnert *et al*. (2016).

Index	Formula
CARTER4	$R710 / R760$
DATT	$(R850 - R710) / (R850 - R680)$
DATT2	$R850 / R710$
DATT3	$D754 / D704$
DATT4	$R672 / (R550 * R708)$
D1	$D730 / D706$
D2	$D705 / D722$
MI	$(R780 - R710) / R7(80 - R680)$
MCARI	$((R700 - R670) - 2 * (R780 - R710)) / (R780 - R680))$
MCARI/OSAVI	$MCARI / ((1 + 0.16) * (R780 - R710) / (R800 - R670 + 0.16))$
mND705	$(R750 - R705) / (R750 + R705 - 2 * R445)$
MTCI	$(R754 - R709) / (R709 - R681)$
REP _ Li	$700 + 40 * (RE - R700 / R740 - R700)$ where $RE = (R670 + R780) / 2$
VOG	$R740 / R720$
VOG2	$(R734 - R747) / (R715 + R726)$
VOG3	$D715 / D705$
VOG4	$(R734 - R747) / (R715 + R720)$
NDVI	*(NIR - Red) / (NIR + Red)*
NDRE	*(NIR – Red edge) / (NIR + Red edge)*
GNDVI	*(R840-R560) / (R840 + R560)*

MCARI: Modified chlorophyll absorption ratio index; NIR: Near-infrared; Red: Red band; Red edge: the region of the spectrum between red and near-infrared.

assumptions were met, an analysis of variance (ANOVA) was performed. If these assumptions were not met, the Kruskal-Wallis test was conducted to determine potential differences between treatments. Finally, the Tukey test was applied to the data with a significance level of alpha=0.05.

Reflectance data was smoothed using the Savitzky-Golay filter (Savitzky & Golay, 1964) with a second-order polynomial and a seven-band window. Subsequently, the first derivative was calculated. Other transformations of reflectance data were explored, such as Range Normalization (RN), where each row is divided by its range (*i.e.*, the difference between the maximum and minimum values), ensuring that the curve segment is normalized to 1 (Camo, 2006). Finally, Pearson's linear correlation coefficients were calculated, and regression analyses were conducted.

Results and discussion

The Mn concentration in the leaves throughout the different phenological states increased due to the applications made through fertigation (Fig. 1). In the "palmiche" stage, all treatments started with similar concentrations of Mn in leaves, with values ranging from 142 to 149 mg kg^{-1}, except for T1, which presented the lowest value (104 mg kg^{-1}). From the "rice" stage and throughout all subsequent stages, differences in Mn concentrations were observed due to the treatments. These results suggest that rose plants responded to fertilization, as treatments affected Mn concentrations in leaves.

In the "palmiche" stage, there were differences between the control treatment (without Mn) and the other treatments, forming two groups, one ("group a") included treatments T2, T3, T4, and T5, while the other ("group b") corresponded to T1 (without Mn). There were no significant differences between the treatments in "group a" while T1 showed significant differences compared to treatments in "group a".

In the "rice" stage, the treatments formed three groups: "group a" (T4 and T5), "group b" (T3), and "group c" (T1 and T2). This suggests that treatments had a clearer impact on Mn concentrations in the leaves at this stage, with low Mn doses related to lower foliar contents of Mn and high Mn doses related to higher concentrations of Mn.

From the " chickpea" stage onwards (including "scratch color" and "straight sepals"), four response groups were observed, indicating that Mn doses affected foliar Mn concentration, as shown in Figure 1.

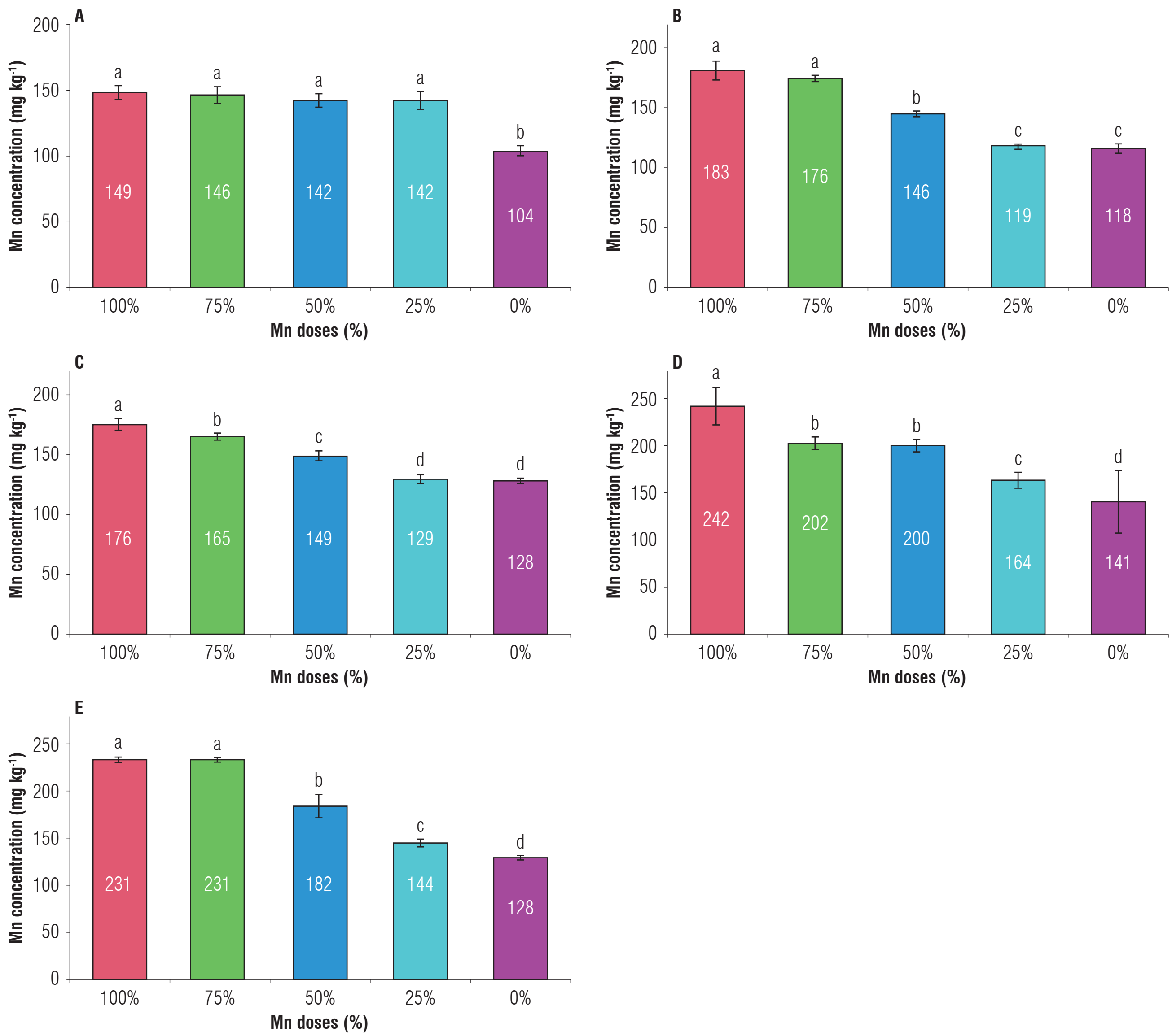

FIGURE 1. Multiple comparisons of the sample mean for the Mn concentration in rose leaves according to different Mn doses (T1: 0% Mn, T2: 25% Mn, T3: 50% Mn, T4: 75% Mn, T5: 100% Mn of the commercial dose, which is 2 mg L^{-1}) and phenological stages: A) palmiche - induction of the floral primordium, B) rice - flower bud less than 4 mm in diameter, C) chickpea - peduncle with an average length of 4 cm, D) scratch color - the color of the petals could be observed, and E) straight sepals. Averages with different letters indicate significant differences according to the Tukey test (alpha = 0.05). Values are expressed as mean ± SE.

The ANOVA indicated highly significant differences in foliar Mn content due to the treatments. The Tukey test showed that as the plants grew, the Mn content in leaves accumulated differentially based on the applied doses. This accumulation became more noticeable starting from the "chickpea" stage, which presented four statistically significant response groups.

Effects of treatments on spectral responses

Figure 2 illustrates the reflectances of the different treatments across the five phenological stages, highlighting the areas of the spectrum with the greatest differences (500-630 nm, 1500-1750 nm, 2000-2400 nm). In Figure 2A, corresponding to the phenological stage of "palmiche," T2 shows the highest reflectance, followed by T3, T1, T5, and

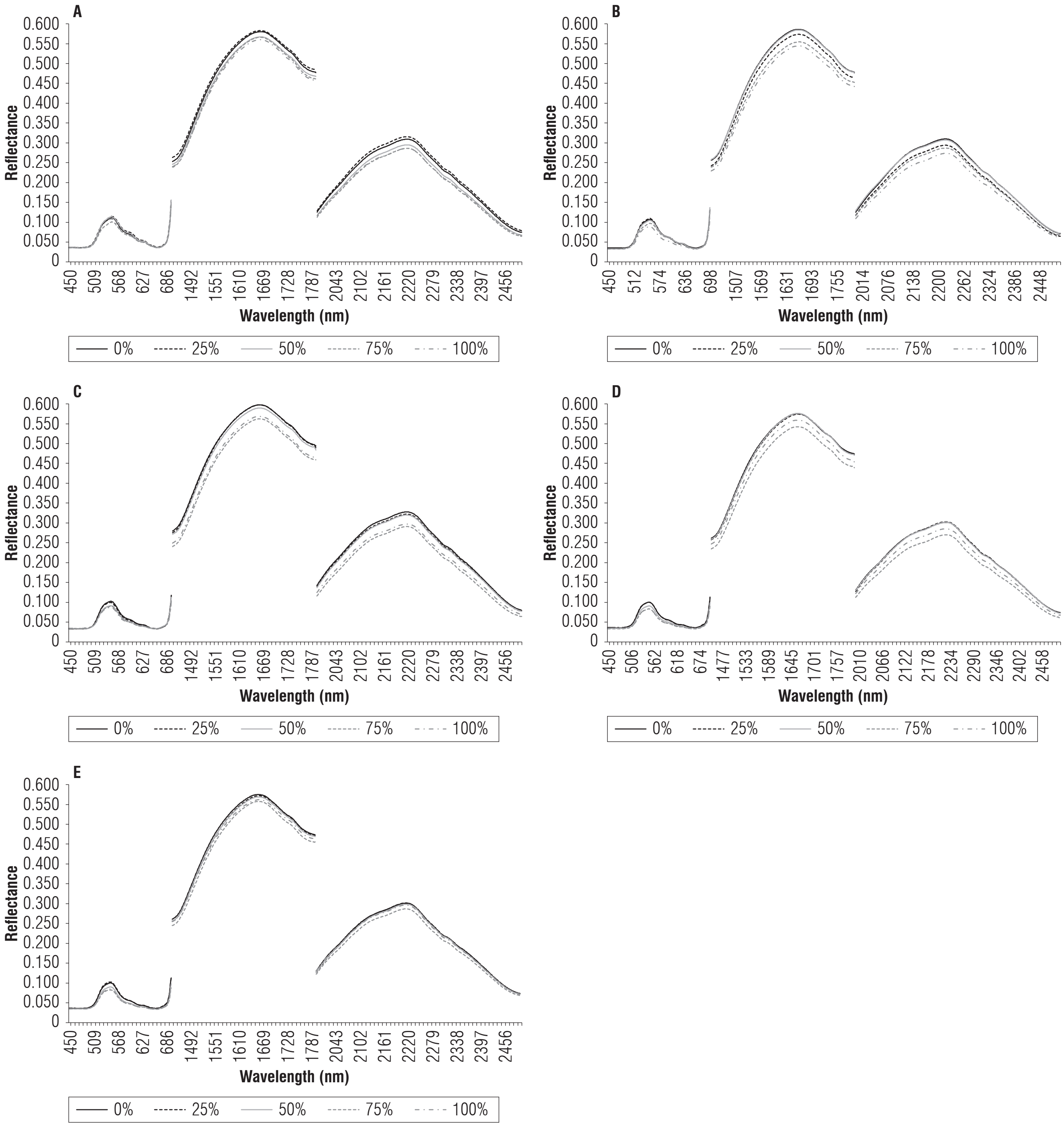

FIGURE 2. Relative reflectance in rose leaves with Savitzky-Golay preprocessing combined with range normalization. A) palmiche - induction of the floral primordium, B) rice - flower bud less than 4 mm in diameter, C) chickpea - peduncle with an average length of 4 cm, D) scratch color - the color of the petals could be observed, and E) straight sepals.

T4, respectively. This pattern suggests an inverse relationship between reflectance and foliar Mn content, with lower Mn content associated with higher reflectance. A similar pattern is observed in Figure 2B for the "rice" stage, where T2 presents the highest reflectance, followed by T1, while T3, T4, and T5 show decreasing values.

The inverse relationship between reflectance and Mn content in leaves becomes more pronounced in the "chickpea" stage (Fig. 2C), where the reflectance values clearly decrease as the Mn content increases. This pattern persists in the subsequent phenological states, "scratch color" and "straight sepals" (Figs. 2D and 2E). In summary, lower concentrations of Mn in the leaves were consistently related to higher reflectance.

Figure 3 presents the first derivative of reflectance in the spectral regions most sensitive to the varying

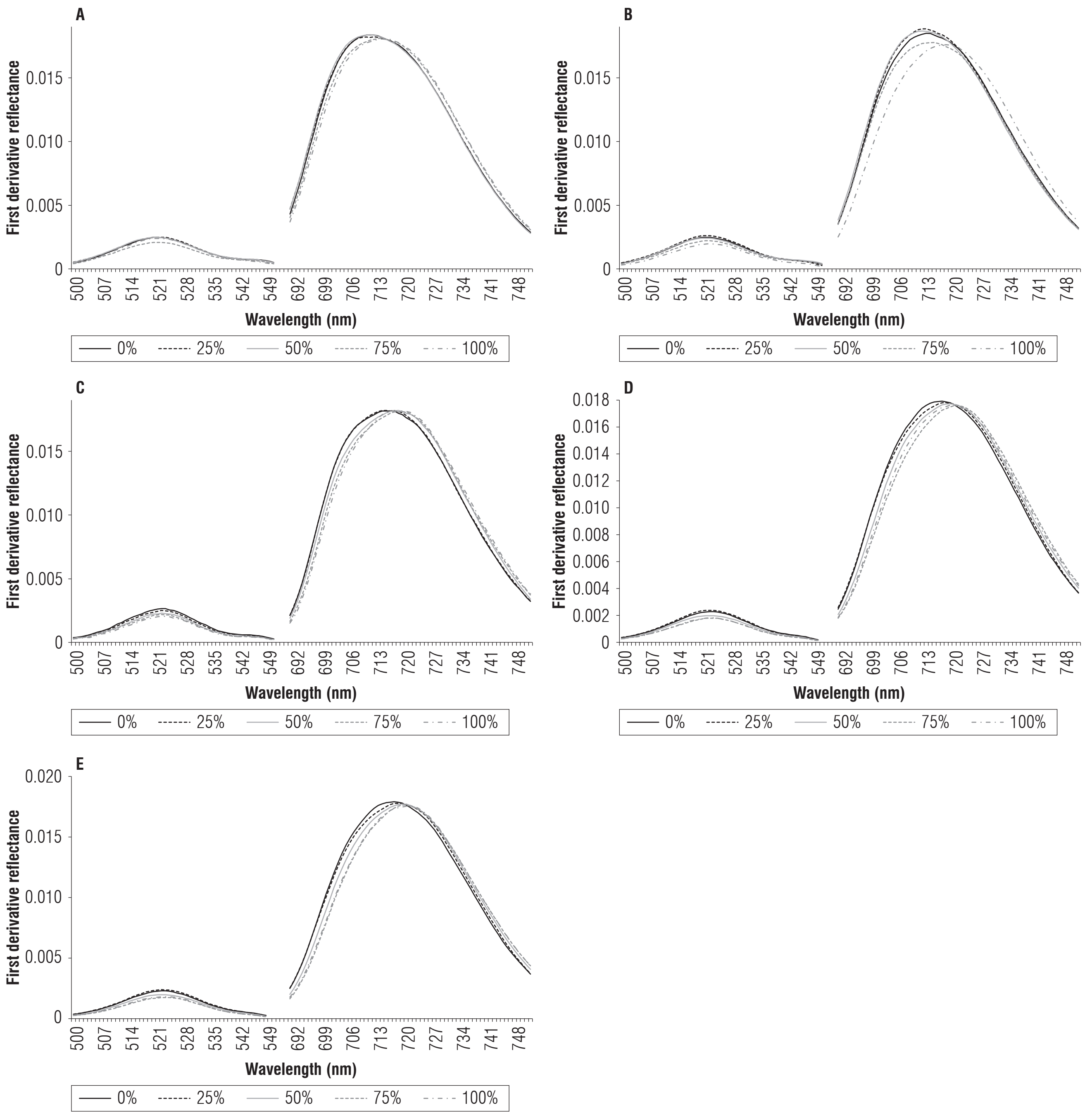

FIGURE 3. Median of the first derivative (n=500, 50 plants x 10 leaflets). A) palmiche - induction of the floral primordium, B) rice - flower bud less than 4 mm in diameter, C) chickpea - peduncle with an average length of 4 cm, D) scratch color - the color of the petals could be observed, and E) straight sepals.

concentrations of Mn in the leaves across the sampled phenological stages. The areas between 500-550 nm and 680-750 nm are particularly highlighted. In the 500 nm to 550 nm region, an inverse relationship is observed between the values of the first derivative of reflectance and the Mn content in the leaves. Conversely, in the 680 nm to 750 nm region, the effect of Mn concentrations is manifested as a rightward shift of the curves, with the treatments with the lowest Mn doses reaching their peak first. As the foliar Mn concentrations increase, the curves shift progressively to the right.

The phenological stage that most clearly demonstrates these results in the 500 nm to 550 nm region is "chickpea" (Fig. 3C). In this case, the highest value of the first derivative corresponds to treatment T1, which had the lowest Mn content in the leaves, followed by T2, T3, and T4. In contrast, T5, which received the highest Mn concentration, showed the lowest value of the first derivative. This clearly illustrates the inverse relationship between the foliar Mn content and the values of the first derivative of reflectance, emphasizing how a lower Mn content is associated with higher reflectance in this phenological stage.

In the 680 nm to 750 nm region, the most relevant phenological stage was "scratch color" (Fig. 3D), due to the behavior of the curves based on foliar Mn concentration. In this area of the spectrum, the values of the first derivative are similar for all treatments; however, a rightward shift is observed, starting with T1 at 715 nm and ending with T4 at 720 nm. This suggests that, in this sampling, the treatments do not significantly affect the values of the first derivative. Instead, as the Mn content increases, the maximum values shift to the right within the spectrum.

Differences in Mn concentration in the leaves had an impact on reflectance. Higher doses of Mn were associated with lower reflectance, whereas treatments with lower doses showed higher reflectance values. These differences were observed from the "palmiche" phenological stage and became more evident progressing from the "chickpea" to "straight sepals" phenological stages. Although there are no specific studies on Mn in this context, these results can be compared with research on other elements, such as nitrogen (N). For instance, Schepers *et al*. (1996) found a strong inverse relationship between the N concentration in corn leaves and reflectance values at 550 nm, attributing this to the N present in chlorophyll (Peng *et al*., 2017). Chlorophyll, as an absorbing pigment, is associated with reflectance reduction, as leaves with higher chlorophyll levels absorb more light in the visible region (Mulyadi *et al*., 2017). These findings can be linked to those obtained in this study, as Mn, as mentioned earlier, is essential in the water oxidation system in photosystem II and plays an important role in chlorophyll synthesis (Rashed *et al*., 2019).

Vegetation spectral indices

The results of the ANOVA for vegetation spectral indices indicate that in the "palmiche" phenological stage, 17 out of the 20 analyzed indices showed highly significant differences (P<0.01). In the "rice" phenological stage, 18 indices exhibited highly significant differences, while in "chickpea," 17 indices showed highly significant differences, and one index had significant differences. For the "scratch color" phenological stage, 16 indices displayed highly significant differences and 2 had significant differences. In the "straight sepals" phenological stage, 17 indices had highly significant differences, and 1 had significant differences. These results, summarized in Table 3, indicate that the treatments had a significant effect on the vegetation spectral indices, suggesting that these indices may estimate the Mn nutritional status in rose cultivation.

Table 4 displays the results of the Tukey test for vegetation spectral indices. In the "palmiche" phenological stage, the NDRE, DATT3, and DATT4 indices showed statistically significant differences in foliar Mn content, forming three groups. For the "rice" phenological stage, 18 indices exhibited highly significant differences, while MCARI and MCARI.OSAVI were not significantly different. The NDRE, DATT4, and D1 indices stood out for forming three significant groups. In the "chickpea" phenological stage, the DATT4 index showed the most significant differences, forming four groups, positioning it as a potential index for estimating Mn in leaves. Other indices such as GNDVI, CARTER4, DATT, D1, D2, MI, mND705, MTCI, VOG2, VOG3, and VOG4 also formed three groups. In the "scratch color" phenological stage, the D1, D2, VOG, VOG2, VOG3, and VOG4 indices stood out, forming four significant groups, making them potential indices for estimating Mn concentration in leaves in this stage. Other indices, including GNDVI, CARTER4, DATT, DATT2, DAFF4, MI, MCARI, MCARI.OSAVI, mND705, MTCI, and REP_Li, formed three groups. Finally, in the "straight sepals" stage, the D1 and D2 indices formed four significant groups, while NDVI, GNDVI, CARTER4, DATT, DATT2, DATT3, DATT4, MI, mND705, MTCI, REP_Li, VOG, VOG2, VOG3, and VOG4 formed three groups. NDRE and MCARI showed no significant differences and MCARI.OSAVI formed two groups. As the plants progressed through the phenological stages, statistical differences increased among the spectral indices.

TABLE 3. Summary of analysis of variance for spectral indices.

Variable	GL	Palmiche	Rice	Chickpea	Scratch color	Straight sepals
NDVI	4	0.05806 *	0.000651 ***	0.189	5.29e-06 ***	0.000281***
NDRE	4	2.48e-05 ***	3.33e-13 ***	4.76e-06 ***	0.0129 *	0.518
GNDVI	4	0.000673 ***	1.23e-08 ***	6.58e-11 ***	1.8e-13 ***	1.66e-13 ***
CARTER4	4	2.3e-06 ***	5.89e-11 ***	2.09e-08 ***	6.03e-13 ***	<2e-16 ***
DATT	4	1.18e-06 ***	7.78e-12 ***	3.72e-08 ***	1.08e-12 ***	<2e-16 ***
DATT 2	4	5.76e-07 ***	5.76e-07 ***	1.15e-08 ***	5.77e-14 ***	<2e-16 ***
DATT 3	4	<2e-16 ***	4.28e-14 ***	1.7e-08 ***	2.92e-05 ***	4.28e-14 ***
DATT 4	4	<2e-16 ***	1.32e-05 ***	4.39e-10 ***	8.01e-14 ***	1.22e-14 ***
D1	4	6.58e-07 ***	2.77e-14 ***	3.43e-08 ***	0.302	2.7e-12 ***
D2	4	4.37e-06 ***	4.37e-06 ***	6.11e-08 ***	0.2503	1.93e-13 ***
MI	4	1.35e-06 ***	2.16e-11 ***	2.45e-12 ***	2.45e-12 ***	2.86e-16 ***
MCARI	4	0.137466	0.053	0.0536	0.000163 ***	0.0378 *
MCARI.OSAVI	4	0.186827	0.186827	0.01267 *	1.09e-05 ***	0.00181 **
mND705	4	1.6e-06 ***	9.35e-11 ***	2.1e-08 ***	1.28e-12 ***	<2e-16 ***
MTCI	4	3.09e-07 ***	8.41e-09 ***	8.41e-09 ***	3e-13 ***	3.08e-16 ***
REP _ Li	4	4.08e-06 ***	1.57e-10 ***	6.7e-08 ***	7.43e-13 ***	<2e-16 ***
VOG1	4	1.08e-06 ***	3.82e-13 ***	2.75e-08 ***	7.24e-13 ***	3.65e-14 ***
VOG2	4	3.21e-07 ***	1.59e-13 ***	1.87e-08 ***	2.16e-13 ***	3.46e-15 ***
VOG3	4	4.55e-07 ***	6.63e-13 ***	9.87e-09 ***	0.0341 *	7.24e-16 ***
VOG4	4	3.21e-07 ***	1.59e-13 ***	3.21e-07 ***	2.16e-13 ***	3.46e-15 ***

*Significant difference at the 0.05 level, ***highly significant difference at the 0.01 level according to ANOVA.

TABLE 4. Multiple comparisons of the sample mean for vegetation indices in rose crop.

INDEX	Palmiche					Rice					Chickpea					Scratch color					Straight sepals				
	Treatments (% Mn)																								
	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
NDVI	ab	b	a	ab	ab	b	a	ab	b	b	a	a	a	a	a	b	b	a	a	a	ab	bc	c	ab	a
NDRE	ab	abc	a	bc	c	ab	a	a	b	c	a	a	ab	b	b	a	ab	ab	b	ab	a	a	a	a	a
GNDVI	ab	b	b	a	ab	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	ab
CARTER4	a	a	a	b	b	a	a	a	a	b	a	ab	bc	c	c	a	a	b	c	bc	a	a	b	c	c
DATT	b	b	b	a	a	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
DATT2	b	b	b	a	a	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
DATT3	c	c	b	a	a	b	b	b	b	a	b	b	b	a	a	a	a	a	a	a	c	c	b	a	a
DATT4	c	bc	b	a	a	bc	c	c	b	a	d	cd	bc	ab	a	c	bc	b	a	a	c	bc	b	a	a
D1	b	b	b	a	a	bc	c	c	b	a	c	bc	ab	a	a	d	cd	bc	a	ab	d	cd	bc	ab	a
D2	a	a	a	b	b	a	a	a	a	b	a	ab	bc	c	c	a	ab	bc	d	cd	a	ab	bc	cd	d
MI	a	a	a	b	b	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
MCARI	a	a	a	a	a	ab	a	ab	b	ab	a	ab	ab	ab	b	a	ab	ab	bc	c	a	a	a	a	a
MCARI.OSAVI	a	a	a	a	a	ab	a	ab	b	b	a	ab	ab	ab	b	a	a	ab	bc	c	a	ab	ab	b	b
mND705	a	a	a	a	a	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
MTCI	a	a	a	a	a	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
REP _ Li	a	a	a	a	a	b	b	b	b	a	b	b	ab	a	a	c	c	b	a	ab	c	c	b	a	a
VOG	a	a	a	a	a	b	b	b	b	a	b	b	ab	a	a	d	cd	bc	a	ab	c	bc	b	a	a
VOG2	a	a	a	a	a	a	a	a	a	b	a	ab	bc	c	c	a	ab	bc	d	cd	a	a	b	c	c
VOG3	a	a	a	a	a	b	b	b	b	a	c	bc	ab	a	a	d	cd	bc	a	ab	c	c	b	a	a
VOG4	a	a	a	a	a	a	a	a	a	b	a	ab	bc	c	c	a	ab	bc	d	cd	a	a	b	c	c

Different lower-case letters (a-d) represent significant differences between treatments according to the Tukey test ($P \leq 0.05$).

Simple linear regression for Mn concentrations in leaves

In the different phenological stages, linear regression analyses between the Mn concentration and the spectral indices showed statistically significant differences. During the "palmiche" phenological stage, the DATT4 index exhibited the most significant correlation, with a value of 0.6, indicating a strong relationship. Therefore, this index has a high potential for estimating Mn content in leaves during this phase.

In the "rice" phenological stage, significant correlations were also observed between Mn concentration and several spectral indices. As shown in Figure 4A, the DATT4 index exhibited the highest correlation, with a value of 0.6, followed by the D1 index in Figure 4B, with a correlation of 0.541; both were classified as strong correlations. Additionally, in Figure 4C, the NDRE index exhibited a strong negative correlation.

These results suggest that the DATT4, D1, and NDRE indices have the potential to estimate the Mn nutritional status in the rose crop during the "rice" phenological stage.

Figure 5 shows the linear regressions of the three indices with the highest correlations for estimating the Mn nutritional status in the "chickpea" phenological stage. In Figure 5A, the GNDVI index exhibited the highest correlation, with a coefficient of 0.646. In Figure 5B, the DATT4 index presented a correlation coefficient of 0.63, while in Figure 5C, the mND705 index recorded a coefficient of 0.571. These results suggest that these three indices have significant potential for estimating the Mn nutritional status in the rose crop during the "chickpea" phenological stage.

Figure 6 presents the linear regressions of the three indices that achieved the highest correlation coefficients in the "scratch color" phenological stage. In Figure 6A, the GNDVI index had the highest correlation, reaching 0.52. In Figure 6B, the DATT index showed a correlation of 0.481, while in Figure 6C, the DATT2 index exhibited a correlation of 0.488. These findings suggest that the GNDVI and DATT indices have the potential for estimating the Mn nutritional status in the rose crop during the "scratch color" phenological stage, while the DATT2 index showed a moderate correlation.

Figure 7 presents the linear regressions of the three indices with the highest correlations for estimating Mn content in the "straight sepals" phenological stage. In Figure 7A, the

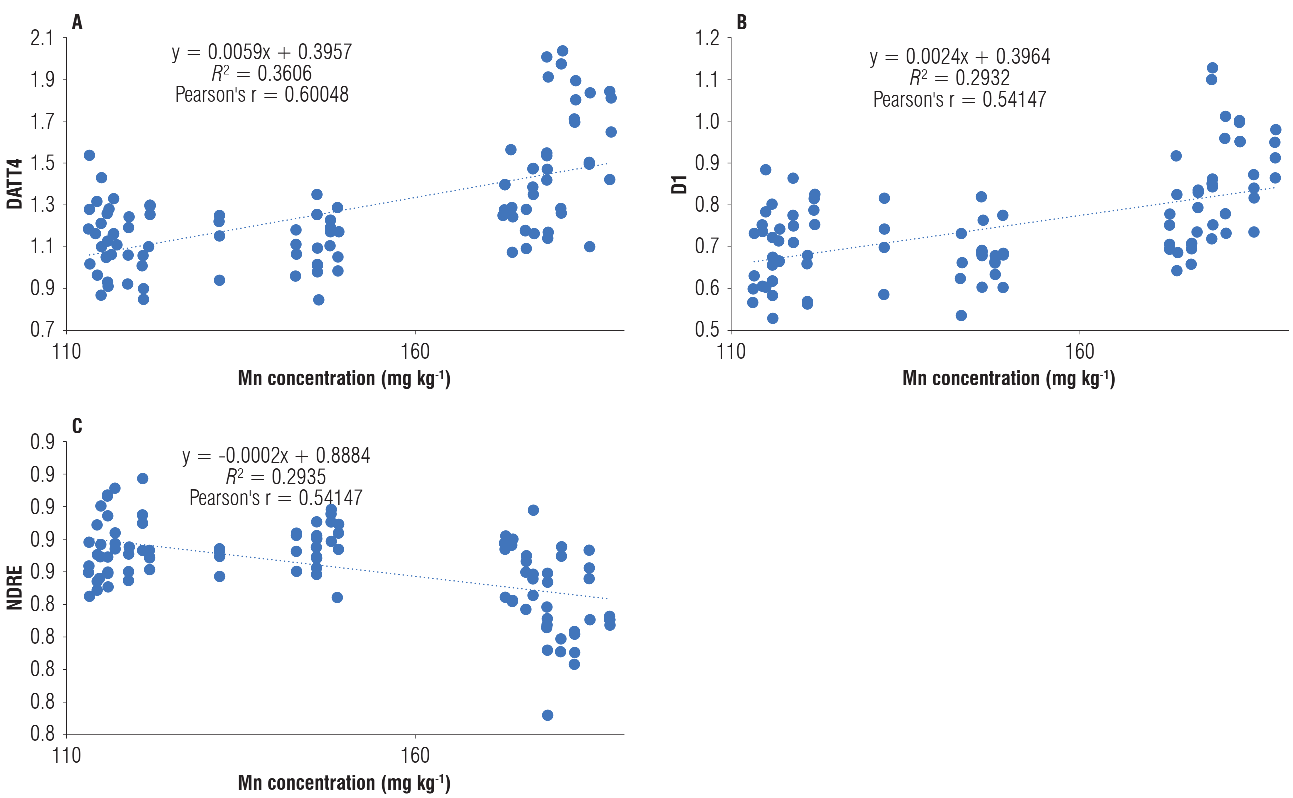

FIGURE 4. Linear regressions between the Mn concentration in rose leaves and spectral indices. A) DATT4 index, B) D1 index, C) NDRE index.

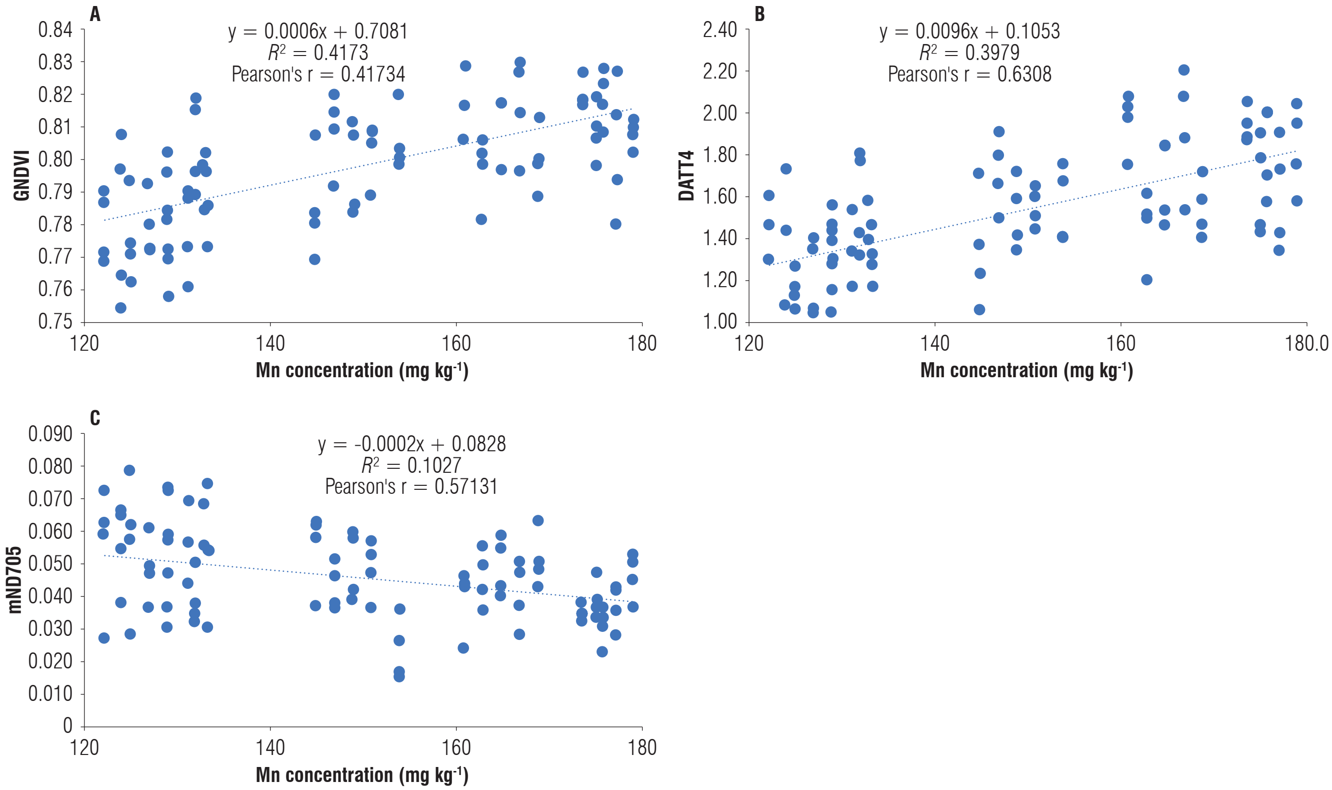

FIGURE 5. Linear regressions between the Mn concentration in rose leaves and spectral indices. A) GNDVI index, B) DATT4 index, C) mND705 index.

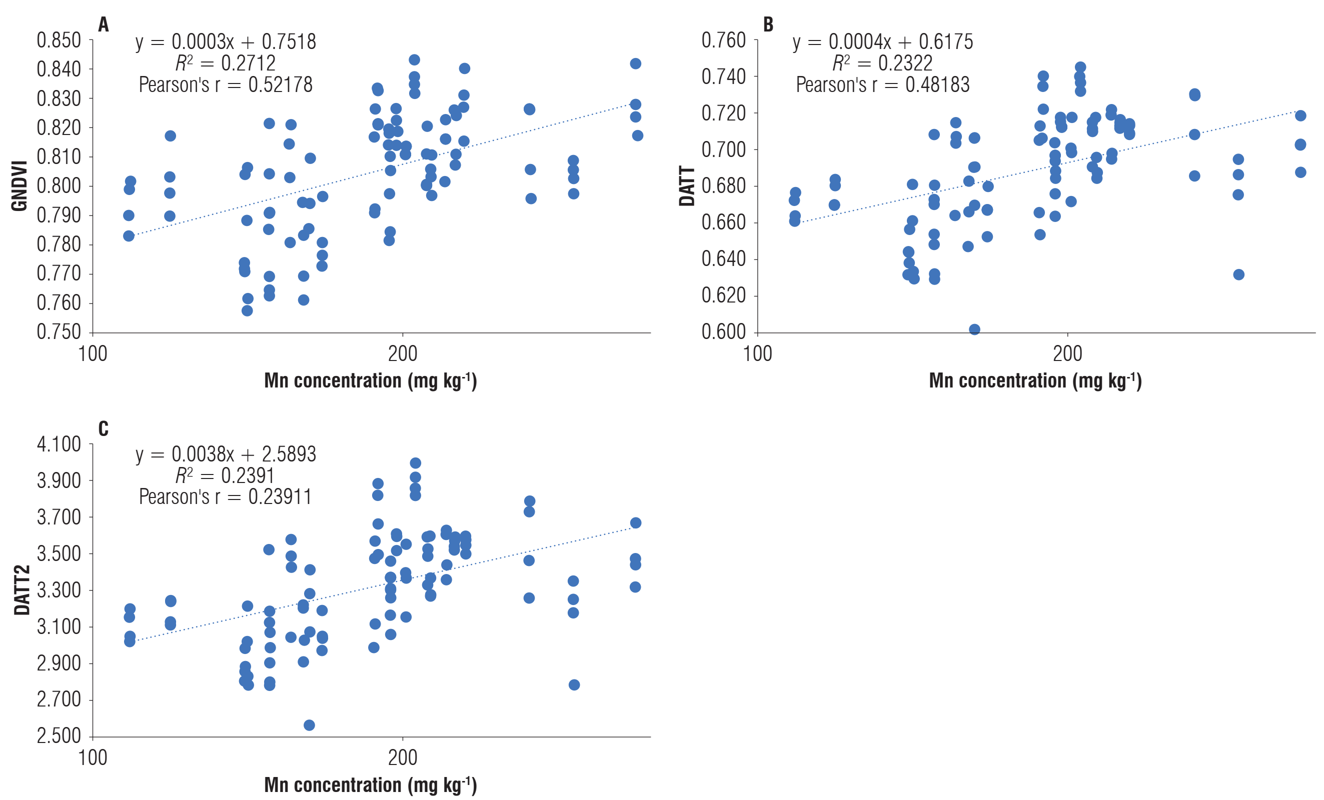

FIGURE 6. Linear regressions between the Mn concentration in rose leaves and spectral indices. A) GNDVI index, B) DATT index, C) DATT2 index.

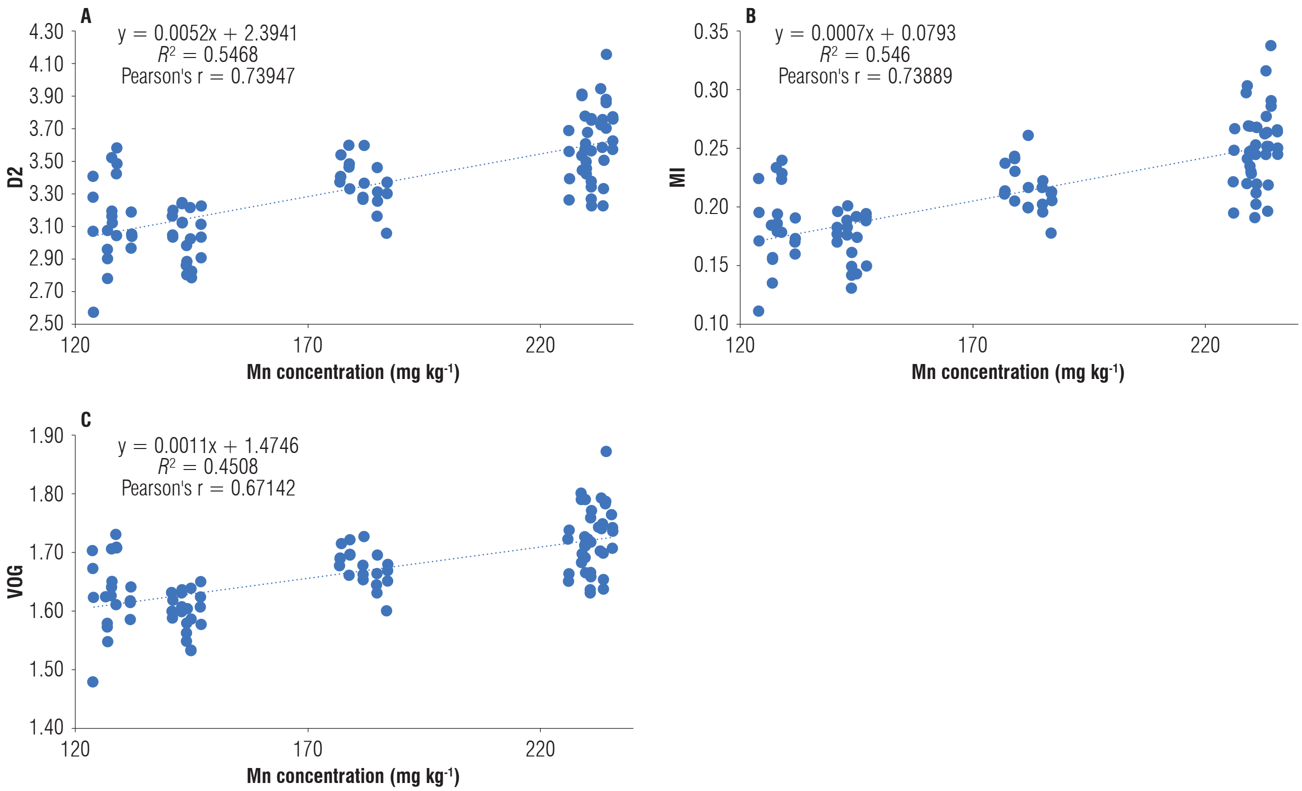

FIGURE 7. Linear regressions between the Mn concentration in rose leaves and spectral indices. A) D2 index, B) MI index, C) VOG index.

D2 index had the highest correlation, reaching 0.739. In Figure 7B, the MI index presented a correlation of 0.738. In Figure 7C, the VOG index showed a correlation of 0.671. These results suggest that all three indices have a high potential for estimating Mn content in leaf tissue during the "straight sepals" phenological stage.

Based on the results presented, it can be inferred that the effect of different Mn doses on rose crop is reflected in the spectral indices, enabling the identification of potential indices for estimating the Mn concentration in leaves using reflectance measurements obtained with the FieldSpec® 4 spectroradiometer. These indices vary depending on the phenological stage of the plants.

For the "palmiche" phenological stage, the DATT4 index stood out, based on the first derivative of reflectance at 706 nm and 730 nm, showing a correlation of 0.6. In the phenological stages of "rice," "chickpea," and "scratch color," the GNDVI index, calculated from reflectance at 560 nm and 840 nm, exhibited correlations of 0.646, 0.664, and 0.664, respectively. In the "straight sepals" phenological stage, the VOG index, calculated using reflectance values at 720 nm and 740 nm, showed a notable correlation of 0.74.

All of these correlations were highly significant, suggesting that these indices have promising potential for estimating Mn concentrations in rose crops, depending on the phenological stage of the plants. The GNDVI index demonstrated outstanding performance, showing strong correlations in the evaluated stages, except for the "palmiche" phase. This index, calculated from reflectance at 560 nm and 840 nm, underscores the importance of this spectral range for estimating Mn concentration in leaves.

Similar findings were reported by Marin *et al.* (2021) in studies on nitrogen estimation in coffee crops, where the GNDVI index allowed the determination of crop areas with nutrient deficiencies (Marin *et al.*, 2021). Additionally, in the case of tomato crop, Padilla *et al.* (2015) found that the GNDVI index proved to be an effective indicator of nitrogen status in plants (Padilla *et al.*, 2015).

The GNDVI (Green Normalized Difference Vegetation Index) showed remarkable performance in advanced phenological stages of rose development, where the pigmentation of leaves is predominantly green. The GNDVI considers reflectance at wavelengths of 560 nm and 840 nm, making it particularly sensitive to the presence of chlorophyll, which has absorption peaks in the red and blue regions of the spectrum (Gamon & Surfus, 1999). In these stages, light absorption by chlorophyll is sufficient to establish significant correlations with the Mn concentration in the leaves. In this study, the GNDVI presented high correlations in the phenological stages of "chickpea" and "scratch color", with correlations of 0.64 and 0.52, respectively.

However, in the "palmiche" and "rice" stages, where the leaves exhibit reddish pigmentation characteristics due to the high presence of anthocyanins and are considered more immature states, the GNDVI showed reduced performance. The presence of anthocyanins may interfere with light absorption at relevant wavelengths, hindering the precise estimation of Mn concentration (Gould & Quinn, 1999). In this context, the DATT4 index proved to be more effective, achieving a significant correlation of 0.6 in both stages

Conclusions

The treatments applied in this study significantly impacted reflectance values. A clear trend was observed in which higher doses of Mn in rose crop resulted in lower reflectance values, while lower dose treatments exhibited notably higher values.

The GNDVI index stands out as a highly effective indicator for estimating Mn concentration in rose leaves across various phenological stages, except in the "palmiche" stage. This index, which considers reflectance at 560 nm and 840 nm, demonstrated significant and strong correlations with Mn levels in rose leaves during the "rice," "chickpea," and "scratch color" phenological stages. Notably, in the "straight sepals" stage, a correlation of 0.739 and an R^2 of 0.546 were achieved. These findings are consistent with previous research on other crops, where GNDVI has been successfully used to assess the nutrient status of plants.

In contrast, the "palmiche" phenological stage exhibited a different dynamic, with the DATT4 index being the only one to show a significant correlation of 0.6 for estimating the Mn concentration. This variation may be related to specific characteristics of the plants at this stage, such as their red coloration, which contrasts with later phenological stages where the stem color turns green. This underscores the need to adapt methodologies according to phenological stages.

The comparison between spectral reflectance and traditional analysis suggests that the spectral reflectance methodology offers several advantages. This methodology allows for *in situ* application, is non-destructive, and provides rapid results. Unlike the traditional approach, which requires a greater amount of labor and samples to obtain results, thus increasing costs, the proposed method optimizes operations and reduces the need for personnel. Each reflectance reading generates data that identifies different nutrient behaviors within the same lot, facilitating the precise evaluation of Mn concentration in leaves. Notably, this procedure does not use chemical reagents, contributing to a more sustainable and efficient practice in the production of cut flowers.

This study establishes an important relationship between spectral reflectance and Mn foliar content in roses, as well as the relevance of the GNDVI index as a promising tool for estimating Mn status in cut flower production. These findings are valuable for monitoring and managing plant nutrition, significantly contributing to precision agriculture and data-driven agronomic decision-making, enhancing sustainability and efficiency in the cut flower industry.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

LJMM designed the conceptual approach and objectives; OFM supervised the experiments, managed data collection and maintenance, performed the statistical analyses and computer codes, coordinated funding; AFT carried out the field and laboratory experiments, and created effective visual representations of the data and findings; LJMM and OFM designed and developed the research methodology, including data collection methods and equipment, verified the accuracy and reliability of the research results through a rigorous validation process; OFM wrote the initial draft. All authors participated in the critical review and approval of the final version of the manuscript.

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Evaluation of electrical conductivity and pH in a nutrient solution with recirculating system in rose crop

Evaluación de la conductividad eléctrica y el pH de una solución nutritiva con sistema de recirculación en el cultivo de rosa

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ABSTRACT

Soilless culture systems with drainage recycling require continuous monitoring of electrical conductivity (EC) and pH, which are basic indicators of the chemical state of the solution that determine the extent to which recycling of nutrients is possible. These indicators are influenced by the physical, chemical, and microbiological properties of the substrates, as well as evapotranspiration, substrate temperature, and the stage of plant development. A rose crop cv. 'Charlotte' was established in three different substrates composed of mixtures of coconut fiber (CF) and burned rice husk (BRH). An automatic drainage recycling system was implemented with three percentages of nutrient recycling (0, 50, and 100%) to record the changes in EC and pH over 8 weeks of cultivation. This bifactorial experiment was carried out under a split-plot design in randomized complete blocks, where the main plot corresponded to the recycling percentage factor and the subplot to the substrate factor. The EC was significantly higher when recycling the nutrient solution in the following substrates: 35% BRH and 65% CF (35BRH) and 65% BRH and 35% CF (65BRH) at 1, 2, and 3 weeks after pruning (WAP). It was also higher for 100% BRH (100BRH) and 65BRH at 7 and 8 WAP. At 6 WAP, recycling at 50% and 100% had a significant effect on the EC values independent of the substrate. This could be caused by the release of ions and higher water retention, typical of CF, and the high adsorption of ions by the BRH. For pH, the trend was acidification, which was significant for the 100BRH treatment without recycling between 0 and 4 WAP. This could be related to changes in the absorption of ions such as NO_3^- and the activity of nitrifying microorganisms facilitated by the properties of the CF.

Key words: horticulture, cut flowers, recirculation drainage, soilless culture, substrate.

RESUMEN

Los sistemas de cultivo sin suelo con reciclaje de drenajes requieren del seguimiento continuo de variables determinantes como la conductividad eléctrica (CE) y el pH, indicadores básicos del estado químico de la solución que determinan hasta dónde son posibles los eventos de reciclaje. Estas variables son influenciadas por las propiedades físicas, químicas y microbiológicas de los sustratos, la evapotranspiración del cultivo, la temperatura de los sustratos, y el estadio de desarrollo de las plantas sembradas. Un cultivo de rosa cv. 'Charlotte' se estableció en sustratos compuestos por tres diferentes mezclas de fibra de coco (FC) y cascarilla de arroz quemada (CAQ), donde se implementó un sistema automático de reciclaje de drenajes con tres porcentajes de reciclaje de nutrientes (0, 50 y 100%), con el objetivo de conocer los cambios en CE y pH a lo largo de 8 semanas de cultivo. Este experimento bifactorial se llevó a cabo bajo un diseño de parcelas divididas en bloques completamente al azar, donde la parcela principal correspondió al factor porcentaje de reciclaje y la subparcela al factor sustrato. A las 1, 2 y 3 semanas después de la poda (SDP) la CE fue significativamente mayor al reciclar la solución en los sustratos: 35% CAQ con 65% FC (35CAQ) y 65% CAQ con 35% FC (65CAQ) y a las 7 y 8 SDP lo fue para 100% CAQ (100CAQ) y 65CAQ. A las 6 SDP hubo un efecto significativo de 50% y 100% de reciclaje independiente del sustrato. Lo anterior pudo ser causado por liberación de iones y alta retención de agua, propias de la FC y la alta adsorción de iones por la CAQ. Para el pH, la tendencia fue la acidificación, siendo significativa para el tratamiento 100CAQ sin reciclaje entre 0 y 4 SDP, lo que posiblemente se relaciona con los cambios en la absorción de iones como el NO_3^- y la actividad de microorganismos nitrificantes, facilitada por las propiedades de la FC.

Palabras clave: horticultura, flores de corte, recirculación de drenaje, cultivo sin suelo, sustrato.

Introduction

Colombia is the second largest exporter of flowers in the world, after the Netherlands, with a 20% market share. Roses dominate this export, making up 19.1%, with the USA as the primary destination, comprising 79.7% of the total export value (ICA, 2024).

Received for publication: July 5, 2024. Accepted for publication: August 30, 2024. Doi: 10.15446/agron.colomb.v42n3.115607

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A modern cultivation technique in roses uses suspended container beds. To prevent salinization of the growth media, drainage must account for 30 to 50% of the applied fertilizer solution. This increases water consumption and negatively impacts the environment, with fertilizers being lost when drained into the ground. This could be minimized with their recycling (Blok, 2023). Key variables in soilless culture systems are the temperature and aeration of the rhizosphere, the pH of the nutrient solution, the NH_4^+:NO_3^- concentrations and ratio, the size of the container or root volume, the medium of growth, and the electrical conductivity (EC) of the solution (Kafkafi, 2001).

Maas *et al*. (1986) modeled plant tolerance to salts by defining: 1) salinity threshold value (STV), which is the maximum salinity value at which there is no significant decrease in growth or yield, and 2) the decrease in yield due to salinity (DYS), which indicates the percentage decrease in yield for each unit increase in EC above the STV. Sonneveld *et al*. (1999) concluded that the absorption of sodium and chloride by the plants increased as their concentrations in the root environment increased, potentially benefitting salinity management, depending on the species. For carnation (*Dianthus caryophyllus* L.), STV was 4.3 dS m^{-1}, with a DYS of 3.9% per dS m^{-1}, whereas for rose (*Rosa* sp.), STV was 2.1 dS m^{-1} and DYS 5.3% per dS m^{-1}. Other authors mention EC values in soil extract no higher than 1.5 dS m^{-1} for rose (Cabrera *et al*., 2017).

EC values above the STV decrease yield mainly due to osmotic effects, which are influenced by the composition of the nutrient solution (Stamatakis *et al*., 2003). Salinity affects the quality of cut flowers, decreasing stem diameter and length as well as firmness and vase life (De Kreij & Van Den Berg, 1990). Under saline conditions, gerbera (*Gerbera* sp.) and rose react by reducing the number of flowers, while carnation and bouvardia (*Bouvardia* sp.) adjust flower weight instead (Sonneveld *et al*., 1999).

In addition to EC and pH, the preparation of the initial fertilizer solution must consider nutrient concentration relationships and water quality. The pH in the root zone for most hydroponic cultures is between 5.5 and 6.0; pH values between 5.0-5.5 and 6.5-7.0 would not cause problems in most cultures (Signore *et al*., 2016). However, pH values greater than 7.0 could cause problems in the absorption of P, Fe, and Mn, as well as possible symptoms of Cu and Zn deficiency (Meselmani, 2022).

The pH in a substrate system varies during the growing season due to the reduced container volume, especially when the substrate has a low buffering capacity as in the case of low reaction substrates, while in organic substrates pH is more stable. The buffering capacity of the nutrient solution is typically very low and almost only determined by phosphate concentrations (Sonneveld & Voogt, 2009). In periods of high growth rate and sufficient light intensity, anion absorption normally exceeds cation absorption, due to a high absorption of NO_3^- used in plant metabolism. This difference in ion absorption rate is mitigated by the release of HCO_3^- and OH^- to the rhizosphere by the roots (Neumann & Ludewig, 2023), increasing the pH of the root zone. However, under conditions of low light intensity, this situation is reversed, with decreases in NO_3^- absorption and increases in the cation:anion absorption ratio; the rapid uptake of cations is compensated by the release of H^+ by the roots (Nietfeld & Prenzel, 2015).

Excess nitrate in the recycled nutrient solution increases dry mass allocation to leaves at the expense of buds and inflorescences. The pH values of the nutrient solution between 3.0 and 4.0 increase the absorption of P and its concentration in leaves, reducing the sucrose biosynthesis and negatively affecting the yield in flower production. Bar-Yosef *et al*. (2009) and Masood *et al*. (2023) found that appropriate mixtures of NO_3^-, NH_4^+, and urea reduce the harmful effects produced by changes in pH, including ionic imbalances and competition for absorption of Ca, P, and Mn, among other elements.

The pH in the solution can be controlled by modifying the NH_4^+:NO_3^- ratio in the recycled solution, applying the required nitrogen in nitric form and applying an acid. The salt load in the system is reduced by eliminating the need for additional acid; however, NH_4^+ could inhibit the absorption of Ca^{2+}, Mg^{2+} and K^+ by the roots and negatively affect root development (Bar-Yosef, 2008; Coleto *et al*., 2023).

This research aimed to determine the effects of drainage recycling at 0%, 50%, and 100% in three organic substrate mixtures: 100% burned rice husk (100BRH), 65% burned rice husk and 35% coconut fiber (65BRH), and 35% burned rice husk and 65% coconut fiber (35BRH). The focus was on assessing pH and electrical conductivity (EC) values of the nutrient solution from 0 to 8 weeks after pruning (WAP) in rose plants cv. 'Charlotte' grown in a commercial plastic-covered system.

Materials and methods

Plant material and growth conditions

The research was carried out at the Center for Agricultural Biotechnology of SENA located in the municipality of

Mosquera (Cundinamarca, Colombia) (4°41' N, 74°13' W, 2516 m a.s.l.), with an annual average temperature of 12.6°C and precipitation of 670 mm. The site corresponds to lower montane dry forest (bs-MB) life zone (Guzmán González, 1996).

A traditional wooden plastic cover was used (AgroClear® plastic, Productos Químicos Andinos, Colombia) with five spans of 65 x 6.8 m each. The rose cv. 'Charlotte' was grafted onto the rootstock 'Natal Briar' and grown in 33 raised beds of 15 x 0.8 m. 8 L pots were placed for a planting density of 7 plants m^{-2}.

The fertilizer formula, in mg L^{-1}, was as follows: 170 total N (15% NH_4^+); 35 P; 150 K; 110 Ca; 60 Mg; 82 S; 1 Mn; 0.5 Zn; 0.5 Cu; 3 Fe; 0.5 B; and 0.1 Mo. This formula was prepared according to the commercial formulas used in the region and adjusted according to the characteristics of the water. The phytosanitary management was carried out according to standard practice for this crop. Organic substrates consisted of a mixture of burned rice husk (BRH), with a burning degree between 70 and 100%, and washed coconut fiber (CF) in different proportions (Tab. 1): 100% burned rice husk (100BRH), 65% burned rice husk and 35% coconut fiber (65BRH), 35% burned rice husk and 65% coconut fiber (35BRH).

An automatic drainage recycling system (ADRS) was implemented to recycle drainages at three levels: 0%, 50%, and 100%. The ADRS is detailed in Cuervo *et al.* (2012) and Cuervo-Bejarano *et al.* (2011).

Data analysis

The experiment consisted of nine treatments, achieved by combining the three levels of recycling percentages with the three types of substrates, each repeated three times (Tab. 2). Each experimental unit corresponded to a raised bed. A bifactorial experiment was carried out under a split-plot design with randomized complete blocks. The main plot was based on the recycling percentage factor, and the subplot was determined by the substrate factor.

The statistical model used for the analysis was:

$$Y_{ijk} = \mu + \alpha_i + \delta_k + \eta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (1)$$

with: $i = 1, 2, 3$; $j = 1, 2, 3$; $k = 1, 2, 3$

where:

μ = effect of the overall mean;

α_i = effect of the *i-th* level of the recycling percentage factor;

δ_k = effect of the *k-th* block;

η_{ik} = effect of random error on the main plot (recycling percentage per block);

β_j = effect of the *j-th* level of the substrate factor;

$(\alpha\beta)_{ij}$ = effect of the *ij-th* interaction between the two factors (recycling percentage per substrate;

ε_{ijk} = effect of random error in the subplot;

Y_{ijk} = observation in the *k-th* block of the *i-th* level of the percentage recycling factor and the *j-th* level of the substrate factor.

TABLE 2. Treatments evaluated in rose cv. 'Charlotte' grown in substrates with automatic drainage recycling.

Treatment	Substrate	Percentage of drainage recycling
100BRH-0R*	100BRH	
65BRH-0R*	65BRH	0
35BRH-0R*	35BRH	
100BRH-50R	100BRH	
65BRH-50R	65BRH	50
35BRH-50R	35BRH	
100BRH-100R	100BRH	
65BRH-100R	65BRH	100
35BRH-100R	35BRH	

100BRH = 100% burned rice husk; 65BRH = 65% burned rice husk and 35% coconut fiber; 35BRH = 35% burned rice husk and 65% coconut fiber; R – percentage of drainage recycling.
*Treatments that did not enter the automatic drainage recycling system.

TABLE 1. Chemical properties of the substrates used in the experiment.

Substrate	pH	EC	OC	N	P	Ca	K	Mg	Na	Cu	Fe	Mn	Zn	B	S
		(dS m^{-1})				(%)						(mg kg^{-1})			
100BRH	5.53	6.82	27.2	0.51	0.06	0.11	0.01	0.04	0.03	4.4	225	136	54	28	481
65BRH	5.31	6.52	23.6	0.39	0.08	0.4	0.01	0.06	0.08	13.4	433	87	50	34	470
35BRH	5.18	5.18	6.04	26.6	0.5	0.06	0.16	0.01	0.17	19.1	704	66	47	-	548

EC – electrical conductivity, OC – organic carbon.

Drainage from each experimental unit was collected in a 20 L container and the EC (dS m^{-1}) and pH were recorded daily between weeks 0 and 8 after pruning (WAP) using a portable Oakton AN 23 (Cole-Parmer, USA). The statistical software R (R Core Team, 2020) was used for performing the analysis of variance (ANOVA) and the subsequent Tukey's comparison test ($P<0.05$) for multiple comparisons (de Mendiburu, 2023). Scientific visualizations were made using the ggplot2 package v3.4.2 (Wickham, 2016).

Results and discussion

The chemical characteristics of the recycled nutrient solution varied, possibly due to the interactions with the physical and chemical characteristics of the organic substrate, the microbiological dynamics, the roots exudates, and the climatic conditions, among others. Emphasis was placed on the concentration of ions and their potential environmental impact, in addition to their influence on EC, a critical factor determining the useful life of the solution to be recycled.

Electrical conductivity (EC)

An increasing trend of the EC was observed over time. Initially, the substrates with higher percentages of CF increased the EC of the drainages, especially when recycling occurred. However, over time, these effects on EC diminished and no significant differences were observed (Tab. 3). At 1, 2, and 3 WAP, the EC was significantly higher ($P<0.05$) in the drainages from the treatments that contained CF (35BRH and 65BRH). At 6 WAP, a significant effect of the substrate was not determined; however, a significant effect of the recycling (50% and 100%) was observed.

At 7 WAP and 8 WAP, the trend reversed. The substrates with CF had no effect on EC, and at higher BRH contents the EC significantly increased in the drained solutions (Tab. 3). This behavior may be associated with the release of ions from the CF, such as Na^+, K^+, NH_4^+, NO_3^-, Fe^{3+}, and S (Tab. 1). However, it is also possible that over time the CF has become saturated with ions (Okafor *et al.*, 2012; Song *et al.*, 2014), resulting in minimal adsorption and increased leaching. The low EC values may be explained by high plant demand for mineral elements that contribute significantly to the EC and that are being absorbed during the vegetative growth phases; therefore, the content of these ions in the solution was reduced (Bugbee, 2004; Van Der Sar *et al.*, 2014). While EC values during the evaluated period did not exceed those reported for DYS, they did exceed those for STV (Sonneveld *et al.*, 1999).

When BRH undergoes the carbonization process, its surface area and mineral fixation capacity increase, which makes it an appropriate material for the adsorption of heavy metals, Ca^{2+}, Fe^{3+}, Cu^{2+}, and Zn^{2+} (Kuan *et al.*, 2011; Phonphuak & Chindaprasirt, 2015) and as an acidity corrector in soils (Islabão *et al.*, 2014; Kath *et al.*, 2018). In this study, significant evidence was found that higher BRH content in the substrate and higher percentage of drainage recycling leads to increases in the EC in the drainages over time (Tab. 3), possibly due to the degradation of the material or the increase in the adsorption of cations that saturate the exchange sites, decreasing the cation exchange capacity. It is possible that BRH adsorbs Ca^{2+} and Mg^{2+} from the nutrient solution, which agrees with what was found by Vélez-Carvajal *et al.* (2014) in a carnation crop planted in the same mixtures of BRH and CF.

Results showed that the Ca^{2+} contents in plant tissue were below the values recommended by Cabrera and Perdomo (2003), which range between 1.0 and 2.0%. The substrate

TABLE 3. Tukey's multiple comparison test for electrical conductivity (EC). Mean EC values (dS m^{-1}) ± standard deviation are presented for the treatments evaluated each week after pruning (WAP) in the automatic drainage system. Different letters indicate a significant difference at $P<0.05$.

WAP	Treatment								
	100BRH-0R	100BRH-50R	100BRH-100R	35BRH-0R	35BRH-50R	35BRH-100R	65BRH-0R	65BRH-50R	65BRH-100R
0	1.92±0.27a	2.17±0.29a	2.06±0.36a	2.14±0.2a	2.49±0.38a	2.42±0.35a	2.42±1.3a	2.44±0.54a	2.56±0.22a
1	2.45±0.27c	2.62±0.43bc	2.62±0.35bc	2.61±0.23bc	2.92±0.29abc	3.01±0.44ab	2.58±0.28bc	3.13±0.41a	3.16±0.45a
2	2.22±0.17e	2.89±0.23bc	2.83±0.46bcd	2.4±0.19cde	2.95±0.2b	3.49±0.97a	2.35±0.22of	3.3±0.31ab	3.08±0.16ab
3	2.57±0.19c	2.82±0.25abc	2.89±0.29abc	2.74±0.12bc	2.99±0.21ab	3.15±0.24a	2.7±0.35bc	2.99±0.22ab	3.11±0.17ab
4	2.05±0.4a	2.68±0.59a	2.59±0.35a	2.23±0.41a	1.96±0.44a	2.65±0.5a	2.48±0.37a	3.0±1.1a	2.6±0.6a
5	2.51±0.3a	2.75±0.3a	3.05±0.62a	3.02±1.01a	2.83±1.05a	3.18±0.99a	2.67±0.29a	3.13±0.76a	2.94±0.4a
6	2.4±0.49d	3.43±0.89abc	3.96±0.98a	2.65±0.54bcd	3.17±0.57	3.33±0.8abcd	2.49±0.32cd	3.67±0.68a	3.61±0.72ab
7	2.25±0.38d	4.19±0.7abc	4.83±1.03a	2.43±0.41d	3.76±0.59c	4.06±0.51bc	2.36±0.27d	4.24±0.86abc	4.51±0.43ab
8	2.28±0.25d	4.62±0.79ab	5±0.92a	2.67±0.57d	3.77±0.65c	4.16±0.71bc	2.23±0.27d	4.24±0.48bc	4.46±0.82ab

100BRH = 100% burned rice husk; 65BRH = 65% burned rice husk and 35% coconut fiber; 35BRH = 35% burned rice husk and 65% coconut fiber; R – percentage of drainage recycling.

contents in initial conditions were 0.06, 0.4 and 0.11% for 35BRH, 65BRH and 100BRH, respectively. At 8 WAP, these values were higher for the 35BRH and 65BRH substrates, reaching 3.0 to 3.8%, while the Ca^{+2} concentrations in the nutrient solution were 110 mg L^{-1}.

pH

The pH trend of the drainages showed acidification (Tab 4). The ANOVA and the Tukey´s mean comparison test indicated that between 0 and 4 WAP, the pH values were significantly lower (P<0.05) for 100BRH-0R compared to the other treatments. The trend indicates that the drainage becomes more alkaline as the CF fraction and the percentage of drains increase. Assuming that the exchange sites in the CF are being saturated by Ca^{2+}, an accumulation of H^+ could occur in the drained solution. There were significant effects for the percentage of recycling and the type of substrates on pH (P<0.05). The lower the percentage of recycling, the greater the tendency to acidify the drainages (0R > 50R > 100R). In the same way, the lower the CF content in the substrates, the greater acidity of the drainages (100BRH > 65BRH > 35BRH) (Tab. 4).

These results are consistent with those of Mesa *et al.* (2011), Vélez *et al.* (2014), and Vélez-Carvajal *et al.* (2014), who reported increases in EC and a decreasing trend in pH during the transition between vegetative and reproductive stages for a carnation crop grown on BRH and CF-based substrates with drainage recycling.

Decreases in pH may be related to the reduction in the active absorption of NO_3^- and the increase in the absorption of NH_4^+ by the plants (Bugbee, 2004; Chapagain & Hoekstra, 2003; Sonneveld & Voogt, 2009), as well as to the nitrification by microorganisms, whose metabolic activity decreases the pH of the medium (Arp *et al.*, 2007; Avrahami & Conrad, 2003). Nitrification requires a medium with greater aeration, as is the case of substrate mixtures with lower CF contents (Londra *et al.*, 2018; Udayana *et al.*, 2017) and lower recycling percentages. In addition, the mixtures of organic materials used as substrates for crops not only improve physical and chemical properties (Awang *et al.*, 2009) but also promote metabolic activity such as urea hydrolysis, nitrite, and ammonia oxidation, and increasing respiration rate (Grunert *et al.*, 2016).

Finally, Yepes and Flórez (2013), in a manual recycling system for a one-harvest-cycle crop of roses, did not find EC and pH values exceeding those that are considered to have a negative impact on production; however, an increasing trend in the EC and pH values was observed. Treatments with 100BRH and 0% drainage recycling had the lowest pH values.

Conclusions

A trend of increasing EC was observed. At 1, 2 and 3 WAP, the EC was significantly higher when recycling the solution in 35BRH and 65BRH, and at 7 and 8 WAP, it was higher for 100BRH and 65BRH. At 6 WAP there was a significant effect of recycling at 50% and 100% on EC independent of the substrate. This behavior can be caused by the release of ions and high-water retention, characteristic of the CF and the high adsorption of ions by the BRH. Between 0 and 4 WAP, the pH was significantly lower for the 100BRH treatment without recycling, which is possibly related to changes in the absorption of ions, such as NO_3^-, by the plants and to the activity of nitrifying microorganisms facilitated by the properties of CF.

TABLE 4. Tukey's multiple comparison test for pH. Mean pH values ± standard deviation are presented for the treatments evaluated each week after pruning (WAP) in the automatic drainage system. Different letters indicate a significant difference at P<0.05.

WAP	Treatment								
	100BRH-0R	100BRH-50R	100 BRH-100R	35BRH-0R	35BRH-50R	35BRH-100R	65BRH-0R	65BRH-50R	65BRH-100R
0	7.3±0.2e	7.71±0.19bc	7.89±0.16ab	7.56±0.22cd	7.71±0.22bc	8±0.18a	7.37±0.28de	7.73±0.23bc	7.84±0.16ab
1	7.19±0.1d	7.47±0.13abc	7.68±0.19a	7.38±0.15bcd	7.53±0.19ab	7.69±0.23a	7.23±0.15cd	7.53±0.17ab	7.65±0.12a
2	7.21±0.14c	7.44±0.12abc	7.55±0.23ab	7.48±0.25abc	7.49±0.15abc	7.69±0.18a	7.33±0.17bc	7.56±0.18ab	7.54±0.11abc
3	7.19±0.18bc	7.26±0.15bc	7.38±0.29ab	7.21±0.11bc	7.38±0.18ab	7.57±0.19a	7.11±0.17c	7.33±0.26abc	7.45±0.1ab
4	7.07±0.51c	7.36±0.2abc	7.49±0.35ab	7.35±0.34abc	7.43±0.42abc	7.68±0.23a	7.19±0.21bc	7.54±0.11ab	7.46±0.23ab
5	6.91±0.33a	7.24±0.18a	7.13±1a	7.19±0.95a	7.04±1.05a	7.31±0.95a	7.08±0.16a	7.45±0.14a	7.36±0.17a
6	6.84±0.19a	7.04±0.22a	7.14±0.46a	7.12±0.3a	6.83±0.2a	7.13±0.27a	6.93±0.18a	7.15±0.18a	7.08±0.23a
7	7.04±0.24a	6.77±0.14a	6.9±0.34a	7.17±0.24a	6.72±0.14a	7.01±0.13a	7.04±0.14a	7.1±0.21a	6.94±0.19a
8	6.9±0.15a	6.68±30.2a	6.84±0.27a	7.09±0.25a	6.73±0.16a	6.93±0.26a	7.09±0.16a	7.04±0.26a	6.9±0.2a

100BRH = 100% burned rice husk; 65BRH = 65% burned rice husk and 35% coconut fiber; 35BRH = 35% burned rice husk and 65% coconut fiber; R – percentage of drainage recycling.

The observed increase in EC and decrease in pH can be attributed to the physical and chemical properties of the substrates, including factors such as cation exchange capacity and mineralization rate. Additionally, microbiological activity involved in the degradation of the growth media, particularly in substrates with higher CF content, could play a significant role. While it is essential to consider the mineral nutrition of the plants and the appropriate percentage of recirculation, it is equally important to account for the substrate composition when formulating the fertigation solution, as this can influence both EC and pH levels.

Acknowledgments

The authors are grateful for funding from the Ministry of Agriculture and Rural Development (Colombia), Colciencias, and Ceniflores, as well as donations from the companies Suata Plants S.A., Productos Químicos Andinos S.A., and Brenntag Colombia S.A.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

WJCB and VJFR: conceptualization, funding acquisition research, writing – original draft, visualization, writing, and editing. SEMM: formal analysis, visualization, writing, and supervision editing. All authors have read and approved the final version of the manuscript.

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Allelopathic potential of *Artemisia absinthium* L. on seed germination and seedling growth of various plant species

Potencial alelopático de *Artemisia absinthium* L. sobre la germinación de semillas y el crecimiento de plántulas de varias especies de plantas

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ABSTRACT

In plant-plant interactions, the emission of secondary metabolites can have significant effects, potentially serving as a tool for weed management. The study of plant-derived substances offers an environmental alternative to traditional production processes. The aim of the research was to evaluate the allelopathic potential of the aqueous extract of *Artemisia absinthium* L. on the germination of seeds of five species: *Calendula officinalis* L., *Taraxacum officinale* L., *Bidens pilosa* L., *Senecio vulgaris* L., and *Brassica juncea* L. The experiment involved a factorial design with five species, five extract concentrations and five replicates, for a total of 125 experimental units (EU). The aqueous extract of *A. absinthium* was prepared from dried foliage at a concentration of 1/50 (w/v) and applied in varying proportions (0, 25, 50, 75, and 100%) according to the respective treatments. In each EU, 20 seeds of the corresponding species were placed and grown under controlled conditions for 21 d, during which germination was monitored. The results indicate that *A. absinthium* has significant potential as an inhibitor of seed germination and seedling growth in *T. officinale* and *B. pilosa*. In *S. vulgaris* and *B. juncea,* the response was highly dose-dependent. In *C. officinalis*, no inhibition was observed in the evaluated parameters. The results indicate that *A. absinthium* extract offers a sustainable alternative to weed management.

Key words: allelopathy, weed management, secondary metabolites, plant-plant interactions, bioactive compounds.

RESUMEN

En las interacciones planta-planta, la emisión de metabolitos secundarios puede tener efectos significativos, los cuales son herramientas potenciales para el manejo de malezas, por lo que el estudio de estas sustancias de origen vegetal ofrece una alternativa medioambiental a los procesos de producción tradicionales. La investigación tuvo como objetivo evaluar el potencial alelopático del extracto acuoso de *Artemisia absinthium* L. sobre la germinación de semillas de cinco especies: *Calendula officinalis* L., *Taraxacum officinale* L., *Bidens pilosa* L., *Senecio vulgaris* L. y *Brassica juncea* L. El experimento tuvo un diseño factorial con cinco especies, cinco concentraciones de extracto y cinco repeticiones, para un total de 125 unidades experimentales (UE). El extracto acuoso de *A. absinthium* se obtuvo a partir de follaje seco a una concentración de 1/50 (p/v), y se aplicó en proporciones variables (0, 25, 50, 75 y 100%) según los respectivos tratamientos. En cada UE se colocaron 20 semillas de la especie correspondiente, que se cultivaron en condiciones controladas durante 21 d durante los cuales se monitoreó la germinación. Los resultados indican que *A. absinthium* tiene un potencial significativo como inhibidor de la germinación de semillas y el crecimiento de plántulas en *T. officinale* y *B. pilosa*. Para el caso de *S. vulgaris* y *B. juncea*, la respuesta fue altamente dependiente de la dosis y en *C. officinalis* no se observó inhibición en los parámetros evaluados. Los resultados señalan al extracto de *A. absinthium* como una alternativa sostenible en el manejo de malezas.

Palabras clave: alelopatía, manejo de malezas, metabolitos secundarios, interacciones planta-planta, compuestos bioactivos.

Introduction

In plant-plant relationships, an interaction between the individual plants develops, a process by which the plants respond to a limiting condition. The main components of this interference are competition and allelopathy (Weidenhamer *et al*., 2023). Competition arises from the need to acquire limiting resources such as water, light, and nutrients (Craine & Dybzinski, 2013), whereas allelopathy involves the production or emission of biochemicals by a plant or its parts which influence the germination, growth, or reproduction of other plant species. This emission can harm sensitive species and plays a crucial role in ecological succession and dominance, contributing to

Received for publication: July 23, 2024. Accepted for publication: August 28, 2024.
Doi: 10.15446/agron.colomb.v42n2.115942

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distinct vegetation patterns (Latif *et al*., 2017; Schandry & Becker, 2020).

In crop production, due to the limited resources available to plants, weed management is crucial because weeds can decrease productivity and quality (Radhakrishnan *et al*., 2018). Various strategies exist for weed control, with chemical control using herbicides being the most widespread; thus, there is a need for more sustainable alternatives for weed management (Dayan *et al.,* 2009; Green & Owen, 2011). Therefore, there is increasing interest in identifying compounds that do not remain active in the environment for long periods, capable of controlling weeds and reducing the selection pressure that leads to herbicide-resistant weeds (Hasan *et al*., 2021).

Among the alternatives, the use of plant extracts emerges as a promising option. Bioactive compounds present in these extracts can control weed populations, with some functioning as natural herbicides that offer the advantage of being biodegradable and avoiding long-term contamination (Khamare *et al*., 2022).

The allelopathic effect typically results from the simultaneous action of several compounds, often including diverse metabolites such as phenols, terpenes, and alkaloids (Khamare *et al*., 2022; Zohaib *et al*., 2017). Numerous studies have reported that these bioactive compounds can modify germination patterns (Chenyin *et al*., 2023), affect growth and development (Jabran *et al*., 2015), and reduce plant biomass production (Zhang *et al*., 2021; Zohaib *et al*., 2017). These results vary among plants, as species differ in their sensitivity to these substances, and the types and amounts of these compounds emitted vary according to the species and the plant´s physiological state (Cheng & Cheng, 2015; Radhakrishnan *et al*., 2018).

Artemisia absinthium L., commonly known as wormwood, is a perennial herbaceous species of European origin from the family Asteraceae (Judžentienė, 2016), introduced to the Americas by the Spaniards during the conquest (Beltrán-Rodríguez *et al*., 2017). It has documented uses in medicine and the food industry (Hbika *et al*., 2022; Judžentienė, 2016; Li *et al*., 2021). Extracts of *A. absinthium* have demonstrated antibiotic, antiparasitic, anticancer, and allelopathic properties (Anibogwu *et al*., 2024; Judžentienė, 2016). These extracts can inhibit or delay the germination of sensitive seeds, which is associated with alterations in cell membrane permeability, affecting water and essential nutrient absorption. They can also influence cell division, reduce the synthesis of photosynthetic pigments and photosynthesis, and promote the formation of reactive oxygen species (Bharati *et al*., 2014; Choudhary *et al*., 2023; Hasan *et al*., 2021; Lee *et al*., 2013; Pouresmaeil *et al*., 2020).

Herbaceous plants, *Calendula officinalis* L., *Taraxacum officinale* L., *Senecio vulgaris* L., and *Bidens pilosa* L. from the Asteraceae family and *Brassica juncea* L. from the Brassicaceae family, are dicotyledonous species. *C. officinalis* is a species known for its high production of bioactive compounds, making it commonly used in traditional medicine in various countries of Latin America and Europe (Khalid & Silva, 2012). *T. officinale*, *S. vulgaris*, and *B. pilosa* are characterized by wide distribution in agricultural areas, high seed production per plant, rapid germination, and highly competitive ability (Dotor & Cabezas, 2016; Froese & Van Acker, 2003). These traits enable them to rapidly colonize various agroecosystems and effectively compete for resources, making them problematic weeds in intensive vegetable or fodder crops, such as carrot (Dotor & Cabezas, 2016). As abundant plants in agricultural systems, they are also subject to selection pressures from constant herbicide application (Green & Owen, 2011).

Although the allelopathic effects of *Artemisia* species have been documented, this study provides new insights by focusing on the species-specific responses of five distinct plants. Additionally, this study aims to contribute to the knowledge of non-chemical weed management by evaluating, under laboratory conditions, the effect of the aqueous extract of *A. absinthium* on the germination of seeds of *C. officinalis*, *B. juncea*, *B. pilosa*, *T. officinale,* and *S. vulgaris*.

Materials and methods

Plant material

The plant material of *A. absinthium*, which included old and new leaves and stem, was collected in February from an orchard at Finca San Rafael located in Facatativá, Cundinamarca, Colombia (4°48’17.2” N, 74°16’47.9” W), where it occupied an area of 1.5 m^2 and was mainly surrounded by *Cenchrus clandestinus* (Hochst. ex Chiov.). The material was placed in paper bags and dried for 15 d at room temperature (20±2°C). Seeds of *B. juncea*, *B. pilosa*, *T. officinale, S. vulgaris,* and *C. officinalis* were collected from free-growing plants located in the greenhouse area of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogotá (4°38’12.4” N, 74°05’18.1” W).

Extract of *A. absinthium* leaves

For the preparation of the extract, 10 g of dry foliage (moisture content of 10±5%) of *A. absinthium* was placed in 500 ml of distilled water and heated to 50°C for 3 h in a beaker. The mixture was then filtered and the extract was stored in dark conditions at room temperature (15±2°C) for 3 d.

Identification and quantification of bioactive compounds in *A. absinthium* extract

Aliquots of *A. absinthium* extract were analyzed by LC-MS (Liquid Chromatography-Mass Spectrometry) in a Bruker Impact II system at the Laboratory of Chromatography and Q-TOF Mass Spectrometry of the Universidad Nacional de Colombia, Bogotá campus. The LC used a Bruker Intensity Solo 2 C18 column, with a mobile phase Water-Formic Acid 0.1% and Acetonitrile-Formic Acid 0.1%. The elution was performed with a Gradient 5-95% B in 10 min with a flow rate of 0.250 ml min^{-1}. MS operated in MS/MS scanning mode, in the range of 50 to 1300 m/z, with a spectrum rate of 4 Hz. For the identification of the extract compounds, the chromatogram was run in Bruker MetaboScape® version 6.0.2 (Bruke Daltonics) metabolomics software, using the Bruker HMDB Metabolite Library 2.0, Bruker MetaboBASE® Personal Library 3.0, and MoNA-export-MassBank libraries.

Treatments

A factorial design was used, with seeds of 5 plant species, 5 doses of *A. absinthium* extract, 5 replicates per treatment, and 20 seeds per experimental unit, for a total of 125 experimental units. Fifty ml vials were used, to which were added 25 ml of solutions at 25, 50, 75, and 100% of the stock extract solution (called treatments 25, 50, 75, and 100, respectively) and the control treatment (0% of stock extract solution, only distilled water). A glass fiber was placed inside the vial to allow the seeds to float, on which 20 seeds of the corresponding species were deposited, ensuring their contact with the solution to be evaluated. The experimental units were placed under germination conditions maintained at 20±2°C throughout the entire duration of the experiment, with 12 h of light per day.

Germination variables

The number of germinated seeds was recorded at 4 d intervals; the criterion to consider germination effective was the emission of the radicle with a minimum size of 4 mm (Ranal & Santana, 2006). Based on the information collected, the germination percentage was defined as

$$G\,(\%) = \Sigma\left(\frac{N}{N_S}\right) * 100$$

where N= number of germinated seeds and Ns= total number of seeds (Escobar Escobar & Cardoso, 2015).

The radicle length was determined by measuring the radicle length of 5 seedlings per experimental unit. For this purpose, a graduated caliper was used.

Statistical analysis

The homogeneity of variances and normality were tested using Bartlett's test and the Shapiro-Wilk test. These tests were followed by analysis of variance (ANOVA) and Tukey's mean comparison test ($P \leq 0.05$) using R Studio software version 2023.03.1 and SigmaPlot 12.0 software.

Results and discussion

Characterization of the chemical compounds in the aqueous extract of *A. absinthium*

The preparation of an aqueous extract of *A. absinthium* was proposed as a straightforward and cost-effective method for obtaining plant extracts. However, due to uncertainties regarding the efficacy of this extraction method in isolating bioactive compounds, a metabolomic analysis was conducted using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS).

The HPLC-MS analysis of the extract revealed the presence of 37 components. The bioactive compounds present in the extract suggest a significant level of secondary metabolism in this species, including synthesis of monoterpenes, alcohols, alkaloids, and both protein and non-protein amino acids (Tab. 1). Among these, the compounds artemisinin, limonene, β-myrcene, methyl eugenol or thujone, camphor and 1,8-cineole are found in greater proportions, as indicated by peaks in their characteristic retention times (Tab. 1). Other compounds, such as proline, isoleucine, betaine, leucine, pipecolic acid, phenylalanine, tyrosine, dioctyl phthalate, D-pipecolic acid, dimethyl sulfoxide, 4-hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine, 3-[5-(methoxymethyl)-1,2,4-oxadiazol-3-yl]-N-(2-methylpropyl) pyrrolidine-1-carboxamide, pentaethylene glycol, triethylene glycol, were also identified (Tab. 1).

TABLE 1. Chemical composition of the aqueous extract of *Artemisia absinthium.*

Identification or name of compound	Rt (min)	Molecular formula of the compound	Molecular mass of the compound (g mol^{-1})	Annotation source
Betaine	0.99	$C_5H_{11}NO_2$	117.148	A
L-Proline	1.01	$C_5H_9NO_2$	115.13	B
Pipecolic acid	1.14	$C_6H_{11}NO_2$	129.157	B
D-Pipecolic acid	1.37	$C_6H_{11}NO_2$	129.16	A
4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine	1.42	$C_{11}H_{23}NO_2$	201.309	C
Xanthine	1.44	$C_5H_4N_4O_2$	152.11	C
L-Tyrosine	1.64	$C_9H_{11}NO_3$	181.19	B
L-Isoleucine	1.73	$C_6H_{13}NO_2$	131.17	A
L-Norleucine	1.88	$C_6H_{13}NO_2$	131.17	B
Leucine	1.89	$C_6H_{13}NO_2$	131.17	C
Triethylene glycol	1.98	$C_6H_{14}O_4$	150.175	A
L-Phenylalanine	3.08	$C_9H_{11}NO_2$	165.192	C
Pentaethylene glycol	3.77	$C_{10}H_{22}O_6$	238.281	A
3-[5-(methoxymethyl)-1,2,4-oxadiazol-3-yl]-N-(2-methylpropyl) pyrrolidine-1-carboxamide	4.05	$C_{13}H_{22}N_4O_3$	281.343	A
Artemisinin	4.34	$C_{15}H_{22}O_5$	282.332	C
α-Santonin	4.69	$C_{15}H_{18}O_3$	246.3	A
Leucodin	5.3	$C_{15}H_{18}O_3$	246.306	A
Camphor	6.38	$C_{10}H_{16}O$	152.23	A
1,8-cineole	7.31	$C_{10}H_{18}O$	154.249	A
Limonene	8.94	$C_{10}H_{16}$	136.24	C
Cetrimonium	10.90	$C_{19}H_{41}N$	284.5	A
Dioctyl phthalate	13.03	$C_{24}H_{38}O_4$	390.556	A
Pyridine	13.04	C_5H_5N	79.101	B

RT - Retention time of compounds in the column (min). Annotation source: A: Bruker MetaboBASE Personal Library 3.0; B: Bruker HMDB Metabolite Library _ 2.0; C: MoNA-export-MassBank.

Seed germination

Germination percentage

Figure 1 illustrates that across the various species evaluated at the end of the test, the control treatments (those not exposed to the *A. absinthium* extract) exhibited germination exceeding 90%. The highest proportion of germinated seeds was observed within the first 10 d following the initiation of the trial, with germination (G%) exceeding 60% of the control treatment. These results indicate adequate viability of the seed lots used in the trial and align with findings reported by authors such as Barrera (2015) and Dotor and Cabezas (2015) regarding the high G% of these species.

Seeds treated with the *A. absinthium* extract had a reduction in G%, with varying results depending on the dose and species, with negative correlations for *B. pilosa* (r=-0.89), *T. officinale* (r=-0.75), *S. vulgaris* (r=-0.95) and *B. juncea* (r=-0.97) (Fig. 1). In *B. pilosa* and *T. officinale*, a decrease in G% was observed across all treatments, with significant statistical differences ($P \leq 0.05$) between the control and the other treatments, indicating high sensitivity to extract exposure (Fig. 1). For *S. vulgaris* and *B. juncea*, the decrease in G% was highly dose-dependent (Fig. 1). For both these species, seeds exposed to 25% extract exhibited G% statistically similar to the control during the first 10 d, with germination exceeding 40% in *S. vulgaris* and 60% in *B. juncea*. By the end of the trial, these species recorded germination values of 60±6%, indicating a maximum 20% of the seeds germinated between 11 and 30 d. In the treatments exposed to a 50% extract, *B. juncea* showed rapid germination during the first 10 d, reaching values close to 40% followed by a cessation of germination. *S. vulgaris* stopped germination by 17 d, recording a maximum value of 30±4%. Additionally, the treatments 50 and 75% extract concentrations effectively inhibited germination, maintaining G% values below 10 and 20% in *S. vulgaris* and *B. juncea*, respectively.

In *C. officinalis*, the results indicate an absence of inhibitory effects on germination, as all treatments exhibited germination patterns comparable to the 100% in the control (Fig. 1).

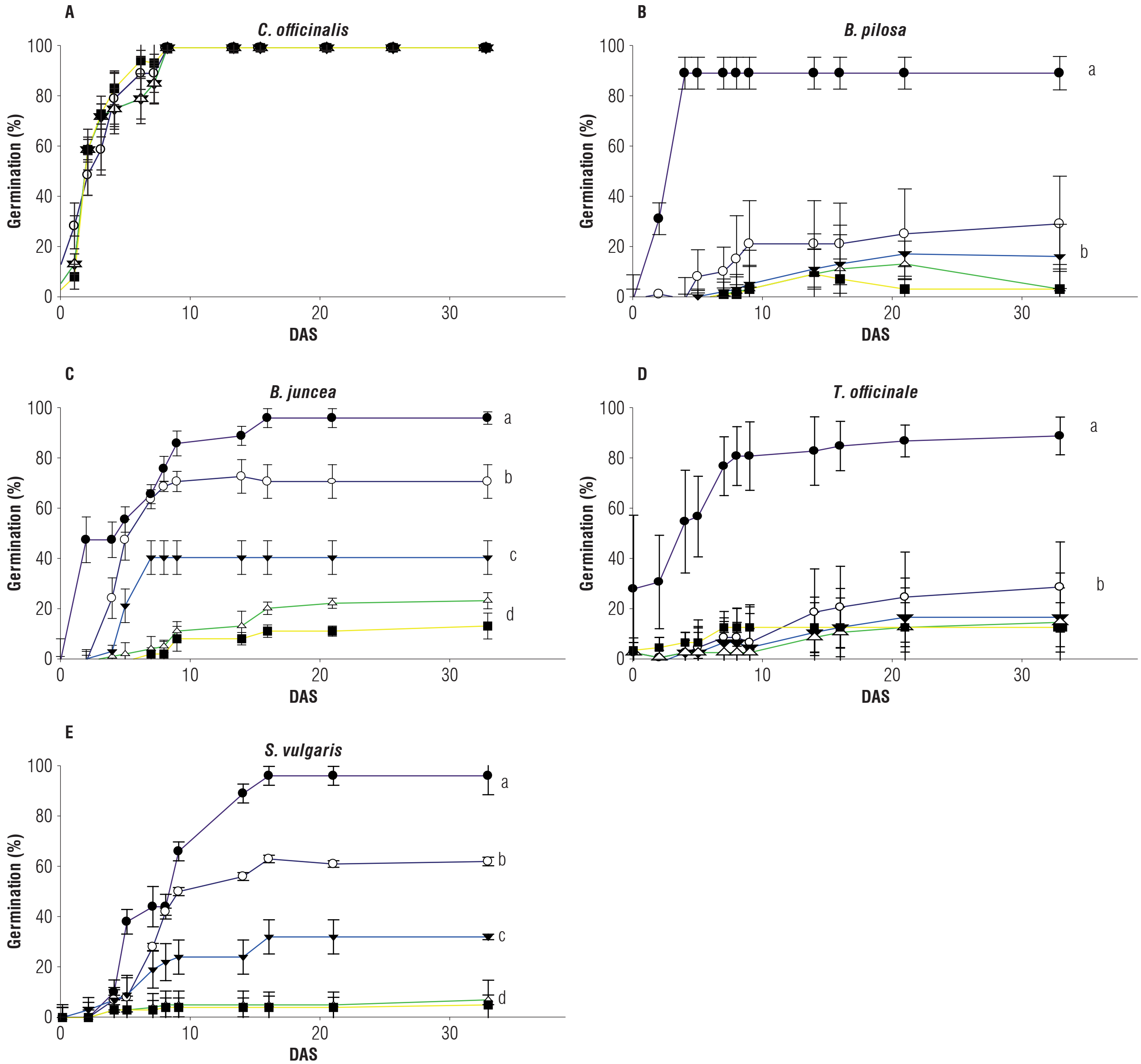

FIGURE 1. Germination (%) in relation to the day after sowing (DAS) in seeds of *Calendula officinalis, Bidens pilosa*, *Senecio vulgaris*, *Taraxacum officinale,* and *Brassica juncea* under different concentrations of *Artemisia absinthium* leaf extract. (----) extract at 0% (control), (----) extract at 25%, (----) extract at 50%, (----) extract at 75%, and (----) 100% extract concentration. The bars correspond to the standard deviation. Different lowercase letters indicate significant differences between treatments according to the Tukey mean test ($P \leq 0.05$).

Root length

Figure 2 displays the average radicle length of the different species, with values expressed as a percentage relative to the control treatment (extract at 0%). This means that the radicle length in the control is set at 100%, and the values for other treatments represent the radicle length as a percentage of that control.

In *B. pilosa*, the greatest reduction was observed in the seeds exposed to 100% of the extract, where the radicle reached only 40% of the control treatment length. Treatments with concentrations of 75% and 50% had lengths of 42% and 50%, respectively. For this species, the 25% treatment did not show statistical differences compared to the control. In *S. vulgaris*, a reduction in radicle length was also observed, with values statistically different from the control for all treatments, with values close to 47% in treatments 50%, 75%, and 100%, and 70% in treatment 25%. A similar statistically significant result was observed in *T. officinale,* with the highest percentage recorded in the 25% treatment

(42%), followed by the 50% treatment (38%), 75% treatment (10%), and 100% treatment (5%). In *B. juncea*, although there is a statistical difference between the control and the treatments, this species exhibited the highest radicle length values in the treatments 25% and 50%, with values of 90% and 85%, respectively. In the treatments 75% and 100%, radicle length was recorded at 40% and 12% compared with control, respectively (Fig. 2). There was also a negative correlation with dose, with values of *r*=-0.84 in *T. officinale*, *r*=-0.83 in *S. vulgaris*, *r*=-0.80 in *B. juncea*, and *r*=-0.79 in *B. pilosa*. The findings suggest that the seed exposure to *A. absinthium* extract influences the germination process, germination speed, and radicle growth.

The results for *C. officinalis* indicate absence of inhibitory effects on the root length, as all treatments exhibited similar results to the 100% in the control treatment (Fig. 2).

The extensive chemical composition of the aqueous extract of *Artemisia* genus has been reported by several authors,

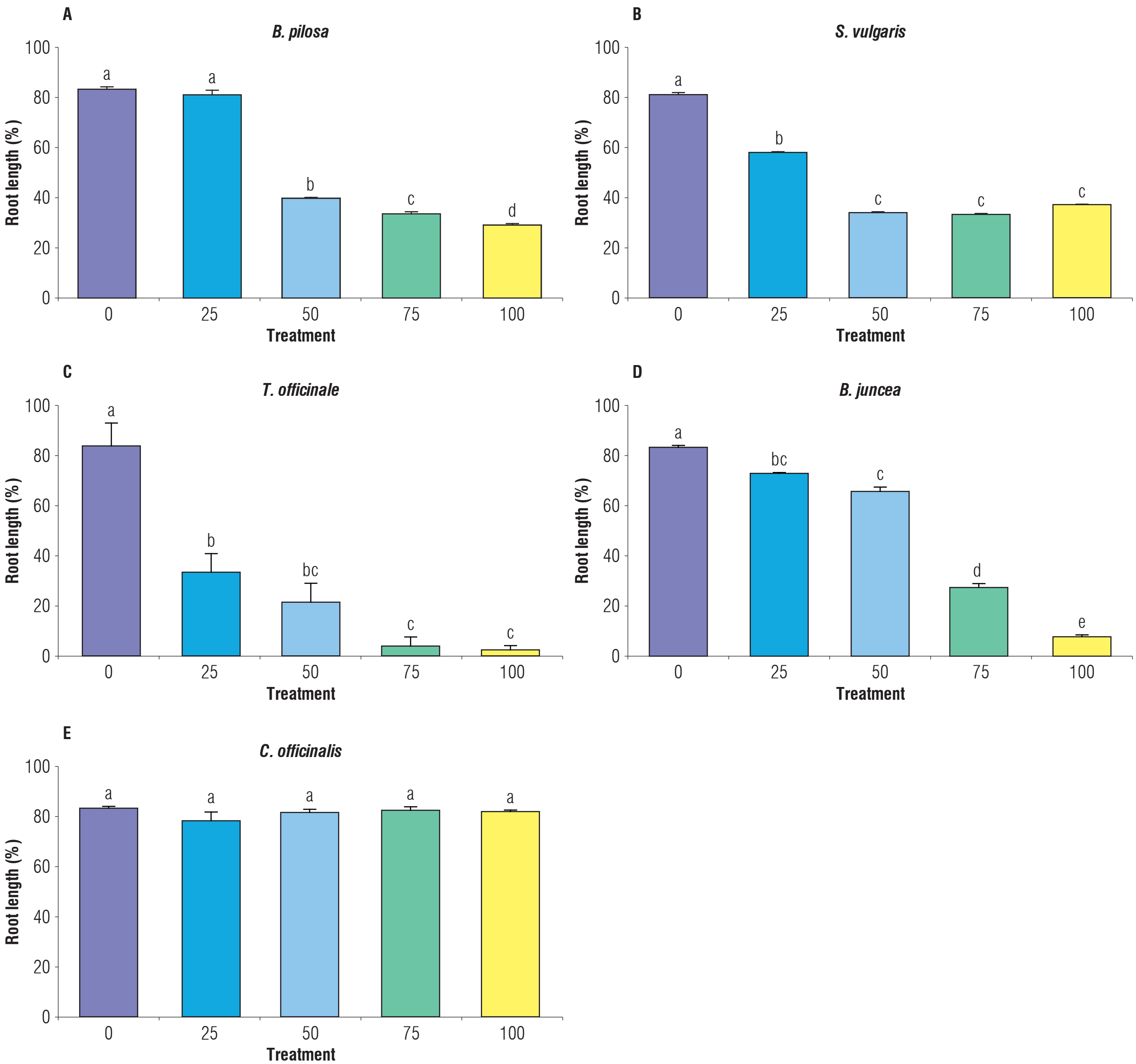

FIGURE 2. Root length expressed as a percentage relative to the control (0% extract) in seedlings of *C. officinalis, B. pilosa, S. vulgaris, T. officinale*, and *B. juncea* under different concentrations of *Artemisia absinthium* leaf extract. (-) extract at 0%, (-) extract at 25%, (-) extract at 50%, (-) extract at 75%, and (-) 100% extract concentration. Different lowercase letters indicate statistically significant differences between treatments according to the Tukey's test ($P \leq 0.05$), and the bars correspond to standard deviation.

including Basher *et al*. (1997), Li *et al*. (2021), Mirjalili *et al.* (2007), and Singh *et al*. (2009). These compounds have been described by numerous authors as insecticides (Ninkuu *et al*., 2021), antioxidants (Lee *et al*., 2013), and antibiotics (Ninkuu *et al*., 2021), among other functions. The results of the metabolomics analysis also indicate that multiple compounds in the extract have been associated with allelopathic responses, among which stand out artemisinin, limonene, β-myrcene, methyl eugenol or thujone, camphor, and 1,8-cineole (Li *et al*., 2021; Pouresmaeil *et al*., 2020).

In relation to the effect of the extract on seed germination, the extracted metabolites affected seed germination and seedling growth in some of the evaluated species (Figs. 1 and 2). The results show different types of responses to the exposure to the *A. absinthium* extract. On one hand, there was an inhibition of germination, with high sensitivity responses observed in *B. pilosa* and *T. officinale*. In contrast, *B. juncea* and *S. vulgaris* showed a dose-dependent sensitivity, while *C. officinalis* showed no sensitivity.

The inhibition in seed germination by allelopathic substances has been studied by numerous researchers, although the mechanisms causing the observed responses are not completely understood. Authors such as Bharati *et al.* (2014), Hasan *et al.* (2021), Pouresmaeil *et al*. (2020), Radhakrishnan *et al.* (2018), and Singh *et al.* (2009) suggested that these effects are the result of synergistic interactions among compounds. These authors report several modes of action that directly affect the germination process, such as inhibition of α-amylase activity, water uptake, alteration of gibberellic acid content, alteration of glycolysis enzyme activity, and interference in respiration. Additionally, exposure to bioactive compounds, such as linalool and cineole alcohols, generates germination inhibition (Cheng & Cheng, 2015; Li *et al*., 2021). Nikolova *et al*. (2023) reported the inhibitory activity on the germination of *Lolium perenne* seeds exposed to aqueous extracts of *Artemisia lerchiana* and *Artemisia santonicum* in doses 2 μl ml^{-1} and 5 mg ml^{-1}, respectively, which is associated with the presence of 1,8-cineole in the essential oil of the extract. On the other hand, Pouresmaeil *et al.* (2020) indicated the presence of α-thujone, camphor, 1,8-cineole and ß-thujone in the essential oil of *Artemisia fragrans* L., demonstrating that exposure to this essential oil inhibited the germination and growth of *Convolvulus arvensis*.

Regarding plant growth and development, a study conducted by Dayan *et al.* (1999) demonstrated that artemisinin not only inhibits plant growth but also specifically affects root growth in a concentration-dependent manner. The researchers suggest that this result may be attributed to the interference with mitosis, particularly through the disruption of microtubule formation, which consequently hampers cell division. This response was also reported by Verdeguer *et al*. (2020) who found that monoterpenes, such as limonene and pulegone (camphor) affected microtubule assembly in microorganisms. Similarly, Li *et al.* (2021) identified that one of the potential modes of action of the genus *Artemisia* may be attributed to the presence of 1,4-cineole and 1,8-cineole. These compounds have been reported as inhibitors of mitotic activity, leading to the inhibition of root growth (Li *et al*., 2021). This cellular damage was also noted by Chaimovitsh *et al.* (2017), who indicated that limonene accumulates in the tissues of transgenic *Arabidopsis thaliana* plants. This accumulation is hypothesized to generate stronger anti-microtubule agents and disrupt the normal membrane activities. Pouresmaeil *et al.* (2020) reported the inhibition of pigment biosynthesis, disruption of photosystem II, and induction of oxidative stress in cotyledonal leaves. Shao *et al.* (2018) pointed out that the extracts contain bioactive components such as 1,8-cineole. *Seriphidium terrae-albae* extracts showed phytotoxicity in species such as *Amaranthus retroflexus* and *Poa annua*, reducing the length of both the aerial part and the root, which is consistent with our findings regarding radicle length. Additionally, monoterpenes, such as camphor or 1,8-cineole, can affect cell proliferation and DNA synthesis in apical and root meristems, thereby decreasing root elongation in *Brassica campestris* (Koitabashi *et al*., 1997; Nishida *et al*., 2005).

In seeds of *B. juncea* and *S. vulgaris*, the dose-dependent effect of *A. absinthium* could be due to the seed capacity to conjugate or metabolize the bioactive compounds and to contain the oxidative stress that these can generate (Tian & Deng, 2020). This response could be attributed to the antioxidant metabolites present in seeds, which been quantified in multiple species, including *B. juncea* (Tian & Deng, 2020), *Brassica oleracea* (Tarasevičienė, *et al*., 2018), *S. vulgaris,* and *Senecio inaequidens* plants (Conforti *et al*., 2006), and *Zizania latifolia* (Chu *et al*., 2020), among others.

Since this is a metabolic response, the containment of the allelopathic effect depends on the seed capacity to counteract the effects of the substances to which they are exposed (Pouresmaeil *et al*., 2020). The results indicate that the seeds can partially resist the allelopathic effect of the metabolites (Fig. 1). However, when this inhibition is overcome, higher percentages of the G% inhibition are achieved (Fig. 1). These responses explain the negative correlation between exposure dose and G%. A dose-dependent response for G%

has been reported in studies of the allelopathic effect of the genus *Artemisia* by Li *et al*. (2021) in *Brassica pekinensis*, *Lactuca sativa*, and *Oryza sativa*, and by Pouresmaeil *et al*. (2020) in *C. arvensis*, among others.

The observed response in *C. officinalis* is associated with the presence of anatomical barriers of the seed coats and cell membranes (Victoria *et al*., 2007), which protect the seed from adverse external conditions (Radchuk *et al*., 2014). These barriers could limit the penetration of the *A. absinthium* extract into the seeds. Anatomically, *C. officinalis* seeds are larger and heavier compared to the other seeds used in this study, which may result in thicker seed coats (Victoria *et al*., 2007) that could further restrict the entry of bioactive compounds. Additionally, it is possible that the *C. officinalis* seeds can counteract the effect of the extract, as they contain more than 5% oxygenated fatty acids, which exhibit high antioxidant activity (Avato & Tava, 2022; Badami *et al*., 1965).

Conclusions

This study provides insights on the potential use of readily available plants in non-chemical weed management.

The extraction method used in this study proved to be efficient, as it had a bioactive effect on the seed germination of the studied species. Therefore, this method represents a replicable field methodology, which could be easily adopted by growers. Further studies on the response of these compounds under field conditions are recommended.

In an agricultural context, understanding the effects of allelopathic substances will enable advances in the development of natural herbicides, including the potential for selectivity by using specific seeds as inhibitors of the allelopathic effect or as protective agents to promote the growth and development of crops, promoting sustainable production practices. Additionally, using natural herbicides derived from plants, such *A. absinthium*, could reduce farmer's reliance on synthetic herbicides, offering environmental benefits by decreasing the amount of potentially harmful chemicals used in agriculture.

Acknowledgments

We thank the Universidad Nacional de Colombia for its support during the completion of this study. Special thanks are extended to all the university community for their technical assistance and valuable feedback throughout the research process.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

CBC and MDR designed the experiments, CBC and MDR carried out the laboratory experiments, CBC and MDR contributed to the data analysis, CBC and MDR wrote the article. All authors reviewed the final version of the manuscript.

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Challenges in the avocado production chain in Latin America: A descriptive analysis

Desafíos de la cadena productiva del aguacate en América Latina: un análisis descriptivo

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ABSTRACT

The avocado market has experienced significant growth in recent decades, with Latin America emerging as a key player in production and export. This article provides an overview of the main challenges facing the avocado production chain in the region, examining crucial aspects such as production trends, supply dynamics, evolving demand patterns, and price fluctuations. By addressing these challenges and capitalizing on emerging opportunities, the Latin American avocado industry can enhance its competitiveness, sustainability, and resilience in the global market. This review provides valuable insights and actionable strategies for stakeholders across the avocado production chain, from producers and exporters to policymakers and researchers, offering key working areas for the sustainable development of the avocado industry.

Key words: agribusiness, agricultural productivity, competitiveness, farm management, *Persea americana* Mill.

RESUMEN

El mercado del aguacate ha experimentado un crecimiento significativo en las últimas décadas, emergiendo América Latina como un actor clave en la producción y exportación. Este artículo ofrece una visión general de los principales desafíos que enfrenta la cadena de producción de aguacate en la región, examinando aspectos cruciales como las tendencias de producción, la dinámica de la oferta, la evolución de los patrones de demanda y las fluctuaciones de precios. Al abordar estos desafíos y capitalizar las oportunidades emergentes, la industria latinoamericana del aguacate puede mejorar su competitividad, sostenibilidad y resiliencia en el mercado global. Esta revisión proporciona información valiosa y estrategias viables para las partes interesadas en toda la cadena de producción del aguacate, desde productores y exportadores hasta formuladores de políticas e investigadores, ofreciendo áreas de trabajo clave para el desarrollo sostenible de la industria del aguacate.

Palabras clave: agronegocios, productividad agrícola, competitividad, gestión agrícola, *Persea americana* Mill.

Introduction

World avocado production has grown rapidly in recent years, increasing from an annual world production of 4.07 million t in 2011 to 8.69 million t in 2021. In the same period, Latin America contributed significantly to global avocado production, with Mexico, Colombia, and Peru adding 1.68 and 4.20 million t in 2011 and 2021, respectively (FAOSTAT, 2022). In 2022, the avocado market was estimated at USD 14.55 billion globally and is projected to reach USD 26.04 billion by 2030 (Grand View Research, 2022).

Latin America is the leading region in global avocado production. Mexico leads both in production and exports, while Colombia, Peru, and Chile are also significant players in the market (Schwartz *et al.*, 2018). These countries share three characteristics: (i) expansion of orchards in recent years, (ii) agroclimatic conditions suitable for the crop, and (iii) projected growth in exports (Arima *et al.*, 2022; Hass Avocado Board, 2022; OECD & FAO, 2021). Given this situation, the region is expected to maintain its leadership in avocado exports in the coming years (Arias *et al.*, 2018). It is estimated that by 2030, 74% of world avocado production will be concentrated in Latin America and the Caribbean (OECD & FAO, 2021).

This rapid growth poses challenges for the avocado market in terms of sustainable production (Sommaruga & Eldridge, 2021). These challenges include the incorporation of small farmers into international markets (Ospina Parra *et al.,* 2023), the role of institutions in supporting agribusiness development (Pérez & Gómez, 2022), the expansion of development of technological applications for the fruit and its subproducts in the food, pharmaceutical, and cosmetics

Received for publication: April 18, 2024. Accepted for publication: July 10, 2024. Doi: 10.15446/agron.colomb.v42n2.113982

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industries (Araújo *et al*., 2018), the growth of new orchards considering environmental and social factors (Denvir *et al*., 2022; Madariaga *et al*., 2021), the adoption of technologies in primary avocado production (Cáceres-Zambrano *et al*., 2023), and the technical challenges related to the crop's phytosanitary and nutritional management (Ramírez-Gil *et al*., 2017). The aim of this article was to describe the main challenges facing the avocado production chain in Latin America. From a competitive perspective, identifying an industry's challenges can lead to strategies to improve business performance (Porter, 1990).

The article is organized as follows: the methodology used for this integrative literature review; an overview of the avocado market, including production, supply, demand, and price trends; the nine key challenges identified for the avocado production chain in Latin America; and, finally, the summary of the main findings, discussing their implications and suggesting directions for future research and industry development.

Methodology

This study employed an integrative literature review methodology, following the guidelines proposed by Snyder (2019). The main purpose was to synthesize and classify the challenges facing the avocado production chain in Latin America. The research question focused specifically on identifying and analyzing these challenges. The search strategy was not systematic, allowing for a more flexible and broad exploration of relevant literature. Various sources were included, such as research articles and other published texts related to avocado production, imports, and exports. The analysis of the collected information was qualitative, enabling a deep interpretation of emerging themes. This approach facilitated the synthesis of findings and the identification of nine key challenges affecting the avocado industry in Latin America. The main contribution of this study is a comprehensive classification of these challenges, providing an integrated view of the issues facing the sector and establishing a foundation for future research and sustainable development strategies in the avocado industry.

Overview of the avocado market

The global avocado market has experienced significant growth in recent decades, with Latin America emerging as a key player in production and export. This section provides a comprehensive overview of the avocado market, examining crucial aspects such as production trends, supply dynamics, evolving demand patterns, and price fluctuations. By analyzing these key market indicators, we aim to establish a solid foundation for understanding the context in which the challenges facing the Latin American avocado industry have developed. This market analysis will help to frame the subsequent discussion of specific challenges and provide insight into the forces shaping the industry's future.

The avocado market

World avocado production increased by 920% from 1961 to 2019, growing from 0.71 to 7.3 million t, with Mexico leading the supply (Díaz Castellanos, 2021). Global avocado imports have been determined mainly by consumption in the USA and the European Union, with China emerging as a potential market in coming years (Arias *et al*., 2018). World imports have grown by around 172% in the past decade (Cruz-López *et al*., 2022). The Hass avocado is the most marketed internationally; its dominance has been attributed to consumer preference and the variety's resistant peel, which gives it the ability to survive the transportation process (Chaparro & Janzen, 2022). The average price per ton of avocados in the last two years was one of the highest for the main tropical fruits, reaching a maximum price of USD 3400 per t in March 2022 (FAO, 2023). However, this situation changed over the first nine months of 2023, with the average price dropping to USD 2063 per t, approximately 20% below the average for the same period in 2022 (FAO, 2024).

Supply

Avocados are grown throughout the world in the tropical and subtropical climates (Hurtado-Fernández *et al*., 2018). According to the Agricultural Outlook 2021–2030, avocado production is expected to reach 12 million t by 2030, making the avocado the second most commercialized tropical fruit globally, after bananas, with 3.9 million t exported (OECD & FAO, 2021).

Production

After Mexico, Colombia, and Peru are the primary leaders in production (Fig. 1). Mexico's share of global avocado production in 1961 was 15% and rose to 31% in 2019; in the same period, Colombia and Peru shares rose from 2% and 3%, respectively, to 7% each (Díaz Castellanos, 2021).

By 2021, global avocado exports were estimated to have grown to 2.5 million t, with Mexico the lead exporter, with 60% of the total (FAO, 2022). The country's exports are primarily sent to the USA (85%) and Canada (8%) (Cruz-López *et al*., 2022). Mexican avocado exports grew to USD 3 billion in 2021 (Statista, 2021). Mexico has significantly expanded avocado planting, production, and exportation, increasing from an annual production of 0.11 million t

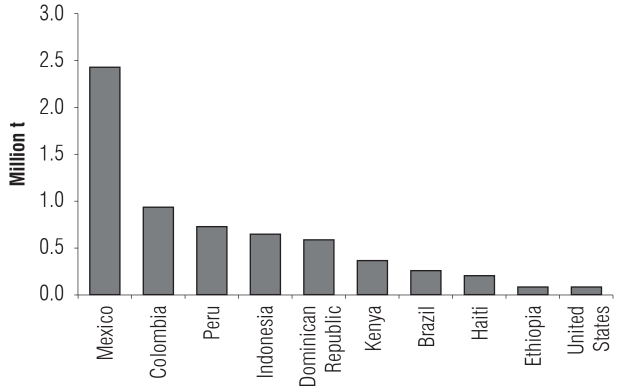

FIGURE 1. Avocado production and primary producing countries. Prepared by the authors using FAO (2022) data.

in 1961 to 2.3 million t in 2019 (Díaz Castellanos, 2021). Mexican dominance can be attributed to its geographic location (proximity to the main import market) and its comparative competitive advantage related to cost efficiency and availability of production factors – labor and capital (Cruz-López *et al.*, 2022). Furthermore, Mexico can produce fruits in all seasons, focusing on high-quality Hass avocado production (FAO, 2023).

Avocado production in Colombia has grown in recent years, from around 490,000 in 2017 to 597,000 t in 2019 (Orrego *et al.*, 2021). In terms of exports, about 92% is sent to the European market, mainly the Netherlands, the United Kingdom, Spain, Belgium, France, and, to a lesser extent, to the USA (Hass Avocado Board, 2022). Colombia is poised to become a leading actor in the exportation of Hass avocados, with projections for an increase from 200,000 t in 2022 to 700,000 t by 2030 (Hass Avocado Board, 2022). The value of Colombian exports in 2021 reached USD 204.6 million, making Colombia the world's sixth largest avocado exporter (Statista, 2021). Its export potential is attributed to: (i) the pacification in agricultural production areas, *i.e.*, territory that was formerly under the control of illegal armed groups that now is dedicated to avocado production; (ii) the availability of land at relatively low cost; (iii) the country's agroclimatic advantages, including yield potential, year-round production availability, natural soil fertility, and the ability to irrigate avocado crops with rainwater; and (iv) the country's strategic geographic location, which facilitates serving key import markets in Europe and the USA (Hass Avocado Board, 2022).

In Peru, Hass avocado orchards grew from 22,499 ha in 2015 to 50,699 ha in 2021 (ProHass, 2022), positioning the country as the second global exporter by volume, with 26% of global exports in 2022 (FAO, 2023). The export value of Peruvian Hass avocados reached USD 1 billion in 2021 (Statista, 2021). Peruvian Hass avocado exports increased from 74,723 t in 2012 to 483,017 t in 2021; 57% of exports targeted Europe – mainly the Netherlands, Spain, and England, followed by the USA with 18% (ProHass, 2022).

Chile is the third largest avocado exporter in the world. The value of the country's exports was USD 213.8 million in 2021 (Statista, 2021). The main destinations for Chilean avocado exports were the Netherlands with 46.83%, Argentina with 13.97%, the United Kingdom with 12.38%, and Spain with 8.87% (Oficina de estudios y políticas agrarias, 2022). Its strong export capacity has been attributed, in part, to the role that institutions have fostered in meeting import market requirements, mainly for the USA, Europe, and more recently, China, as well as to favorable international prices and exchange rates offered (Guevara *et al.*, 2021; Schwartz *et al.*, 2018).

Yield

Avocado yield is typically measured as production per ha (Ramírez-Gil *et al.,* 2017), though it can also be quantified by total fruit weight, fruit count, or individual fruit weight (Rojas-Rodríguez *et al.*, 2023). In Latin America and the Caribbean, the Dominican Republic's performance was superior among the evaluated countries (Fig. 2). Some elements that may explain this behavior are the country's institutional effort via extension services, support for technology adoption and phytosanitary protection capacity building, and favorable agroclimatic conditions (González *et al.*, 2009). It is necessary to investigate whether the strategies implemented by this country could be implemented in Latin American countries to improve productivity. Following the Dominican Republic, Brazil showed the second

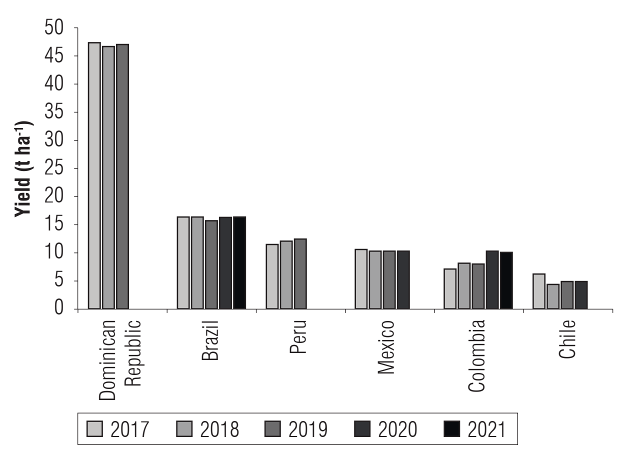

FIGURE 2. Avocado yield (t ha^{-1}) in Latin America and the Caribbean. Prepared by the authors using Statista (2021) data.

highest performance in avocado production, which may be related to the efforts underway to face technological and technical production challenges (Moraes *et al*., 2022). Peru's performance has been attributed to the implementation of best practices in production (ProHass, 2022). In the case of Mexico, despite some productive areas showing high performance levels, others have been held back by high production costs (Cruz-López *et al*., 2022). In Colombia, efforts have been made to improve production; however, low levels of competitiveness have also been evident, particularly in productivity (Hass Avocado Board, 2022). Additionally, Chilean production has been reported to be losing efficiency (Guevara *et al*., 2021).

Demand

The world import volume of avocados increased from 0.0021 million t in 1961 to 2.7 million t in 2019, an increase of 128,571% (Díaz Castellanos, 2021). By 2019, world imports rose to USD 7.2 billion (Orrego *et al*., 2021). In 2022, world imports were 2.3 million t (FAO, 2023). The increase in consumption of this fruit has been attributed to its health, nutrition, and flavor benefits (Ballen *et al*., 2022). Additionally, Cheikhyoussef and Cheikhyoussef (2022) stated that the fruit's properties enable it to be used not only in the food industry but also in the pharmaceutical and cosmetics industries. The main import markets for avocados are the USA and the European Union.

The USA has been the main global importer of avocados since 2005, displacing the dominance held by France from 1970 to 2000 (Díaz Castellanos, 2021). In 2021, the USA accounted for 46% of global avocado imports (FAO, 2022). In 2022, the USA imported approximately 1 million t (FAO, 2023). The US market is supplied mainly with avocados from Mexico and Peru, along with local production from California (Chaparro & Janzen, 2022). Ballen *et al*. (2022) stated that the USA demand for avocados grew from 0.53 million to 1.14 million t from 2009 to 2018, with the Hass variety constituting around 97% of the total avocado supply in the country. Ambrozek *et al*. (2018) indicated that per capita consumption increased 344% from the 1990s to the 2014–2016 period, increasing from 0.73 to 3.2 kg. This increase in demand has been attributed, in part, to Hass Avocado Board promotional activities/programs related to the nutritional and health benefits of avocado consumption.

The European Union is the second most important avocado market in the world. In 2021, it accounted for 27% of global imports, equivalent to 0.8 million t (FAO, 2023). According to the Centre for the promotion of imports from developing countries (CBI) of the ministry of foreign affairs of the Netherlands, imports increased from 0.52 million t in 2017 to 0.8 million t in 2021, with an average per capita consumption in Europe in 2021 of 1.4 kg (CBI, 2023), led by Norway (2.87 kg) followed by the Netherlands (2.85 kg) and Denmark (2.65 kg) (Statista, 2023). The Netherlands is the main importer in the European Union, with 9% of global imports (Guevara *et al*., 2021). The value of exports for the Netherlands was USD 1.2 billion in 2021, which places it as the second largest exporter in monetary terms (Statista, 2021). This is because the country basically operates as a re-export platform to other markets in the region (Arias *et al*., 2018).

Xiong and Song (2018) stated that China is becoming a significant avocado importer; from 2012 to 2016, the value of imports was equivalent to USD 60 million per year. These authors found that the increase in imports to China responded to the country's economic growth and changing consumption preferences (growing interest in avocados). Despite the potential growth of the Chinese market, its share of avocado consumption in the Asia–Oceania region is 12%, below that of Indonesia, which accounts for around 50% of consumption. China's per capita consumption is the lowest in the region at 0.2 pounds (Huang *et al*., 2023).

Price

The average price of a t of exported avocado was one of the highest for tropical fruits, reaching a peak of USD 3400 per t in the 2020–2022 period (FAO, 2023). International avocado prices have shown an upward trend in the past few years, which is attributed to demand being higher than supply (Arias *et al*., 2018). However, this trend may reverse if production surpasses demand, leading to a drop in prices (Huang *et al*., 2023). In fact, prices experienced a significant decline in 2023, primarily due to a substantial increase in global supply, which outpaced demand (FAO, 2024).

International avocado prices are sensitive to multiple factors, including climate, pests, seasonal supply from some countries, the degree of intermediation, fruit size, and the target market (Arias *et al*., 2018). Avocado prices in importing markets vary significantly compared to prices in countries of origin. In Mexico, a kilo of avocados destined for the USA costs between USD 1.00 and 2.50 (Herrera-González *et al*., 2020). The average prices in Colombia and Peru for Hass avocados paid to the producer were around USD 0.97 per kg and USD 0.99 per kg, respectively (Orrego *et al*., 2021). Conversely, in the USA, the average price is around USD 3 per kg, with prices as high as USD 8 per kg (FAO, 2023).

A review of historical and current trends in the global avocado market, as reported by the FAO (2024), reveals a significant shift in price dynamics. In contrast to the upward price trends observed over the previous decade, the current market situation suggests a potential stagnation or even decline in international avocado prices. This reversal is primarily attributed to the substantial growth in global supply, with Mexico playing a pivotal role. The FAO report highlights those Mexican avocado exports are expected to reach approximately 1.5 million t in 2023, a 27% increase from the previous year. This surge in supply, outpacing demand growth, has exerted downward pressure on prices, resulting in the lowest average export unit values seen in nearly a decade. This development underscores the need for careful consideration of market dynamics in planning future avocado production expansions.

Likewise, the avocado market is experiencing significant shifts in global trade patterns. While Mexico remains the dominant supplier to the USA market, there have been notable changes in the role of other exporters. Chile, once a major supplier to the USA, has been redirecting its exports to the European Union and China, diversifying its market presence. This shift presents opportunities for other producing countries to fill the supply gap in the USA market.

Simultaneously, there is a growing trend in organic Hass avocado production and trade. According to USDA Foreign Agricultural Service's Global Agricultural Trade System (GATS) data, imports of organic Hass avocados have been increasing in both quantity and value. Specially, USA imports of organic Hass avocados grew from about 49,000 metric t valued at more than USD 138 million in 2019 to more than 60,000 metric t valued at approximately USD 164 million in 2023, indicating promising growth in demand for organic avocados (USDA, 2024).

Challenges for the avocado production chain

The avocado market is experiencing significant growth, driven by relatively favorable prices that have spurred both the production and exports of the fruits. Latin America stands out as the dominant region for avocado supply. However, this favorable scenario also poses a series of challenges throughout the production chain that must be addressed to guarantee business sustainability. These challenges were identified from a market analysis and literature review, which are described in this section of the article. These challenges start with the need for sustainable production and industrial transformation of the fruits, extending to the complexities of commercialization in international markets, and the need for consumption markets to adapt in response to sustainability efforts in the productive chain.

Strengthening management capacity among producers

Access to external markets is a challenge for avocado producers, especially in terms of developing management capacity that would enable them to take advantage of these opportunities effectively. The management capacity of farmers can be defined as (i) possessing suitable personal characteristics and (ii) decision-making ability to face problems and opportunities in a timely and sound manner. In other words, it is the ability to run the farm business in an efficient way to obtain desired farm outcomes (Rougoor *et al*., 1998). It is expected that a farmer with high management capacity will obtain better farm results in terms of productivity, profitability, and efficiency (Taramuel-Taramuel *et al*., 2023).

Changes in the global avocado market stemming from rapid growth in demand, the expansion of orchards in Latin America, and quality and food safety requirements imposed by food and non-food markets in Europe and the USA necessitate a transition from a traditional farming approach to sustainable farming management. In this change, the producer must see him or herself not only as a farmer but also as a manager, treating production as a business involving not only the technical aspects of farming but also strategic and commercial management, innovation, and social and environmental responsibility. For this, public and private interventions targeted at strengthening the management capacity of avocado producers must be promoted, as they could improve business productivity and profitability (Taramuel-Taramuel *et al*., 2023).

Greater government involvement in agribusiness development

Most countries in Latin America are middle-income, and the state can play an essential role in the development of the avocado production chain. First, R&D investment in the avocado sector needs to be strengthened, given that this expense is associated with increases in agricultural productivity (Fuglie *et al*., 2020; Taramuel *et al*., 2021). Second, it is indispensable to strengthen public technical extension and farm management programs since they are positively correlated with better technical crop performance (Mariano *et al*., 2012). Third, the role of the state in providing infrastructure is essential in strengthening the avocado export industry, for example, in the development of logistics from avocado production sites to the port of export (Hass Avocado Board, 2022). Fourth, the promotion of associativity has been recognized as a mechanism for rural development, especially for small farmers, to gain greater market advantages (Gutiérrez, 2014). Fifth, access to public subsidies can also sustain avocado business development, particularly when prices fall, or adverse climatic events cause problems. Pérez and Gómez (2022) emphasized the critical role played by institutions, including government agencies and trade unions, in the consolidation of the avocado export chain via (i) negotiation of avocado access to international markets, (ii) promotion of small and medium producer associations and cooperatives, and (iii) provision of public agricultural extension services.

Integration of small avocado producers into global markets

Higher prices in import markets are an opportunity that could potentially be leveraged by small and medium avocado producers from Latin America. However, admission to these markets may be conditioned on quality standards, food safety, and other technical requirements which contrast to national markets (Amare *et al*., 2019). To face this challenge, strategies could include (i) strengthening organizing processes, (ii) providing technical assistance, (iii) adopting certifications, (iv) improving fruit quality, and (v) using contract-based agricultural schemes (Ospina Parra *et al*., 2023). In addition, tools for market management, including digital tools, can improve farmer's marketing activities (Romero-Sánchez & Barrios, 2022).

Pérez and Gómez (2022) discussed two strategies – vertical integration and associativity – targeting avocado export markets. Vertical integration is usually adopted by large organizations that implement actions such as (i) export-type (Hass) avocado production, (ii) fruit packaging, (iii) international commercialization, (iv) diversification of agricultural production and investments, and (v) implementation of sustainable production practices. Associativity consists of collaboration among small and medium avocado producers, focusing on entry into national and external markets by strengthening the commercialization of agricultural products via certifications and a supply chain infrastructure that grants access to international markets.

An emerging opportunity for small avocado producers lies in the growing demand for organic avocados in global markets. While this transition requires initial investments and certification processes, it can lead to premium prices and access to niche markets. Cooperatives and associations can play a crucial role in helping small producers navigate the organic certification process, achieve economies of scale in production and marketing, and connect with international buyers seeking organic avocados. By tapping into the organic market, small avocado producers can potentially increase their competitiveness and profitability in the global marketplace.

Incorporating technological innovations to improve business operations

One strategy for improving productivity and profitability is the adoption of agricultural innovations (Fuglie *et al*., 2020). In the avocado sector, joining forces to improve efficiency in production and commercialization is essential to contributing to the business's continuity in the market.

It is critical to promote the incorporation of cutting-edge innovations to improve productivity. First, technologies are needed to optimize communication with customers; marketing via digital tools could benefit business operations. Romero-Sánchez and Barrios (2022) propose the adoption of e-commerce in the fruit and vegetable sector as an opportunity to decrease intermediaries in commercialization, lower operational and transactional costs, improve producer–consumer communication, and increase consumer satisfaction and loyalty. Second, López-Pimentel *et al*. (2022) highlighted the importance of strengthening the traceability of the avocado supply chain using blockchain, a key element in reaching international markets. Blockchain, a distributed ledger technology that creates an immutable and transparent record of transactions, offers several benefits for the avocado industry: (i) it enhances traceability by enabling detailed product tracking from farmer to end consumer, (ii) it increases transparency across the supply chain, reducing the risk of fraud and improving consumer trust, and (iii) it facilitates quick and accurate verification of certifications and quality standards (Granillo-Macías *et al*., 2023). The implementation of these innovations is directly related to increasing trust from the end consumer, which could significantly contribute to expanding the avocado market.

The adoption of emerging technologies in avocado agribusiness is an opportunity to improve efficiency throughout the production chain. However, low adoption of technologies has been reported in avocado production systems in Latin America (Cáceres-Zambrano *et al*., 2023; López-Pimentel *et al*., 2022). Therefore, it is necessary to continue implementing policies/programs that integrate emerging technologies in avocado farms, addressing technical and management aspects to foster sustainability and profitability in the avocado sector.

Expansion of industrial applications for the avocado

Avocado pulp and oil have applications in the food, cosmetics, and pharmaceutical industries. Duarte *et al.* (2016) reviewed the fruit's main attributes: (i) high nutritional value due to its containing lipid-soluble vitamins, proteins, potassium, and unsaturated fatty acids; (ii) health benefits due to the bioactive components of the fruits, including omega fatty acids, phytosterols, tocopherols, and squalene, with phytosterols helping lower cholesterol and prevent cardiovascular diseases; and (iii) avocado oil uses in developing perfumes, producing avocado oils commercially, and preparing flour for baked goods.

Despite considerable development of industrial applications for pulp and oil, there is potential to take advantage of waste, such as pits and leaves. Cheikhyoussef and Cheikhyoussef (2022) reported that typically 50-80% of the fruit is used after processing, with the rest considered waste, including the pit, peel, and unprocessed pulp, leading to around 40% of the fruit being discarded or wasted. Avocado waste can be used in the production of animal feed, oil, microbiological crop media, starch, biodiesel, fuel, biopolymers, and other value-added products (Araújo *et al.*, 2018). Thus, it is necessary to continue exploring the nutraceutical and medicinal applications of fruits that may contribute to strengthening the production chain.

Planning for new orchards

Favorable prices and the upward trend in avocado consumption have driven the creation of new groves to meet this demand. However, in some regions, the expansion of avocado has not been organized, neglecting key factors, including climate, soil, infrastructure (access roads), and the exclusion of orchards in protected areas, such as reserves and natural parks (Anacona Mopan *et al.*, 2023).

In Latin America, Grüter *et al.* (2022) assessed the appropriate distribution of avocado production, considering climatic conditions and biophysical and soil requirements. Their analysis revealed that there are highly suitable regions in Honduras, Venezuela, Bolivia, and Brazil. In the case of Mexico, the Dominican Republic, and Peru, the suitability of avocado orchards is limited by climatic factors and, to a lesser extent, by soil requirements. The analysis also shows that in the climatic conditions of 2050, rising temperatures could be beneficial for avocado orchards in the USA, Brazil, Uruguay, Paraguay, and Argentina. In Mexico, future climatic conditions are expected to favor avocado production, while in Peru and the Dominican Republic, changes in climatic conditions could reduce the zones suitable for avocado crops. Ramírez-Gil *et al.* (2018) also called for establishing new orchards in Colombia to respond to suitable conditions, noting that orchards in highly suitable regions with greater levels of technology use had better yields.

The viability of new avocado orchards hinges critically on future price trends. Recent market developments, characterized by significant price declines due to substantial increases in global supply, particularly from Mexico, raise concerns about the economic feasibility of new orchard investments. This situation underscores the importance of careful market analysis and strategic planning in the expansion of avocado production, especially considering the long-term nature of orchard investments and the time lag between planting and full production capacity.

Likewise, a critical challenge for the avocado industry in Latin America is the implementation of sustainability practices throughout the production chain. To address this, the industry should adopt a Triple Bottom Line (TBL) approach, which balances economic, environmental, and social aspects of the business (Elkington, 1998). This holistic strategy not only mitigates the risk of market restrictions but also enhances long-term viability and consumer trust. Successful implementation of TBL in the avocado sector requires transparent practices, rigorous environmental stewardship, and proactive engagement with local communities, ensuring that economic growth aligns with ecological preservation and social well-being.

Responsible water management in primary production

The unplanned expansion of orchards is associated with forest fragmentation, loss of biodiversity, and imbalances in land use and hydrological systems (Denvir *et al.*, 2022). In terms of the avocado's water footprint, Sommaruga and Eldridge (2021) estimated that, on average, 849 m^3 of rainwater and 237 m^3 of surface and ground water per t are needed. These levels of consumption are higher than those reported for the average of all fruits (727 and 147 m^3 t^{-1} for rainwater and surface and ground water, respectively).

In some regions of Latin America, crop expansion has been associated with water scarcity. Panez-Pinto *et al.* (2018) found that water use for avocado production has been far higher than water use for regional human consumption. Madariaga *et al.* (2021) argued that the influence of avocado producers has allowed the expansion of the crop despite demands from communities that have experienced insufficiency in water availability.

Deforestation is a significant consequence of avocado crop expansion, particularly in Mexico's main avocado-producing region Michoacán. Cho *et al.* (2021) attributed 17% of total deforestation in the region to avocado plantation growth between 2001 and 2017. This environmental impact is largely driven by the growing demand for avocados in the USA, facilitated by complex supply chains lacking transparency and environmental accountability. The study employs innovative methods to map these supply chains, linking the USA retailers to Mexican producers and revealing a disconnect between actual environmental damage and industry perceptions. To address the deforestation associated with avocado production, the authors suggest implementing improved supply chain governance, increasing transparency, and developing multi-stakeholder initiatives. Projected crop expansion will contribute to further degradation of hydrological ecosystems (Arima *et al.*, 2022).

Although avocado crop expansion leads to economic opportunities, such as new sources of income (agricultural diversification) and job creation for farmers in Latin America, avocado production needs to be promoted based on balancing the production system with its surroundings, involving responsible use of water and natural resources.

Social impact stemming from crop expansion

To maintain Latin America's leadership in avocado production, it is important for the business to be socially sustainable. Some authors have drawn attention to the social costs of crop expansion. Vega-Rivera and Merino-Pérez (2021) reported that the rapid expansion of the business has had social consequences, some of which include: (i) land seizures, (ii) changes in subsistence livelihoods, including the loss of peasant farming of staple foods, (iii) land use conflicts, (iv) increased violence (armed groups use the avocado as a platform for money laundering), and (v) increased income inequality (a few large agribusiness concentrate profits along the production chain).

Overcoming these challenges requires coordination among social actors. The state should engage in (i) policymaking, in conjunction with avocado producers, to address land concentration through programs for small and medium producers, (ii) greater institutional presence to limit the operation of illegal businesses around avocados, and (iii) regulation of crop expansion in areas with vulnerable populations. In addition, it is necessary to promote corporate social responsibility, in which avocado agribusinesses review their actions towards local communities and implement practices that contribute to society. For example, avocado producing companies should ensure fair and safe labor conditions. These joint actions can minimize the social costs of the crop's rapid growth.

Improving technical crop management

Technical barriers in terms of pests, diseases, and other phytosanitary risks can limit avocado commercialization in international markets. Peterson and Orden (2008) estimated that if the United States had lifted restrictions on the import of Mexican Hass avocados and applied effective phytosanitary measures in 2004, it could have potentially increased net profits for the USA by USD 77.4 million annually.

Technical challenges can negatively impact the economic performance of avocado agribusiness. Ramírez-Gil *et al.* (2017) estimated that the avocado wilt complex generated economic losses of up to USD 420.50 in the nursery stage in a 1-year production cycle, while in the establishment and production stages, these losses could reach USD 2565 per ha in an 8-year period due to plant mortality and decreased production.

Diseases that occur at harvest and postharvest, including anthracnose and stem end rot, can limit commercialization of the fruit in export markets and cause economic losses due to discarded fruit and decreases in the fruit shelf life and quality (Herrera-González *et al.*, 2020). For example, Ramírez-Gil *et al.* (2020) reported that anthracnose and stem end rot cause bruises on the fruits, which leads to the rejection of up to 70.1% of the fruits at export packaging facilities. The cost of discarding fruits due to these diseases is estimated at USD 13.44 per t of production. In summary, crop health issues may limit the production and commercialization of the fruits; therefore, it is necessary to continue working on exploring alternatives to control pathogens, including biological control, to foster avocado production.

One promising strategy is the use of biostimulants in avocado production systems. Recent research by Rojas-Rodríguez *et al.* (2023) has shown that biostimulants can significantly improve yield and preharvest quality in both traditional and organic avocado production systems. Incorporating biostimulants into integrated crop management plans could be a valuable tool for avocado producers to enhance productivity and fruit quality, thereby addressing some of the technical challenges in avocado cultivation.

Conclusions

The avocado production chain has rapidly consolidated in recent decades. The main producers and exporters of this fruit are in Latin America, while imports are driven primarily by the USA and the European Union. The increase in demand is related to the fruit's attributes, making them valuable in the food, pharmaceutical, and cosmetics industries. However, the accelerated growth of the avocado market leads to challenges that must be considered to ensure business sustainability. Nine challenges were identified for avocado agribusiness: (i) strengthening management capacity among primary producers, *i.e.*, strengthening decision-making skills to ensure the business's economic success; (ii) greater government involvement to provide public goods, such as infrastructure, extension promotion, financing, and associativity; (iii) integration of small farmers into global markets to leverage the benefits offered by these markets; (iv) incorporation of technological innovations in business operations, via the adoption of technical and management tools, especially those that are emerging, to increase agricultural income, optimization of resource use, and improvement in commercialization; (v) expansion of industrial applications, in both fruit products and subproducts, to consolidate the business industrial transformation; (vi) planning for new orchards, which involves considering biotic and abiotic factors; (vii) responsible water management in primary production, a key aspect in contributing to production sustainability; (viii) considering social impacts stemming from crop expansion to ensure business viability; and (ix) improving technical crop management, aspects related to crop diseases and pests to prevent limits on its entry into international markets.

The analysis shows that it is possible to achieve sustainable business development by addressing challenges related to social and environmental costs in the primary production of avocados and agro-industrial development in the transformation stage and fostering consumption that supports efforts toward more sustainable agricultural production. Future research should focus on exploring strategies to confront each of the challenges identified, with the goal of the sustainable development of the avocado production chain.

Acknowledgments

The authors thank Professors Liliana Chicaiza and Edison Jair Duque of the Faculty of Economic Sciences at the Universidad Nacional de Colombia for their comments on the earlier version of the paper. This article is part of J. P. Taramuel-Taramuel doctoral research.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

JPTT and DB conceptualized the manuscript. JPTT, IAMR, and DB wrote the manuscript. All authors critically revised the manuscript and approved the final version of the manuscript.

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Evaluation of entrepreneurial intention in agronomic engineering students

Evaluación de la intención emprendedora en estudiantes de ingeniería agronómica

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ABSTRACT

Entrepreneurship has emerged as a crucial factor in economic development, especially in developing nations, where the COVID-19 pandemic has exacerbated socioeconomic challenges, particularly in rural areas. Despite these adversities, countries like Colombia boast abundant natural resources and a dynamic young population, creating a conducive environment for sustainable economic growth. In this context, the main objective of this study was to conduct a comprehensive analysis of the factors influencing entrepreneurial intention in academic settings related to agriculture. To achieve this, a structural equation modeling was conducted on a sample of 200 agronomic engineering students at the Universidad Nacional de Colombia. This analysis identified the positive impact of entrepreneurial self-efficacy and opportunity recognition on entrepreneurial intention. The research focused on understanding entrepreneurial spirit among young individuals, acknowledging its significance as a driver of economic and social development.

Key words: businesses, enterprises, management.

RESUMEN

El emprendimiento se posiciona como un factor crucial para el desarrollo económico, especialmente en naciones en desarrollo, donde la pandemia de COVID-19 ha agravado los retos socioeconómicos, sobre todo en áreas rurales. A pesar de estas adversidades, países como Colombia cuentan con vastos recursos naturales y una población juvenil dinámica, creando un escenario propicio para un crecimiento económico sostenible. En este contexto, el objetivo principal de este estudio es realizar un análisis exhaustivo de los factores que inciden en la intención emprendedora en entornos estudiantiles relacionados con la agricultura. Para lograr este objetivo, se llevó a cabo un modelo de ecuaciones estructurales en una muestra de 200 estudiantes de ingeniería agronómica en la Universidad Nacional de Colombia. Este análisis permitió reconocer el impacto positivo de la autoeficacia emprendedora y el reconocimiento de oportunidades en la intención emprendedora. La investigación se centró en comprender el espíritu emprendedor de los jóvenes, reconociendo su importancia como motor del desarrollo económico y social.

Palabras clave: empresas, gestión, negocios.

Introduction

Entrepreneurship has emerged as a key component in driving economic development and job creation globally. It is defined as the process of establishing a new business, encompassing everything from developing the business plan to implementing strategies and managing associated risks (Muñoz & Dimov, 2023). At a global level, countries are focusing their efforts on supporting entrepreneurship. With a growing demographic and an increasing demand for employment, there is an urgent need to establish new businesses to accommodate this workforce (Zen *et al*., 2023). This phenomenon could have a particularly positive impact on developing nations like Colombia, where the COVID-19 pandemic has triggered a humanitarian crisis that severely affected low- and middle-income communities, especially in rural areas. In Colombia, the contribution of the agricultural sector to the country's GDP decreased from 14% in 1995 to 6% in 2020 (Cámara de Industria y Comercio Colombo-Alemana, Cámara de Comercio de Medellín para Antioquia & Institución Universitaria EUSUMER, 2021). Nevertheless, Colombia has significant opportunities for sustainable growth in rural regions, leveraging its biodiversity, fertile lands, young population, abundant freshwater resources, and access to renewable energy such as wind and solar power (Organization for Economic Co-operation and Development-OECD, 2022). Despite these comparative advantages, rural entrepreneurship in Colombia needs a stronger foundation to promote rural economic growth. However, according to the Global University Entrepreneurial Spirit Students' Survey (GUESSS), entrepreneurial intention has increased in the country, particularly since the COVID-19 pandemic, driven by entrepreneurship policies and the university environment (Martins *et al*., 2021).

Entrepreneurial success largely depends on the skills and characteristics possessed by entrepreneurs. Regardless of the field in which they operate, these individuals exhibit a

Received for publication: April 13, 2024. Accepted for publication: August 30, 2024. Doi: 10.15446/agron.colomb.v42n2.115744

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range of fundamental abilities, among which recognition of opportunities and self-efficacy stand out. Self-efficacy is defined as a person's conviction in their ability to manage and control events and situations affecting daily life (Lopez-Garrido, 2023). This quality, essential in the entrepreneurial context, entails belief in the possibility of establishing and successfully directing a business. On the other hand, recognition of opportunities refers to the capacity to identify business prospects that were previously unknown (Sautet, 2016).

In addition to these skills, various factors, such as gender and entrepreneurial education, influence the entrepreneurial process. Evidence suggests that entrepreneurship may exhibit a gender bias, with women showing less willingness than men to embark on entrepreneurial ventures due to lower self-efficacy and openness to new experiences (Elshaer & Sobaih, 2023). In contrast, higher education plays a crucial role in promoting entrepreneurship and fostering an entrepreneurial culture among young people. Both self-efficacy and recognition of opportunities are influenced by the university environment, which in turn impacts the entrepreneurial intention of students.

According to Liñán and Chen (2009), self-efficacy is a significant predictor of entrepreneurial intention, indicating that students who have confidence in their entrepreneurial abilities are more likely to have entrepreneurial intentions. Furthermore, the perception of opportunities also influences this intention. Fayolle and Gailly (2015) found that students who perceive more entrepreneurial opportunities are more likely to have entrepreneurial intentions.

A study conducted by the Organization for Economic Co-operation and Development (OECD) in 2021 revealed that universities play a significant role in fostering entrepreneurship and creating new businesses. Universities can influence the entrepreneurial intention of students, as these institutions can provide them with the knowledge, skills, and tools necessary to become successful entrepreneurs and create innovative new ventures (Fayolle & Gailly, 2015).

Today, the competition in the job market has increased significantly. This implies that students are increasingly considering entrepreneurship as an option due to the evident saturation of the job market. Barba-Sánchez *et al.* (2022) argue that this trend has led to students demanding new and competitive tools, including entrepreneurial education, from universities. This educational approach is essential for preparing future entrepreneurs and providing them with the necessary resources to establish businesses in a saturated labor market. Additionally, Syed *et al.* (2020) suggest that the drive towards entrepreneurship may be influenced by individuals' passion and interest. Therefore, it can be inferred that there is a growing interest among students in entrepreneurship, which in turn drives the demand for education focused on this area.

Given the central role of agriculture in rural development, agronomic engineering students have a unique opportunity to contribute to Colombia's sustainable growth through entrepreneurship. Understanding the factors that influence their entrepreneurial intention, such as self-efficacy and opportunity recognition, is essential for designing effective educational programs that foster these skills. Therefore, the aim of this research was to assess the entrepreneurial intention of agronomic engineering students and provide a theoretical basis for developing academic programs that enhance their entrepreneurial skills.

Materials and methods

Sample size

The determination of the minimum sample size was based on the work of Westland (2010), who established a metric using Monte Carlo simulations and considered the relationship between the number of latent variables and items. The resulting formula is $n \geq 50r^2-450r+1100$, where r represents the relationship between items and constructs, and n is the sample size. In this study, the model proposed by Hassan *et al.* (2020) was utilized, which incorporates four latent variables evaluated in twenty-two items, with a relationship of 5.5. Based on this model, the necessary minimum sample size was determined to be 137 individuals. However, to ensure the validity and reliability of the study, a sample of 200 agronomic engineering students was selected.

Data collection and description of the measurement instrument

The study enlisted a sample of 200 agronomic engineering students enrolled in the Faculty of Agricultural Sciences at the Universidad Nacional de Colombia, Bogotá campus. Data were collected online via a Google Forms questionnaire administered between May and August 2023. The questionnaire was structured into two sections. The first section focused on demographic information, including age, gender, and family business experience, to understand the social and economic backgrounds, as well as family exposure to entrepreneurship among the respondents. The second section featured the Likert questionnaire adapted from Liñán and Chen (2009) and Ozgen and Baron (2007) (Tab. 1). This section included validated questions designed

TABLE 1. Latent variables and their items to measure entrepreneurial intention in agronomic engineering students.

Construct	Variable name	Item
Entrepreneurial self-efficacy	SELF1	I can control the creation process of a new business
	SELF2	If I tried to start a business, I would have a high probability of success
	SELF3	Starting a business and keeping it functional would be easy for me
	SELF4	I know the necessary practical details to start a business
	SELF5	I am prepared to start a viable business
	SELF6	I know how to develop an entrepreneurial project
Opportunity recognition	OPT1	I see many opportunities to start and grow a business
	OPT2	Finding potential venture opportunities is easy for me
	OPT3	In general, there are many opportunities for new product innovation
	OPT4	I have a special sense of new venture ideas
	OPT5	During my routine day-to-day activities, I see potential new venture ideas
Entrepreneurship education	EDU1	Knowledge about the entrepreneurial environment
	EDU2	Greater recognition of the entrepreneur's figure
	EDU3	The preference to be an entrepreneur
	EDU4	The necessary abilities to be an entrepreneur
	EDU5	The intention to be an entrepreneur
Entrepreneurial intention	INT1	I am ready to do anything to be an entrepreneur
	INT2	My professional goal is to become an entrepreneur
	INT3	I will make every effort to start and run my own firm
	INT4	I am determined to create a firm in the future
	INT5	I am very seriously thinking of starting a firm
	INT6	I have a firm intention to start a company someday

to assess the latent variables under investigation. Each latent variable was represented by a minimum of five questions, totaling 22 observed variables. This was done to determine the relation between the latent variables and the entrepreneurial intention.

Hypotheses

Based on Hassan *et al*. (2020), the following structural model and hypotheses were formulated (Fig. 1):

H1: Entrepreneurial self-efficacy has a positive impact on entrepreneurial intention.

H2: Opportunity recognition has a positive impact on entrepreneurial intention.

H3: Entrepreneurship education positively moderates the relationship between opportunity recognition and entrepreneurial intention.

H4: Entrepreneurship education positively moderates the relationship between entrepreneurial self-efficacy and entrepreneurial intention.

H5: Gender negatively moderates the relationship between entrepreneurial self-efficacy and entrepreneurial intention.

H6: Gender negatively moderates the relationship between opportunity recognition and entrepreneurial intention.

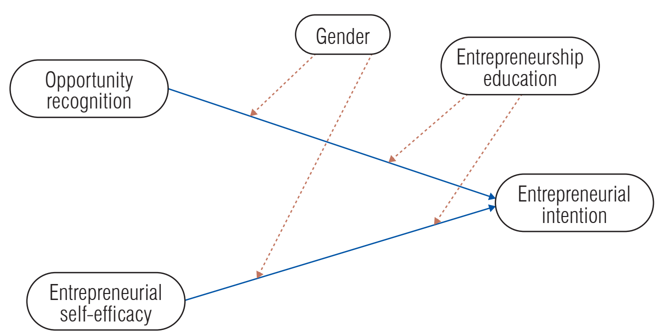

FIGURE 1. Structural model of entrepreneurial intention in agronomic engineering students.

Statistical validation

A structural equation model (SEM) was developed and validated by assessing its internal consistency, goodness of fit, discriminant validity, and convergent validity.

Internal consistency was evaluated using the Cronbach's alpha coefficient, which measures how closely related a set of items are and the degree of internal consistency within the data. Values above 0.7 were considered acceptable, with higher values indicating better model consistency (Panayides, 2013). Goodness of fit was determined using the goodness-of-fit index (GoF). The GoF measures how well the model fits the data by assessing the model's implicit covariance matrix, with a value above 0.5 indicating a good fit of the model to the data (Tian *et al*., 2020). The convergent validity of the model was assessed using the average variance extracted (AVE), with values above 0.5 considered acceptable, indicating that the latent variable is measured by at least 50% of the items (Dabbous & Barakat, 2020). Discriminant validity was determined based on the Fornell-Larcker criterion. This was assessed by comparing the square root of the AVE with the shared variance, with positive values indicating that the latent variables discriminate from each other.

Results and discussion

Among the surveyed students, 62% were men and 38% were women, with an average age of 23.3 years. The students were classified into six socioeconomic levels (strata) to understand the household context and the urban atmosphere within, with Level 1 being the lowest and Level 6 the highest. The results were as follows: stratum 1 comprised 9% of the students, stratum 2-32.8%, stratum 3-46.8%, and stratum 4-11.4%. Strata 5 and 6 did not report any percentage of students. The results are relevant because individuals from wealthier backgrounds are more likely to become entrepreneurs. Boldureanu *et al*. (2020) argue that if someone is born in a better social and economic position, the chances of a better education and networking increase significantly, thus increasing the probability of entrepreneurship.

As regards the family entrepreneurship experience, 57.9% of the students affirmed that someone in their household had had an experience with entrepreneurship. This information is important because, according to Georgescu and Herman (2020), individuals from entrepreneurial families are more likely to start their own business. Al Mamun *et al*. (2019) affirm that the lowest-income households often create businesses to provide themselves with the necessities. Regardless of their socioeconomic status, most of the students surveyed had family entrepreneurship experience, suggesting that those students were more likely to become entrepreneurs.

The analysis of motivations and limitations (Tab. 2) reveals that the reasons for pursuing entrepreneurship vary by gender. Women showed greater motivation for labor independence and self-employment. Bullough and Renko (2017) argue that the entrepreneurial intention of women is focused on self-determination and the pursuit of equity, which aligns with our findings. In contrast, the main motivation for men lies in income expectations. Men tend to be more oriented towards achieving specific goals or status (Brixiová *et al*., 2020). In terms of limitations, both men and women cited a lack of funding as the greatest obstacle. Melugbo *et al*. (2020) found that individuals aged 18 to 35, regardless of gender, have lost confidence in the entrepreneurial ecosystem since the onset of the COVID-19 pandemic. This finding aligns with our results, as funding is an integral part of the entrepreneurial ecosystem.

The model exhibited satisfactory reliability indicators (Tab. 3). The Cronbach's alpha coefficient, which measures the internal consistency of the model on a scale from 0 to 1, showed values above 0.7 for all latent variables, suggesting good internal consistency (Taber, 2018). According to Omar and Zolkaflil (2015), an index exceeding 0.5 indicates strong alignment between the model and the data.

TABLE 2. Motivation and limitation factors for entrepreneurship in agronomic engineering students.

Motivation	Percentage (%)	Limitation	Percentage (%)
Labor independence/self-employment	19.9	Lack of financing	26.3
Existence of a business opportunity	17.1	High risk	13.1
Income expectations	16.5	Market concurrence	11.5
Personal	16.1	Lack of experience	10.9
Create something of your own	15.0	Lack of guaranteed minimum wage	9.8
Unemployment	9.3	Failure fear	8.4
Family tradition	3.5	Tax charges	7.4
Reference model or recognition of successful entrepreneurs	1.6	Lack of entrepreneurial education	6.9
Dissatisfaction with current occupation	1.0	Other	5.7

Furthermore, the goodness-of-fit index (GoF) was 0.63, indicating a good model fit. Lastly, the average variance extracted (AVE) confirmed convergent validity, as all values surpassed the threshold of 0.5, indicating that at least 50% of the variance in the latent variables was explained by the observed variables (Setiawan Wibowo *et al*., 2020).

TABLE 3. Consistency, validity, and reliability indicators for the model of entrepreneurial intention in agronomic engineering students.

Latent variable	Cronbach's alpha	AVE*	GoF**
Entrepreneurial self-efficacy	0.84	0.60	0.63
Opportunity recognition	0.84	0.61	
Entrepreneurial education	0.85	0.61	
Entrepreneurial intention	0.91	0.70	

*Average variance extracted (AVE), ** Goodness-of-fit index (GoF).

The measure of discriminant validity (Tab. 4) relied on the comparison between the square root of the average variance extracted (AVE) and the shared variance among latent variables. This confirms that the latent variables in the model differ from each other. It also indicates that the extracted variances surpass the shared variances among constructs. According to Moreira and Silva (2015), the difference between the average variance extracted and shared variance indicates the presence of discriminant validity between the two latent variables. This observation suggests a clear distinction between the various constructs (Alamer, 2021).

Verification of each hypothesis is presented in Table 5. These results confirm that entrepreneurial education positively moderates the relationship between entrepreneurial self-efficacy and recognition of opportunities, which in turn influences entrepreneurial intention. Likewise, gender negatively moderates the relationship between recognition of opportunities and self-efficacy, which in turn positively influences entrepreneurial intention.

Chien-Chi *et al*. (2020) emphasize that self-efficacy plays a fundamental role in fostering a proactive mindset among students, which encourages development of the practical skills necessary for entrepreneurship. This self-confidence in one's abilities drives entrepreneurial intention by generating a readiness to face challenges and pursue business opportunities. Additionally, evidence suggests that opportunity recognition positively influences the entrepreneurial intention of students by providing a market perspective that reveals unmet needs and opens up the possibility of starting a business.

Furthermore, entrepreneurial education plays a key role in strengthening both self-efficacy and opportunity recognition among students. These educational programs provide young entrepreneurs with the tools and knowledge necessary to initiate and manage a business effectively. By providing a more informed understanding of entrepreneurship, entrepreneurial education nurtures confidence in individual capabilities and empowers students to identify

TABLE 4. Average variance extracted and shared variance in entrepreneurial intention in agronomic engineering students.

Latent variable	√AVE	Entrepreneurial self-efficacy	Opportunity recognition	Entrepreneurship education	Entrepreneurial intention
Entrepreneurial self-efficacy	0.76	0.68	0.45	0.56	0.59
Opportunity recognition	0.78	0.57	0.65	0.48	0.38
Entrepreneurship education	0.78	0.42	0.54	0.51	0.57
Entrepreneurial intention	0.84	0.70	0.48	0.41	0.61

TABLE 5. Model hypotheses to assess entrepreneurial intention among agronomic engineering students.

Hypotheses		*P-value*	Result
H1	Entrepreneurial self-efficacy has a positive impact on entrepreneurial intention.	0.0000001	Accepted
H2	Opportunity recognition has a positive impact on entrepreneurial intention.	0.0000001	Accepted
H3	Entrepreneurship education positively moderates the relationship between opportunity recognition and entrepreneurial intention.	0.00009	Accepted
H4	Entrepreneurship education positively moderates the relationship between entrepreneurial self-efficacy and entrepreneurial intention.	0.0000001	Accepted
H5	Gender negatively moderates the relationship between entrepreneurial self-efficacy and entrepreneurial intention.	0.0351	Accepted
H6	Gender negatively moderates the relationship between opportunity recognition and entrepreneurial intention.	0.00094	Accepted

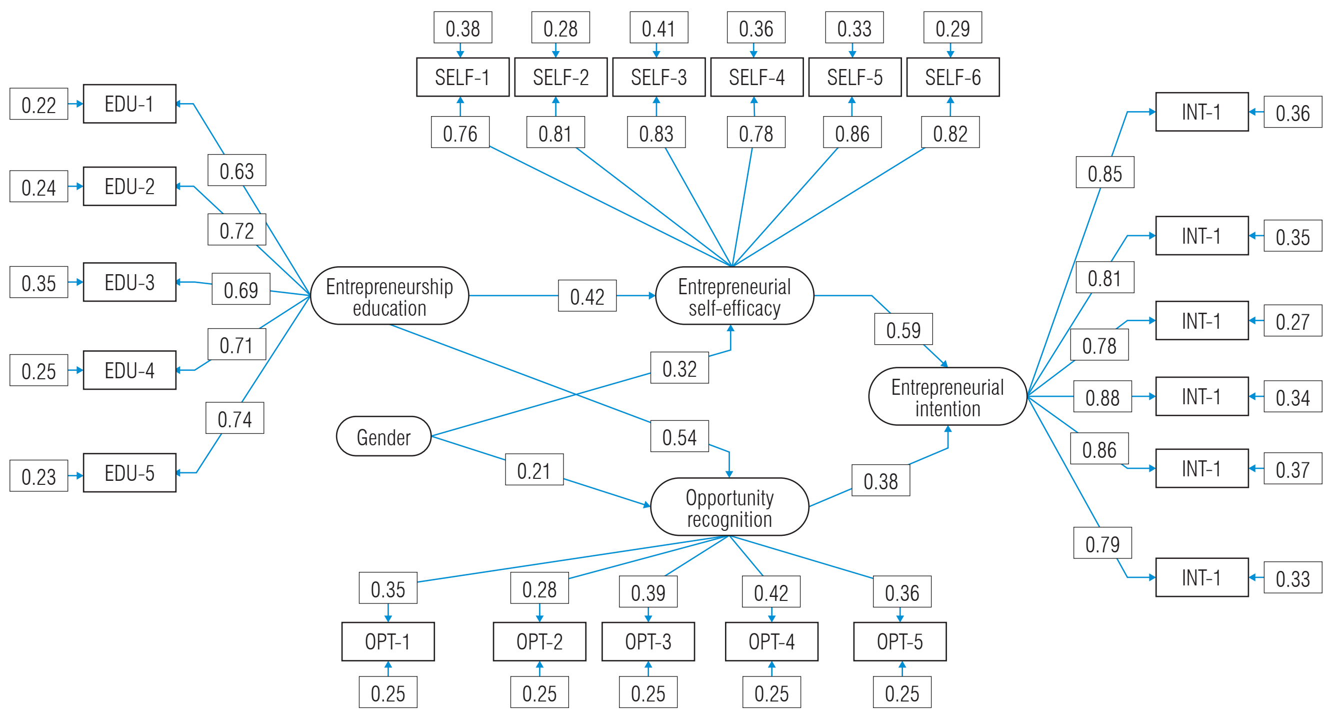

FIGURE 2. Structural equation model of entrepreneurial intention in agronomic engineering students.

and capitalize on emerging opportunities in the market, as highlighted by Wiramihardja *et al.* (2022).

However, a gender disparity in self-efficacy and opportunity recognition has been observed, negatively impacting the entrepreneurial intention of women. Research by Nowiński *et al.* (2019) indicates that women tend to exhibit less willingness towards entrepreneurship than men. This gap can be attributed, in part, to the lack of representation of female entrepreneurs and the existence of a predominantly male entrepreneurial environment, as suggested by Cochran (2019). Overcoming these gender barriers requires measures that promote inclusivity and diversity in the entrepreneurial ecosystem, as well as the promotion of female role models in the business sphere.

This study indicates that most agronomy student's families have had some experience related to entrepreneurship, possibly due to the high unemployment rate and prevalent informal work environment in Colombia. According to Arango and Flórez (2020), Colombia faces structural unemployment stemming from a lack of education. Villanueva and Martins (2022) stated that entrepreneurship in Colombia exhibits high failure rates, aligning with the general perception among students that a lack of financing is the main limitation. This suggests a perceived issue within the entrepreneurial ecosystem. However, according to Meoli *et al.* (2020), entrepreneurship is still seen as an opportunity for social change. Motivations for entrepreneurship vary, though they differ by gender in this study. For women, the primary motivation lies in labor independence or self-employment, while for men, income expectations predominate.

The study also confirmed that the entrepreneurial intention of agronomic engineering students is positively influenced by self-efficacy and opportunity recognition. Furthermore, it demonstrated that entrepreneurial education has a positive effect on both self-efficacy and opportunity recognition. Fietze and Boyd (2017) corroborate the existence of a significant positive influence on entrepreneurial intention. These qualities provide students with the necessary self-management tools and knowledge to enter the entrepreneurial ecosystem. Additionally, the study showed that gender negatively affects self-efficacy and opportunity recognition, implying that entrepreneurial intention is gender-biased, with men presumably showing more interest in the business environment than women. This disparity can be explained by differences in educational opportunities, such as the lower representation of women in STEM (Science, Technology, Engineering, and Mathematics) fields compared to men, as noted by Gomez Soler *et al.* (2020).

The findings highlight the importance of self-efficacy, opportunity recognition, and entrepreneurial education in fostering entrepreneurial intention among agronomic engineering students. Self-confidence in one's abilities and the ability to identify and capitalize on emerging opportunities in the market are key factors driving entrepreneurial spirit. However, addressing gender disparities in entrepreneurship is crucial. Promoting inclusion and diversity in the business ecosystem can create an environment that encourages equitable participation of women and men in entrepreneurial activity. These actions can contribute to building a more inclusive and vibrant entrepreneurial future.

Conclusions

This study provides a comprehensive insight into the entrepreneurial intention among agronomic engineering students, laying the groundwork for designing strategies focused on developing entrepreneurial skills, such as self-efficacy and opportunity recognition, with the aim of fostering entrepreneurship intention. The results suggest that implementing entrepreneurship-focused educational programs could be pivotal in cultivating an entrepreneurial mindset among agronomic engineering students. These programs could encompass practical activities, mentorship, and specialized courses to prepare students for the challenges of the business world, thereby contributing to shaping a more skilled and entrepreneurial generation.

Furthermore, the importance of addressing gender disparities in entrepreneurship through specific policies promoting inclusion and equity, especially to support female entrepreneurship, was emphasized. Public policies can play a crucial role by providing resources and financial support targeted at female entrepreneurs, as well as by eliminating structural and cultural barriers that may hinder their participation in the labor and business market. In this regard, adopting a comprehensive approach that spans education, access to financing and the creation of support networks is essential to create an enabling environment for the development and growth of women-led businesses.

While these findings are promising, it is crucial to acknowledge some limitations of the study. For instance, the research focused exclusively on students from the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia in the city of Bogotá, which limits the generalizability of the results to other student populations or geographical contexts. Therefore, future research could expand the sample and consider different faculties or universities to obtain a broader and more representative understanding of entrepreneurial intention among agronomic engineering students. Additionally, conducting long-term follow-ups to assess the impact of educational programs on the development of entrepreneurial skills and the realization of entrepreneurial activities among students would be beneficial. These additional efforts would help strengthen the theoretical and practical foundation for fostering entrepreneurial spirit in the university context.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

DESC and DB conceptualized the manuscript. DESC, DRS, and DB wrote the manuscript. All authors critically revised the manuscript and approved the final version.

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Effect of operational variables on the extraction of compounds with antioxidant capacity from chicory roots

Efecto de las variables operativas sobre la extracción de compuestos con capacidad antioxidante de raíces de achicoria

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ABSTRACT

Polyphenol solvent extraction from vegetable matrices has gained significant importance in various sectors, including food, pharmaceuticals, and agro-industries. This research focuses on the experimental design of Batch extraction procedures for obtaining polyphenols from dried and milled chicory roots. Air-forced and vacuum-drying techniques were employed to dry fresh chicory roots. The research examined the impact of operational variables on obtaining extracts from dried chicory roots. We developed a comprehensive mathematical model using diffusion transfer principles, taking into account various operational factors. We integrated the model into the optimization tool General Algebraic Modeling System (GAMS) and subsequently validated it through experimentation. The results demonstrated strong agreement between the theoretical and experimental data, with satisfactory values for both the root mean square error and correlation coefficients. The optimal extraction conditions that yielded maximum outputs were 50°C temperature, 1.10 m s^{-1} agitation speed, 50% ethanol concentration, and 20 ml solvent per gram of flour. Moreover, we observed higher diffusivity coefficients for polyphenolic compounds and lower activation energy values for extracts derived from vacuum-dried chicory root flour at 60°C and 25 mm Hg pressure. Overall, the proposed mathematical model effectively predicted the described behavior with satisfactory accuracy.

Key words: drying methods, antioxidant extraction, phenolic content, optimization model.

RESUMEN

La extracción por solvente de polifenoles a partir de matrices vegetales ha ganado gran importancia en diversos sectores, incluidos el alimentario, farmacéutico y agroindustrial. Este estudio se centra en el diseño experimental de la operación de extracción Batch para la obtención de polifenoles a partir de raíces de achicoria deshidratadas y molidas. Se emplearon las operaciones de secado al vacío y por convección forzada de aire para deshidratar raíces de achicoria fresca. Se evaluó el impacto de diversas variables operativas en la obtención de extractos a partir de raíces de achicoria deshidratadas. Se desarrolló un modelo matemático integral utilizando los principios de transferencia por difusión, considerando diversos factores operativos. Dicho modelo se implementó en la herramienta de optimización General Algebraic Modeling System (GAMS) y posteriormente se validó con resultados experimentales de laboratorio. Los resultados demostraron una fuerte concordancia entre los datos teóricos y experimentales, con valores satisfactorios tanto para el error cuadrático medio como para los coeficientes de correlación. Se encontró que las condiciones óptimas de extracción que produjeron resultados máximos fueron para la temperatura de 50°C, velocidad de agitación de 1,10 m s^{-1}, concentración de etanol del 50% y 20 ml de disolvente por gramo de harina. Además, se observaron mayores coeficientes de difusividad para los compuestos polifenólicos y valores de energía de activación más bajos para los extractos derivados de harina de raíces de achicoria deshidratadas al vacío a 60°C y 25 mm Hg de presión. En general, el modelo matemático propuesto predijo eficazmente el comportamiento descrito con una precisión satisfactoria.

Palabras clave: métodos de secado, extracción de antioxidantes, contenido fenólico, modelo de optimización.

Introduction

Polyphenol extracts derived from vegetable products have increased in importance in both the food and pharmaceutical industries due to their applications as food preservatives and their potential impact in mitigating certain diseases (Aguiñiga-Sánchez *et al.*, 2020; Kaur, 2020; Mir *et al.*, 2018; Pateiro *et al.*, 2021; Vega-Galvez *et al.*, 2023; Zhang *et al.*, 2023). Studying the processing of chicory roots enables us to evaluate their use in producing flour and concentrating antioxidants that can enhance various food matrix

Received for publication: December 12, 2023. Accepted for publication: May 06, 2024. Doi: 10.15446/agron.colomb.v42n2.112040

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antioxidant and antimicrobial capacity, improving their preservation. Whether in the form of flour or extract, the products of this process will supply applications as nutraceutical substances and prebiotics. Their consumption may help reduce the risk of cardiovascular diseases, atherosclerosis, and conditions that affect the proper functioning of the colon, thus promoting overall health (Palacios Flores, 2022). Chicory (*Cichorium intybus* L.) root is a notable consumable source of inulin, housing an array of antioxidants such as chicoric, chlorogenic, ferulic, and caffeic acids, along with tannins (Perović *et al.*, 2021). Like many other vegetables and herbs, this root is prone to rapid deterioration (Sánchez-Sáenz *et al.*, 2014). As a result, these materials necessitate drying procedures to facilitate processing within a preplanned annual operational timeframe, requiring equipment that permits substantial extraction as technological limitations dictate.

In this research, we tested convective and vacuum drying. The convection drying method is the most used to preserve products in the food industry. It allowed an evaluation of the behavior of the polyphenolic compounds with the drying parameters tested. The aim of vacuum drying was to study the effects on the antioxidant capacity, thus achieving shorter thermal treatment times since prolonged dehydration times can influence the quality of the final product.

In this context, the initial polyphenol composition and the quality of the flour used for extraction depend on the choice of the drying method and the associated conditions. Subsequently, the extraction process efficacy is influenced by its operational parameters and the selected drying technique (Teffane *et al.*, 2021). Employing mathematical modeling serves as a robust tool to explain the intricacies of involved unit operations. Each successive stage of the process scan is intricately linked to optimize specific objectives, encompassing the minimization of resource utilization, waste generation, or expenses and the maximization of process yields while adhering to predefined quality or composition benchmarks. Such models are effectively implemented through programming techniques, culminating in nonlinear representations. Furthermore, integrating mechanistic mathematical models grounded in fundamental principles yields more realistic process simulation and optimization models.

The kinetics of solid-liquid extraction has been extensively examined in the literature concerning the extraction of antioxidants and polyphenols (Chanioti *et al.*, 2014; Zhou *et al.*, 2017). This exploration involves various operational factors on extraction performance, such as the solid-liquid ratio, solvent selection, particle size, and temperature, among others (Al-Farsi & Lee, 2008; Carciochi *et al.*, 2018; Gubsky *et al.*, 2018; Radha Krishnan *et al.*, 2015; Sun *et al.*, 2011). Several studies in this area have addressed the extraction process by incorporating kinetic models (Amrouche *et al.*, 2019; Chaiklahan *et al.*, 2014; Qu *et al.*, 2010). More complex and comprehensive research has entailed the utilization of mechanistic models. An interesting example is the study by Garcia-Perez *et al.* (2010), where the authors evaluated the impact of drying temperature on antioxidant extraction by applying an optimization model. This model was devised to simultaneously ascertain the initial antioxidant concentration, as well as mass transfer and diffusion coefficients.

This study aimed to identify and optimize the conditions for extracting polyphenols from dried chicory roots using a novel mathematical model that integrates drying and extraction parameters. This approach seeks to enhance extraction efficiency and provide a versatile tool applicable to different antioxidant-rich sources, addressing a gap in existing extraction methodologies.

Materials and methods

Preparation of the samples

Fresh chicory roots (*Cichorium intybus* L.) were obtained from a local farmer from Rosario, Santa Fe, Argentina, during the autumn. The samples were subjected to drying using a convective drying oven (Tecno Dalvo, Model CHC/F/I, Argentina) and a vacuum dryer (ORL, Argentina). Each subsequently dried sample was ground using a dry blade mill (IKA, Germany) and stored under refrigeration in vacuum-sealed packaging. The resultant powdered samples were subjected to sieving to segregate particles that passed through an ASTM 40 sieve, utilizing a Ro-Tap sieve shaker (Tyler, USA). A particle diameter statistic was computed, with 0.26 mm as the mean.

The drying process was carried out in a convective oven and under vacuum. A mathematical model was used to represent the extraction process, incorporating considerations for the drying method and its associated parameters (drying temperature and air velocity or vacuum pressure) and the extraction variables (temperature, hydroalcoholic solvent composition, solid-liquid ratio, and agitation velocity). Consequently, the model established a connection between the operational parameters of both primary unit operations to attain polyphenol-enriched extracts. A series of experimental runs were conducted to determine the mass transfer coefficients. Subsequently, an independent set of experimental runs was employed to validate the proposed model and the calculated mass transfer coefficients. Lastly, the model was optimized to maximize extraction yield. This optimization process yielded potential operating conditions suitable for scaling up the drying and extraction unit operations.

Extraction of phenolic compounds

An experimental design was carried out to study the influence of various drying and extraction variables on the yields of extractions rich in polyphenols. A fractional factorial design was chosen, considering 6 factors and 2 levels for each factor. Consequently, each design comprised 16 individual experimental runs, elucidated in Table 1.

The ranges of the selected variables for the extraction process were aligned with prior studies conducted by other researchers who had extracted polyphenolic compounds from diverse vegetable sources (Jokić *et al.*, 2010; Pinelo

TABLE 1. Fractional factorial experimental design for air forced drying.

Air-forced drying						
E_{ic}	T_d (°C)	v_d (m s^{-z1})	T_e (°C)	v_e (m s^{-1})	Solvent/flour ratio, v/w (X_1)	Ethanol concentration, % (X_2)
$E1_c$	60	0.2	25	0.55	20	70
$E2_c$	80	0.2	25	0.55	30	70
$E3_c$	60	0.7	25	0.55	30	50
$E4_c$	80	0.7	25	0.55	20	50
$E5_c$	60	0.2	50	0.55	30	50
$E6_c$	80	0.2	50	0.55	20	50
$E7_c$	60	0.7	50	0.55	20	70
$E8_c$	80	0.7	50	0.55	30	70
$E9_c$	60	0.2	25	1.10	20	50
$E10_c$	80	0.2	25	1.10	30	50
$E11_c$	60	0.7	25	1.10	30	70
$E12_c$	80	0.7	25	1.10	20	70
$E13_c$	60	0.2	50	1.10	30	70
$E14_c$	80	0.2	50	1.10	20	70
$E15_c$	60	0.7	50	1.10	20	50
$E16_c$	80	0.7	50	1.10	30	50
Vacuum drying						
E_{iv}	T_d (°C)	P_d (mm Hg)	T_e (°C)	v_e (m s^{-1})	Solvent/flour ratio v/w(X_1)	Ethanol concentration, % (X_2)
$E17_v$	60	25	25	0.55	20	70
$E18_v$	80	25	25	0.55	30	70
$E19_v$	60	50	25	0.55	30	50
$E20_v$	80	50	25	0.55	20	50
$E21_v$	60	25	50	0.55	30	50
$E22_v$	80	25	50	0.55	20	50
$E23_v$	60	50	50	0.55	20	70
$E24_v$	80	50	50	0.55	30	70
$E25_v$	60	25	25	1.10	20	50
$E26_v$	80	25	25	1.10	30	50
$E27_v$	60	50	25	1.10	30	70
$E28_v$	80	50	25	1.10	20	70
$E29_v$	60	25	50	1.10	30	70
$E30_v$	80	25	50	1.10	20	70
$E31_v$	60	50	50	1.10	20	50
$E32_v$	80	50	50	1.10	30	50

Td: drying temperature; vd: air drying velocity; Pd: absolute vacuum pressure; Te: extraction temperature; ve: agitation velocity.

et al., 2005; Teffane et al., 2021). The drying temperature range of 60-80°C is reported by Figueira et al. (2004) as suitable for retaining bioactive compounds in chicory roots, further validated in a previous study by Balzarini et al. (2018). The levels of extraction temperature were set at 25°C and 50°C to ensure the stability of polyphenols (Bouchez et al., 2020; Cissé et al., 2012), while the ratios of solvent to flour and the concentrations of ethanol were determined based on those yielding superior extraction efficiencies (Bouchez et al., 2020; Carciochi et al., 2018; Das & Bera, 2013; Dzharov et al., 2016).

All extraction runs were executed employing the precise weight of dried chicory root flour dictated by the experimental design, accompanied by 600 ml of the hydroalcoholic ethanol mixture. A batch extractor within a thermostatic bath (Lauda, Alpha A6, Germany) was equipped with continuous agitation using a propeller agitator with an extraction duration of 90 min. Samples of 5 ml were taken at previously defined time intervals. They were filtered and stored in opaque containers at 3°C ± 1°C.

Total phenolic content (TPC) determination

The Folin-Ciocalteu method with modifications (Boroski et al., 2015) was used to determine polyphenolic compounds in samples of hydroethanolic extract of chicory roots. A 250 µl aliquot of the sample was combined with 250 µl of the Folin-Ciocalteu reagent. After 3 min, 500 µl of a saturated Na_2CO_3 solution (20%) was added. The reaction was allowed to proceed in the dark for 120 min, and the absorbance was subsequently measured at 725 nm. The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of dry sample (mg 100 g^{-1} db), using a calibration curve (R^2=0.998). Total phenolic content was reported as the mean ± standard deviation, and measurements were performed in triplicate.

Initial phenolic content (TPC_0) determination

The initial polyphenol content within the chicory root flours (C_{p0}) was evaluated following the approach presented by Tao et al. (2014), which included specific changes. According to tests, a 50% hydroalcoholic mixture of ethanol was selected as the extraction solvent due to its high efficiency. To initiate the process, 10 g of chicory root flour sample was combined with 400 ml of the aqueous ethanol solution in a 600 ml beaker. The exhaustive extraction of polyphenols was conducted at 50°C with continuous agitation for 48 h. Subsequently, the resulting extract was filtered. Then, the chicory root flours were added to the beaker, and an additional 100 ml of 50% ethanolic solution was introduced. The mixture was treated at 50°C while being agitated for 4 h, followed by another round of filtration. Ultimately, the two filtrates were combined, and the TPC was ascertained utilizing the methodology elaborated in the preceding section.

DPPH radical scavenging activity determination

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Shimada et al. (1992) was used. One ml extract was added to 5 ml of DPPH solution of 0.1 mM. The mixture was shaken for 30 s and allowed to stand for 50 min. Absorbance was measured at 517 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). All samples were tested in triplicate. The results were expressed as the average value ± the standard deviation. The DPPH scavenging activity was determined using Equation 1.

$$\%\text{DPPH} = \left(1 - \frac{\text{Abs}}{\text{Abs control}}\right) \times 100 \tag{1}$$

Statistical analysis

An analysis of variance (ANOVA) was performed, and Duncan's New Multiple Range test was used ($P \leq 0.05$). The SPSS Statistical Analysis Program for Windows (SPSS Inc., Chicago, IL, USA) was used.

Mathematical model

The phenomenological description of the extraction process has undergone extensive discourse in the literature (Crossley & Aguilera, 2007; Garcia-Perez et al., 2010; Kostic et al., 2019; Popescu et al., 2013). In our research, the extraction model was initially employed to compute mass transfer parameters. Subsequently, they were subjected to experimental validation. Subsequently, the mathematical model was integrated with the preceding drying phase to optimize extraction yield while accounting for both drying alternatives. It is worth noting that this model does not aim to determine the superior alternative. Such a determination will be addressed in subsequent research endeavors involving assessing cost functions. Specifically, regarding extraction modeling, it is posited that the solvent permeates the solid to dissolve the extractable components, which diffuse from within the solid into the surrounding bulk liquid. This model considers the diffusion process limiting (Cheung et al., 2013). The model considers the following assumptions based on Fick's second law:

- Every particle was considered spherical, symmetrical, and homogeneous. So, the uniform initial distribution of the active compounds in the matrix was taken;
- The polyphenolic diffusion path was assumed only in the radial direction;

- Only the diffusion of polyphenolics was taken under study;
- The rate of diffusion of polyphenolics from the solid was independent of the time, but it depended on the temperature, explained by Arrhenius equation;
- A perfect mixing between flour and solvent was considered;
- The convective mechanism of transfer was considered from the particle surface to the solvent bulk phase;
- There are no chemical reactions or thermal degradation of the bioactive compounds in the working temperature range.

Equations

Fick's law was implemented to explain the mass transfer of polyphenols within particles using spherical coordinates in one direction and considering the assumptions:

$$\frac{(1-\varepsilon(X_1))}{D_{p,\beta}(T_e,X_2)}\frac{\partial^2 C_{p,\beta}(E_{ic},E_{iv},X_1,X_2,r,t)}{\partial t} =$$
$$(1-\varepsilon(X_1))\frac{\partial^2 C_{p,\beta}(E_{ic},E_{iv},X_1,X_2,r,t)}{\partial r^2} +$$
$$\frac{2}{r}\frac{(1-\varepsilon(X_1))\partial C_{p,\beta}(E_{ic},E_{iv},X_1,X_2,r,t)}{\partial r}, 0 < r < R_p \quad (2)$$

where (mg ml^{-1}) is the polyphenol concentration inside the particle and (m^2 s^{-1}) is the diffusivity coefficient of polyphenols within each particle. is the solvent volume fraction; is the spherical radial coordinate (m), is the time(s), and is the particle radius (m).

In this model, the diffusion coefficient of polyphenols within each particle, $D_{p,\beta}$, was obtained with the Arrhenius functionality:

$$D_{p,\beta}(T_e,X_2) = D_0(T_e) \times e^{\left(\frac{-E_A(X_2)}{R\times T_e}\right)} \quad (3)$$

where D_0 is the Arrhenius factor in m^2 s^{-1}, R represents the universal gas constant in kJ mol^{-1}K^{-1}, T_e is the absolute extraction temperature in K, and E_a is the activation energy of the polyphenol diffusion in kJ mol^{-1}.

The fraction of solvent volume, denoted as ε, was defined according to Equation 4, in which and were the volumes of the solid particle and the solvent phase, respectively.

$$\varepsilon(X_1) = \frac{V_\gamma}{V_\gamma + V_\beta(X_1)} \quad (4)$$

The initial condition was represented by Equation 5, assuming homogeneous initial polyphenol concentration within the particles.

$$C_{p,\beta}(E_{ic},E_{iv},X_1,X_2,r,t) =$$
$$C_{p0,\beta}(E_{ic},E_{iv}), 0 \le r \le R_p, t = 0 \quad (5)$$

The drying process impacts the concentration of polyphenols due to thermal degradation (Shad *et al.*, 2013). Consequently, distinct initial concentrations were attained based on the drying method and conditions elucidated in Table 2.

Equation 6 expressed the boundary condition for the particle center considering no mass transfer. The other necessary boundary condition was represented by Equation 7 for the interfacial polyphenols flux, where was the mass transfer coefficient in the solvent phase, was the interfacial polyphenol concentration and was the concentration in the bulk solvent.

$$\frac{\partial C_{p,\beta}(E_{ic},E_{iv},X_1,X_2,r,t)}{\partial r} = 0, r = 0, t > 0 \quad (6)$$

$$-D_{p,\beta}(T_e,X_2)\frac{\partial C_{p,\beta}(E_{ic},E_{iv},X_1,X_2,r,t)}{\partial r} =$$
$$k_{p,\gamma}(T_e,v_e,X_2)\left(C_{p,\gamma,i}(E_{ic},E_{iv},X_1,X_2,t) -\right.$$
$$\left.C_{p,\gamma}(E_{ic},E_{iv},X_1,X_2,t)\right), r = R_p, t > 0 \quad (7)$$

The equilibrium correlation between polyphenolic concentrations in both phases was depicted by Equation 8, assuming a diluted solution:

$$C_{p,\gamma i}(E_{ic},E_{iv},X_1,X_2,t) =$$
$$K(T_e,X_2)\,C_{p,\beta,i}(E_{ic},E_{iv},X_1,X_2,t), r = R_p, t > 0 \quad (8)$$

where K was the distribution constant.

The mass transfer coefficient $k_{p,\gamma}$ was computed using Equations 9 to 12, which are correlations applicable to fluidized beds of spheres within the Reynolds number range of 2–2,000 (Geankoplis, 1993).

$$S_h(T_e,X_2,v_e) =$$
$$2 + 0.95\left(R_e(T_e,X_2,v_e)\right)^{\frac{1}{2}}\left(S_c(T_e,X_2)\right)^{\frac{1}{3}} \quad (9)$$

$$k_{p,\gamma}(T_e,v_e,X_2) = \frac{S_h(T_e,X_2,v_e)\,D_{p,\gamma}(T_e,X_2)}{2\,R_p} \quad (10)$$

$$S_c(T_e,X_2) = \frac{\mu_\gamma(T_e)}{D_{p,\gamma}(T_e,X_2)\,\rho_\gamma(T_e,X_2)} \quad (11)$$

$$R_e(T_e,X_2,v_e) = \frac{2\,R_p\,\rho_\gamma(T_e,X_2)\,v_e}{\mu_\gamma(T_e)} \quad (12)$$

where S_h, S_c and R_e are Sherwood, Schmit, and Reynolds numbers, respectively.

TABLE 2. Mass transfer parameters root mean square error and R^2 values.

		[T_e (°C), X_2]			
	Drying origin	[25, 50]	[25, 70]	[50, 50]	[50, 70]
$D_{p,\beta}$ (m^2 s^{-1})	Convection	$2.92\ 10^{-12}$	$1.37\ 10^{-12}$	$7.44\ 10^{-12}$	$3.70\ 10^{-12}$
	Vacuum	$1.03\ 10^{-11}$	$1.83\ 10^{-12}$	$1.06\ 10^{-11}$	$2.15\ 10^{-12}$
K	Convection	0.105	0.159	0.316	0.391
	Vacuum	0.248	0.166	0.596	0.416
	v_e (m s^{-1})				
$k_{p,\gamma}$ (m s^{-1})	0.55	$3.64\ 10^{-4}$	$3.51\ 10^{-4}$	$5.44\ 10^{-4}$	$5.24\ 10^{-4}$
	1.10	$5.12\ 10^{-4}$	$4.94\ 10^{-4}$	$7.65\ 10^{-4}$	$7.38\ 10^{-4}$
$D_{p,\gamma}$ (m^2 s^{-1})		$7.59\ 10^{-10}$	$7.28\ 10^{-10}$	$1.25\ 10^{-9}$	$1.20\ 10^{-9}$

		[T_e (°C), X_2]			
	Drying origin	[25, 50]	[25, 70]	[50, 50]	[50, 70]
$D_{p,\beta}$ (m^2 s^{-1})	Convection	$2.92E^{-12}$	$1.37E^{-12}$	$7.44E^{-12}$	$3.70E^{-12}$
	Vacuum	$1.03E^{-11}$	$1.83E^{-12}$	$1.06E^{-11}$	$2.15E^{-12}$
K	Convection	0.105	0.159	0.316	0.391
	Vacuum	0.248	0.166	0.596	0.416
	v_e (m s^{-1})				
$k_{p,\gamma}$ (m s^{-1})	0.55	$3.64\ 10^{-4}$	$3.51\ 10^{-4}$	$5.44\ 10^{-4}$	$5.24\ 10^{-4}$
	1.10	$5.12\ 10^{-4}$	$4.94\ 10^{-4}$	$7.65\ 10^{-4}$	$7.38\ 10^{-4}$
$D_{p,\gamma}$ (m^2 s^{-1})		$7.59\ 10^{-10}$	$7.28\ 10^{-10}$	$1.25\ 10^{-9}$	$1.20\ 10^{-9}$

The diffusion coefficient of polyphenols in the hydroalcoholic phase, denoted as $D_{p,\gamma}$ was evaluated employing the correlation introduced by Wilke and Chang (1955), as depicted by Equation 13:

$$D_{p,\gamma}(T_e, X_2) = 1.173E10^{-16}\left(\varphi(X_2)\,MW_\gamma\right)^{0.5}\frac{T_e+273.15}{\mu_\gamma(T_e)\,V_{m,p}^{0.6}} \quad (13)$$

where ϕ, MW_γ, μ_γ, $V_{m,p}$ were solvent association parameters, molecular weight (kDa) and viscosity (kg m^{-1} s^{-2}) of the solvent phase, and molar volume of polyphenols (m^3 $kmol^{-1}$).

Finally, Equation 14 resumes the mass transfer balance for the whole system:

$$(1-\varepsilon(X_1))\frac{d\langle C_{p,\beta}\rangle(E_{ic},E_{iv},X_1,X_2,t)}{dt} = -\varepsilon(X_1)\frac{dC_{p,\gamma}(E_{ic},E_{iv},X_1,X_2,t)}{dt},\ 0<t<t_f \quad (14)$$

The extraction yield, denoted as Y, depended on the liquid-solid ratio, the ethanol concentration used as the extracting solvent, and the initial polyphenol concentration. This efficiency is characterized by Equation 15 for the concluding extraction duration, t_e, quantifying the mass of extracted polyphenols from the mass of polyphenols initially present in the chicory root flour:

$$Y(E_{ic},E_{iv},X_1,X_2,t_e) = \frac{m_{p,\gamma}(E_{ic},E_{iv},X_1,X_2,t)}{m_{p0,\beta}(E_{id},E_{ip})}100 = \frac{C_{p,\gamma}(E_{ic},E_{iv},X_1,X_2,t_e)m_\gamma}{C_{p0,\beta}(E_{ic},E_{iv})\,m_\beta}100,\ t=t_e \quad (15)$$

The partial differential equations were discretized using the central finite difference method (CFDM) and an implicit scheme. Equations 16 and 17 defined the interval width for the radial (Δr) and temporal grids (Δt) with M = 7 and N = 10.

$$\Delta r = \frac{R_p}{G_R} \quad (16)$$

$$\Delta t = \frac{t_e}{G_t} \quad (17)$$

where G_R and G_t were the number of grid intervals in the radial coordinate and intervals in the temporal grid.

The resolution of the partial differential equations gave the local value of the polyphenol concentration. Local concentrations within the particles were integrated using

Simpson's rule to calculate average polyphenol concentrations, according to Equation 18:

$$\overline{C_{p,\beta}}(E_{ic}, E_{iv}, X_1, X_2, t) = \frac{\int_0^V C_{p,\beta}(E_{ic}, E_{iv}, X_1, X_2, r, t)dV}{\int_0^V dV}, t \geq 0 \quad (18)$$

The root mean square error (RMSE) minimization function was used as an objective function to solve the model. It corresponds to the sum of the differences between experimental and theoretical data.

The GAMS (General Algebraic Modeling System) program and the CONOPT tool were used to implement and solve the model. We obtained 2,283 variables and 2,269 restrictions.

Results and discussion

The development of the experimental design led to a total of 32 extraction runs. The primary subset of the dataset (consisting of 20 runs) was gathered separately to derive the mass transfer parameters. Subsequently, the remaining set of experimental runs (12 runs) was executed to validate the proposed model through the application of the estimated mass transfer parameters.

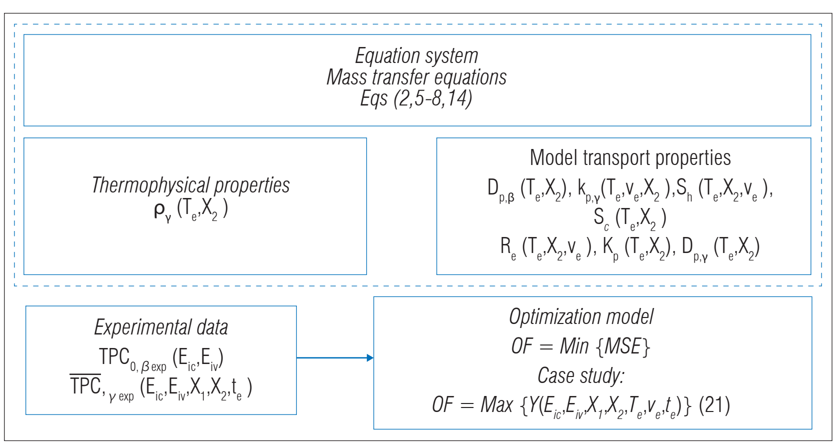
A visual representation of the mathematical model for extracting polyphenolic compounds from chicory root flour is presented in Figure 1.

Estimation of mass transfer parameters

Table 2 presents mass transfer parameter values. $D_{p,\beta}$, $k_{p,\gamma}$ and $D_{p,\gamma}$ were calculated by Equations 4, 10, and 13, respectively, and K was estimated as a model parameter.

The diverse microstructures generated by the drying treatments lead to distinct internal diffusion pathways within the particles. These differences are evident in the variations observed in the internal diffusion coefficients. Remarkably, during the extraction process, polyphenolic compounds exhibited more rapid diffusion from vacuum-dried samples than from air-forced dried samples, even when subjected to the same extraction conditions (Aravindakshan *et al.*, 2021). A lower diffusion coefficient was obtained for convective drying.

The diffusion coefficient of polyphenols, $D_{p,\beta}$, exhibits an increment with rising extraction temperatures (Chaiklahan *et al.*, 2014; Nova *et al.*, 2023; Thaisamak *et al.*, 2019; Vallejo-Castillo *et al.*, 2021). This augmentation was attributed to the molecule enhanced internal energy and increased mobility, leading to a reduction in dynamic viscosity. The activation energy values yielded by the model ranged from 20.3 to 22.4 kJ mol^{-1} for extractions from air-forced convection-dried samples and from 0.7 to 3.5 kJ mol^{-1} for vacuum-dried samples. As presented in Table 2, the highest $D_{p,\beta}$ values were observed for a bath temperature of 50°C and with an ethanol concentration of 50% in the extraction solvent (P≤0.05), regardless of the drying method. Notably, the distribution constant exhibited higher values for samples dried within the vacuum chamber, indicating a heightened extent of polyphenol extraction under these conditions. The polyphenol diffusion coefficients obtained in this study were similar to those reported by other authors for plant matrices. Chaiklahan *et al.* (2014) conducted extractions of bioactive compounds at different temperatures, with values that range from 1.07×10^{-12} m^2 s^{-1} at 50°C to 3.02×10^{-12} m^2 s^{-1} at 90°C. Aramburu *et al.*



OF: Objective function; MSE: means square error; TPC: total phenolic content.

FIGURE 1. Description of the mathematical model of extracting polyphenolic compounds from chicory root flour.

(2020) report diffusivity values of the same order (6.97×10^{-12} m^2 s^{-1} at 25°C).

Regarding the mass transfer coefficients of the solvent phase of Equation 10, the model provides consistent operational conditions for the extraction phase, regardless of the drying technique applied. Thus, $k_{p,y}$ is affected by agitation velocity, temperature, and the hydroalcoholic extraction mixture, while the diffusion coefficient, $D_{p,y}$ hinges on the latter two factors, as evaluated in Equation 13. These associations are detailed in Table 2.

The diffusion coefficient of total polyphenols in the phase of the solvent, the mass transfer coefficient in the solvent phase, $k_{p,y}$, and the distribution constant, K, all exhibited similar upward trends. Similarly, elevated values for the mass transfer parameters were observed at a bath temperature of 50°C and when using an extraction solvent containing 50% ethanol concentration. The mass transfer coefficient demonstrated responsiveness to agitation velocity, with higher values observed as agitation velocity increased. Consequently, higher mass transfer rates are achieved using heightened agitation velocity during extraction, elevated temperatures, and utilization of a 50% ethanol concentration within the extraction solvent. This observation aligns with published studies concerning the extraction of bioactive compounds in soybeans and certain fruits (Jokić *et al.*, 2010; Velić *et al.*, 2011; Zhou *et al.*, 2017).

Model validation

The polyphenol concentrations from a subset of 12 experimental runs were compared with the predicted values, employing the mass transfer parameters previously determined in the model. RMSE and R^2 were calculated to assess the model predictive efficacy, obtaining values ranging between 0.002-0.013 and 0.957-0.997.

Figure 2 shows the discrepancy in the polyphenol extraction contents for samples from different drying methods. Graphs A-B and C-D correspond to samples dried in their forced convection chamber and the vacuum chamber but submitted to the same extraction conditions.

The confidence band (CB) and prediction band (PB) for total phenol contents are also presented in Figure 2. It should be noted that PB is the area where 95% of the experimental data points are expected, where all the obtained observations lie within this area. Similarly, CB is the area where 95% of the regression line was expected, which contains more than 50% of the experimental values for all experiences reported here. The obtained CB and PB fit the experimental data points well, increasing the confidence in the model's predictions. Standard probability plots of the residuals for the different experimental runs were also shown in Figure 2 (E and F). The data points above and below the line are evenly distributed, ensuring that they are typically distributed and that there are no undesirable trends or correlations between them.

The initial phenolic content of the samples dried using the air-forced convection chamber was (451.03 – 645.04) mg GAE 100 g^{-1} d.b., and for the vacuum dryer they were (805.05 – 1344.31) mg GAE 100 g^{-1} d.b. The higher polyphenol content observed with vacuum drying can be attributed to the lower operational pressures, which reduce the boiling point of water and allow for drying at lower temperatures. This minimizes the thermal degradation of polyphenols, which are sensitive to heat, thus preserving a more significant amount of these bioactive compounds. The vacuum conditions create an environment where moisture can be removed efficiently without exposing the samples to high thermal stress, aligning with observations reported in the literature (Shad *et al*., 2013; Wang *et al*., 2003).

The effects of the extraction parameters (hydroalcoholic solvent mixture, temperature, agitation velocity, and solid-liquid ratio) at the final time were examined in Figure 3. For this analysis, the drying process conditions were maintained at the lowest air temperature, air velocity, and absolute vacuum pressure values (60°C, 0.2 m s^{-1}, and 25 mm Hg).

The influence of the ethanol content on TPC is depicted in Figure 3A. The outcomes proved to be statistically significant ($P \leq 0.05$). For extracts derived from chicory roots subjected to both conventional and vacuum drying, the highest values were 428.515 ± 7.059 and 745.909 ± 4.531 mg GAE 100 g^{-1} d.b., employing a solvent concentration of 50% (v/v). Additionally, for an ethanol content of 70% (v/v), the corresponding values were 335.454 ± 2.349 and 698.518 ± 2.282 mg GAE 100 g^{-1} d.b. These findings align with the data of Thach *et al.* (2017) and Zhou *et al.* (2017).

The effect of extraction temperature on TPC was examined at 25°C and 50°C (Fig. 3B). The greatest phenolic compound concentration was achieved at 50°C, with a significant difference observed ($P \leq 0.05$). The elevation of temperature contributed to an augmented TPC, possibly attributed to the heightened diffusion coefficient leading to enhanced solubility of polyphenols in the solvent (Sarkar *et al*., 2017).

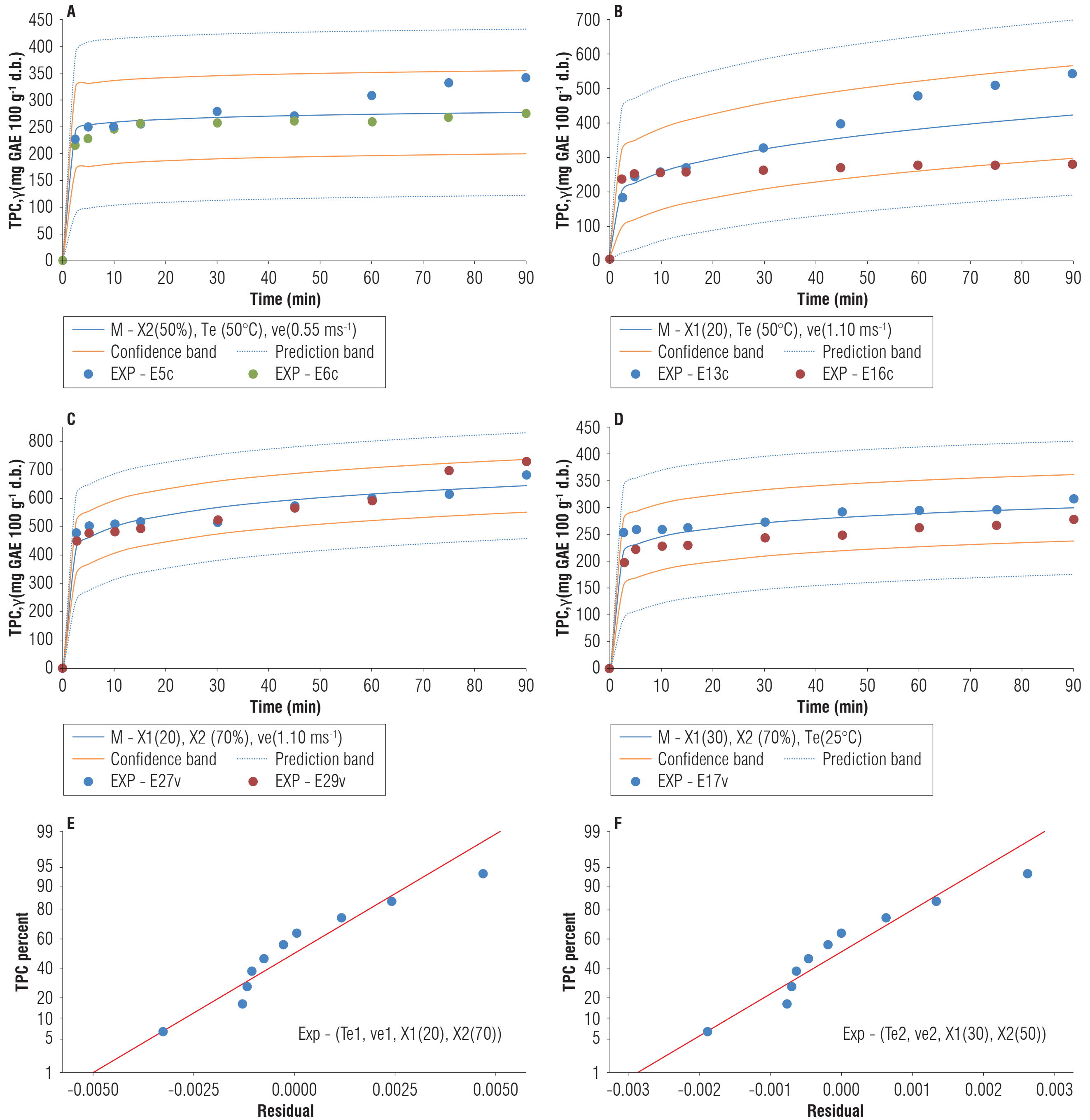


FIGURE 2. Experimental and predicted variation of the total polyphenols content (TPC) in the solvent phase for dehydrated samples using dried in the air-forced convection chamber (A and B) and vacuum chamber (C and D). Standard probability plots of residuals for different extraction operating conditions (E and F). C, convective; v, vacuum; Te, extraction temperature; ve, agitation velocity.

Figure 3C demonstrated a noteworthy effect of agitation velocity ($P \leq 0.05$) on the extracted TPC compounds within the hydroalcoholic bath. The results are consistent with those reported by Teffane *et al.* (2021). Agitation extraction techniques and velocity significantly influence total phenolic extraction (Sarkar *et al.*, 2017; Teffane *et al.*, 2021).

The effect of the liquid-to-solid ratio on the extraction of total polyphenolic compounds from ground chicory root is presented in Figure 3D, comparing ratios of 20:1 and 30:1. The highest TPC extraction was achieved with a ratio of 20:1 ($P \leq 0.05$). It is widely established that the interaction between the solvent and the solid matrix significantly

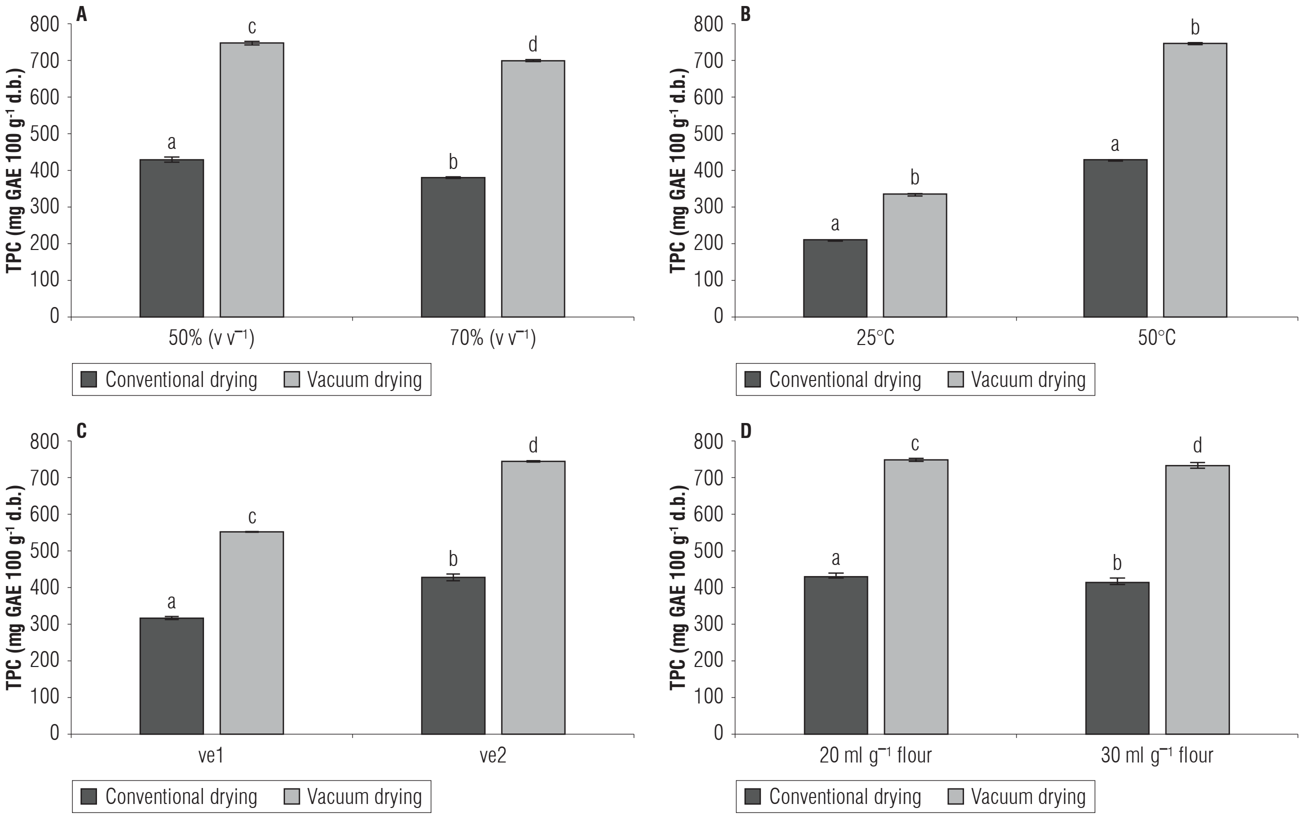


FIGURE 3. Effects of hydroalcoholic solvent mixture (A), temperature (B), agitation velocity (C), and solid-to-liquid ratio (D) on total polyphenol content (TPC) extraction from chicory roots floor. Values are expressed as mean ± SD. Different lower-case letters (a-d) represent significant differences between values according to Duncan's New Multiple Range test ($P \leq 0.05$). v v^{-1}, volume volume^{-1}; ve1, agitation velocity (0.55 m s^{-1}); ve2, agitation velocity (1.10 m s^{-1}).

influences extraction efficiency. Adequate solvent volume is essential to facilitate effective hydration and swelling of the solid phase, ultimately leading to improved recovery yield. Across all cases, parameter values were consistently higher for extracts obtained from chicory root flour subjected to vacuum drying.

The DPPH radical scavenging capacities are presented in Table 3. The maximum antioxidant activity ($P \leq 0.05$) was observed in samples subjected to vacuum drying at 60°C and 25 mm Hg absolute pressure. Elevated antioxidant activity was also evident at a dehydration temperature of 80°C. This phenomenon could be attributed to the development of Maillard reaction compounds, which enhance the antioxidant potential of chicory root flours dried at air temperatures of 80°C or above. Maillard reaction products contain electron donors and hydroxyl groups that function as reducing agents by providing hydrogen atoms to neutralize free radicals, thereby augmenting the overall antioxidant activity of the food (Vhangani & Van Wyk, 2016).

TABLE 3. DPPH of hydroalcoholic extracts of chicory roots dehydrated at 60°C and 80°C by conventional and vacuum drying. Different letters indicate that the values are significantly different according to Duncan's New Multiple Range tests ($P \leq 0.05$).

Treatment	% DPPH
60°C v1	90.876 ± 0.141^{a}
60°C v2	88.591 ± 0.014^{b}
80°C v1	83.552 ± 0.141^{c}
80°C v2	69.327 ± 0.014^{d}
60°C P1	91.035 ± 0.014^{e}
60°C P2	89.497 ± 0.028^{f}
80°C P1	87.151 ± 0.028^{g}
80°C P2	80.104 ± 0.004^{h}

DPPH (2,2-diphenyl-1-picrylhydrazyl).

Optimization model

The validated model maximized the extraction yield (% of dry weight or mg GAE g^{-1} sample). This study facilitated a comparison of operational strategies between the two drying methods and an analysis of their impact on subsequent extraction stages.

The drying method and extraction operating conditions were considered optimization variables in the optimization model, enhancing the model degree of freedom compared to the validation model. Additionally, the initial concentrations of total polyphenols (C_{p0}) for the extraction using the drying method were integrated into the model using Equations 19 and 20. These equations represent response surfaces derived from experimental data as a function of the drying process operating conditions.

$$C_{P0,c} = 931.4 - 4.55 * T_d + 294 * V_d - 5.89 * T_d * V_d \quad (19)$$

$$C_{P0,v} = 3947.56 - 33.4317 * T_d - 22.5380 * P_d + 0.277190 * T_d * P_d \quad (20)$$

The appropriateness of the model linear response surfaces (Equations 19 and 20) was evaluated using the adjusted R^2 equations, yielding the subsequent values: Adj $R^2 = 0.986$ and $R^2 = 0.977$ for forced convection drying, and Adj $R^2 = 0.999$ and $R^2 = 0.998$ for vacuum drying. In this study, the ethanol concentration was maintained at 50%.

The optimization model was subjected to Equations 2 to 20, incorporating the objective function of maximizing the yield of total polyphenol extraction:

$$\text{OF: maximize } Y(E_{ic}, E_{iv}, X_1, X_2, T_e, \nu_e, t_e) \quad (21)$$

Under the objective function of Equation 21, the maximum extraction yields achieved are 63.48% and 71.19% for air-forced and vacuum drying, respectively. These optimal yields are attained by applying the same extraction operating conditions regardless of the drying method. These conditions encompass the highest extraction temperature (50°C), agitation velocity (1.1 m s^{-1}), solvent/flour ratio (30 ml g^{-1}), and maximum extraction time (90 min).

The optimal solution selected dried samples with a higher initial polyphenol concentration, obtained by employing lower air temperature, absolute vacuum pressure, or air velocity during the drying process (60°C and 25 mm Hg or 0.2 m s^{-1}). As a result, achieving maximum yields requires heightened requirements in the extraction stage, involving a higher agitation speed (1.1 m s^{-1}) and the maximum solvent-to-flour ratio (30 ml g^{-1}). The optimal extraction temperature was also consistently resolved at the upper limit (50°C), enhancing the mass transfer rate. This outcome stems from the extraction efficiency being significantly improved with increased agitation velocity and temperature, both of which approached their upper bounds due to technological constraints.

The optimization results underscored the significant impact of several processing variables on polyphenol extraction yields from both the drying and extraction stages. Further analyses involving operational and investment costs are essential to establish a balanced solution that strikes a compromise between quality and costs.

Conclusions

A phenomenological model for extracting polyphenols from chicory root flour was developed and validated, encompassing numerous extraction variables. Moreover, the model accounted for the operational variables employed in the preceding drying stage, which determined the initial concentration of polyphenols for the extraction process.

Both drying methods were included in the results to facilitate the incorporation of cost models in subsequent optimization endeavors. Consequently, this lays the foundation for an advanced optimization model that can effectively determine the appropriate drying method based on cost considerations.

The provided optimal solution captures the interdependencies among the primary critical variables. However, the model will be enhanced in future research by integrating economic and technological constraints, encompassing costs, maintenance, and operational feasibility, to select the optimal operational strategies.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the National Technological University (UTN) and the National Scientific and Technical Research Council (CONICET) of Argentina for this research.

Conflicts of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

MFB conducted the research and formal analysis and prepared the initial draft. MFB and MAR developed the methodology, provided the study materials, reviewed and edited the manuscript, and supervised the planning and execution of the research activity. MAR and MCC managed and coordinated the planning and execution of the research activity and acquired financial support for the project. All authors approved the final version of the manuscript.

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Processing agronomically biofortified BRSMG Caravera rice cultivar

Procesamiento del cultivo de arroz BRSMG Caravera biofortificado agronómicamente

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ABSTRACT

The aim of the research was to evaluate the influence of agronomic biofortification of BRSMG Caravera rice on grain processing by analyzing processing yield (PY) and grain yield (GY), in addition to defining the grain classification for each treatment. The analyzed plants received treatments with NPK + foliar and/or soil fertilization from different fertilizer sources. Some treatments had higher PY values compared to the control plants. For the GY parameter, only treatments with soil fertilization using $ZnSO_4{\cdot}7H_2O$ and two foliar fertilizations using a Bayer Antracol-Zn® product showed higher values. There may be an influence of biofortification on rice processing depending on the treatment. The grains of the BRSMG Caravera variety did not achieve a good classification, as only the grains with the treatment of soil fertilization with $ZnSO_4{\cdot}7H_2O$ were classified as type 4, while the grains in other treatments were classified out of type.

Key words: biofortification, grain yield, income from processing.

RESUMEN

El objetivo del estudio fue evaluar la influencia de la biofortificación agronómica en el arroz BRSMG Caravera en el procesamiento de grano mediante el análisis del rendimiento por procesamiento (P) y el rendimiento de grano (G), además de definir la clasificación de los granos en cada tratamiento. Las plantas analizadas recibieron tratamientos con NPK + fertilización foliar y/o edáfica de diferentes fuentes. Algunos tratamientos presentaron valores de PY superiores a los de las plantas control. Para el parámetro G, solo los tratamientos de fertilización del suelo con $ZnSO_4{\cdot}7H_2O$ y dos fertilizaciones foliares con el producto Bayer Antracol-Zn® mostraron mejores valores. Pudo haber una influencia de la biofortificación en el procesamiento dependiendo del tratamiento. Los granos de la variedad BRSMG Caravera no presentaron una buena clasificación, ya que solo los granos en el tratamiento de fertilización edáfica con $ZnSO_4{\cdot}7H_2O$ fueron clasificados como tipo 4, y los granos de los demás tratamientos fueron clasificadas como fuera de tipo.

Palabras clave: biofortificación, rendimiento de grano, ingreso por procesamiento.

Introduction

Rice (*Oryza sativa*) is an essential component of the diet and livelihood of more than 3.5 billion people (Nathani *et al.*, 2023; Zhao *et al.*, 2020), including in Brazil (Fernades *et al.*, 2024), making it one of the grains with the highest production in the world (Lima *et al.*, 2002). For rice to reach the table of all consumers in the world, it must be produced and processed in large quantities. Paddy processing has the following main objectives: removal of impurities from the field, separation of the husk and grains through peeling and the straw chamber, separation of rice with husk, burnishing, homogenization, and classification (Bragantinni & Vieira, 2004).

The market value of rice is directly influenced by industrial quality, as rice without defects and with a high number of whole grains achieves the best prices (Canellas *et al.*, 1997). Broken grains (grits), rice husks, and bran are the main co-products from rice processing and can be reused; for example, grits can be used to produce pre-cooked starches and flour, bran can be used to produce oil or animal feed, and the husk can be used as an energy source or to produce paper (Lorenzett *et al.*, 2012).

All rice marketed for consumption as grains must be classified into types (1, 2, 3, 4, and 5), with this classification defined by the percentage of broken grains, grit, and the occurrence of defects (Brazil, 2009). Thus, paddy rice is classified into "types" according to its quality. The grains that do not meet the classification requirements for the types mentioned are classified as non-standard or disqualified (Brazil, 2009).

Received for publication: April 28, 2024. Accepted for publication: May 05, 2024. Doi: 10.15446/agron.colomb.v42n2.114148

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Recently, biofortification of food has emerged as an interesting approach to the problem of hidden hunger. Biofortification seeks to nutritionally enrich food directly in the field during its production process, which can be carried out by two methods: genetic biofortification and agronomic biofortification. The first consists of food enrichment through the genetic improvement of crops (transgenic or conventional), while the second is through crop management (mainly fertilization) (Vergütz *et al*., 2016).

Due to the scarcity of studies that relate biofortification with technological aspects, as well as to the importance of processing for the subsequent commercialization of biofortified rice, the aim of the research was to evaluate the influence of agronomic biofortification on the processing of BRSMG Caravera rice. BRS stands for Brazilian Agricultural Research Corporation – Embrapa and MG corresponds to EPAMIG – Agricultural Research Company of Minas Gerais and UFLA – Federal University of Lavras (Brazil). This cultivar was obtained in a breeding program carried out in partnership between Embrapa, EPAMIG, and UFLA. The research analyzed rice processing yield and grain yield, in addition to classifying the rice samples according to the standards of the Ministry of Agriculture, Livestock and Food Supply (MAPA) of Brazil (Brazil, 2009).

Materials and methods

Description of samples

This study analyzed rice grains of the BRSMG Caravera variety, cultivated by EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais), in the municipality of Lambari, state of Minas Gerais (Brazil). Lambari is located at an altitude of 887 m a.s.l. with geographic coordinates of 21°58’ S and 45°20’ W. Throughout the year, the air temperature typically varies from 10°C to 28°C. The climate is humid subtropical. The rice samples included a control treatment, which was not biofortified, and agronomically biofortified plants in 15 different treatments. The 16 treatments were:

A) **Control (NPK):** Application of fertilizer to the soil: nitrogen (N), phosphorus (P) and potassium (K). The fertilizer doses per ha were 32 kg of N, 112 kg of P_2O_5, and 64 kg of K_2O, at sowing, in addition to 112 kg ha^{-1} of urea, resulting in 50.4 kg ha^{-1} of N. The application was at sowing using a seeder in the planting furrow;

B) **NPK + $ZnSO_4·7H_2O$ soil application** (soil application of 50 kg ha^{-1} $ZnSO_4·7H_2O$);

C) **NPK + two foliar applications of $ZnSO_4$** (0.5% $ZnSO_4·7H_2O$ in 800 L ha^{-1});

D) **NPK + ATP ReLeaf® spray** (ReLeaf® 6-18-5 Cereal, ATP, Canada), which contained Fe and Zn, was applied twice as a foliar application; additionally, the product contained N, P, K, B, Cu, Mn, and seaweed extract;

E) **NPK + two foliar applications of potassium iodate (KIO_3):** The amount of iodine applied in each spray was 0.05% KIO_3 in 800 L ha^{-1} equivalent to 400 g ha^{-1} KIO_3;

F) **NPK + two foliar treatments of 0.05% potassium iodate (KIO_3) together with 2% potassium nitrate (KNO_3):** The application was the same as described for treatment E, however using water containing 2% KNO_3 in the preparation of 0.05% KIO_3;

G) **NPK + two foliar treatments of ADOB® 2.0 Zn IDHA– 10%** (ADOB, Poland): The amount of Zn applied with each foliar spray was the same as the one applied in treatment C;

H) **NPK + two foliar treatments of ADOB Basfoliar®** (ADOB, Poland): In this treatment, Basfoliar® 2.0 was applied in a similar way as treatment C. The composition of the product included water-soluble S and Zn;

I) **NPK + two foliar treatments of EPSO Combitop®** (K+S Minerals and Agriculture GmbH, K+S Company, Germany) **together with urea** (the foliar treatment solution contained 0.4% urea): The EPSO Combitop® contained water-soluble Mg, S, Mn, and Zn. The volume at the time of application was 500 L ha^{-1} of spray;

J) **NPK + VALAGRO (Company Valagro, Italy) solution:** This solution contained 1.4% Zn;

K) **NPK + Bayer Antracol-Zn®:** Three kg of Antracol-Zn per ha in 800 L was sprayed twice - once at the ear stage and the second one at the early milk stage. Antracol-Zn is a Zn-containing fungicide, and spraying 3 kg Antracol-Zn provides about 510 g Zn per ha. If the size of the experimental plot is 10 m^2, the amount of Antracol-Zn to be sprayed on plants will be 3 g ml^{-1};

L) **NPK + Foliar Cocktail micro spray.** This product is referred in HarvestZinc Project - IPNI Research (http// research.ipni.net) and contained I, Zn, Fe, and Se;

M) **NPK + Foliar Cocktail micro spray-II.** This product is referred in HarvestZinc Project - IPNI Research (http// research.ipni.net) and contained I, Zn, Fe, and Se in different proportions when compared to the treatment L;

N) **1.5 kg Mg (gypsum) - 10 m^2:** Soil application of a mixed sulfate of Ca and Mg;

O) **3.0 kg Mg (gypsum) - 10 m^2:** Soil application of a mixed sulfate of Ca and Mg;

P) Quimifol Znitro® (Fênix Agro-Pecus Industrial Ltda., Brazil. Product registration: SP-002645-0.000048): Nutrient compound for foliar application. This foliar applied product contained water soluble 10% N and 15.0% Zn.

The planting density in the experiment was approximately 360 panicles m^2 at harvest, with the area of each plot of 8 m^2 totaling 480 m^2 experimental area.

Experimental design

Treatments were arranged in a completely randomized block design, including 15 treatments and control, with four replicates. Each experimental plot consisted of 4 rows of 5 m in length spaced 40 cm apart, totaling 64 plants.

Rice processing

The processing of the rice was carried out using a Suzuki testing device (Model MT96, Rice Processing Machine, Brazil). Rice samples were husked using the equipment husking rollers and passed through rollers three times. Then, husked rice grains were manually separated from rice grains with husk (*i.e.*, the rice grains that remained unhusked even after passing through the husking rollers three times). Next, husked rice grains were burnished for 1 min to separate the bran and germ from rice grains. Finally, grains were classified according to their size using rotary trieur classifiers (Model MT96, Rice Processing Machine, Brazil). The burnished grains were placed in the trieur with alveoli number 2 and rotated for 1 min to separate whole rice grains. The remaining grains were placed in the trieur with alveoli number 1 and 0 to separate the ¾ size rice and ½ size grains, respectively. The remaining rice grains were those of size ¼ and broken grains. After these procedures, all the separate portions were weighed, namely: husk, rice grains with husk, bran with germ, whole grains, ¾ size grains, ½ size grains, and ¼ size grains plus broken grains.

Processing yield and grain yield

Processing yield (PY) is a quality standard that measures the number of polished grains (whole, ¾, ½, ¼ with broken grain) in relation to the weight of paddy rice, expressed as a percentage. Grain yield (GY) is a quality reference that measures the fraction of whole grains among the fractions of broken and whole grains, expressed as a percentage.

Classification of rice grains

Rice samples submitted to agronomical biofortification treatments were classified according to Normative Instruction 6 of February 16, 2009 by MAPA (Brazil, 2009). The purpose of classifying paddy rice into "types" (1, 2, 3, 4, and 5) was to define its quality. The grains that do not meet the classification requirements for these types were classified as non-standard or disqualified (Brazil, 2009).

Statistical analysis

Analysis of variance was performed using the F test. Comparison of means obtained from different treatments was tested by the Scott-Knott test ($P \leq 0.05$). These analyses were performed using Sisvar 5.6 software (Ferreira, 2014).

Results and discussion

Results of the PY quality parameter (Tab. 1) indicate that some treatments increased the number of processed grains in relation to the weight of paddy rice, *i.e.*, there was an improvement in PY for some treatments compared to the control.

TABLE 1. Mean values for processing yield (%) and grain yield (%) for the different agronomic biofortification treatments in BRSMG Caravera rice.

Treatment	Processing yield (PY) %	Grain yield (GY) %
A	64.24 a	38.61 a
B	67.29 b	56.67 c
C	64.03 a	34.89 a
D	63.44 a	33.10 a
E	66.57 b	35.20 a
F	67.16 b	36.50 a
G	65.23 a	33.05 a
H	65.11 a	38.70 a
I	66.28 b	40.68 a
J	63.01 a	34.53 a
K	66.09 b	47.08 b
L	66.50 b	36.23 a
M	63.78 a	38.94 a
N	67.09 b	39.25 a
O	66.64 b	40.86 a
P	63.28 a	30.24 a

The treatment abbreviations are explained in the Materials and methods. Comparison of means from different treatments was tested by Scott-Knott test ($P \leq 0.05$). Means followed by the same letter in the column do not differ from each other according to the Scott-Knott test at 5% probability.

There was a significant effect of treatments and blocks at 1% probability according to the F test. Applying the Scott-Knott test at 5% probability showed that treatments B, E, F, I, K, L, N, and O differed from the control. These treatments had a mean PY value of 66.7%, higher than the control treatment (64.24%), indicating results were satisfactory.

In the soil fertilization treatments, the application of $ZnSO_4 \cdot 7H_2O$ (treatment B) showed better PY results compared to foliar application (treatment C). The foliar application of potassium iodate (KIO_3) (treatments E and F) showed significantly positive and statistically similar results. Treatment F combined the foliar application of KIO_3 with 2% KNO_3. Treatment I, containing Zn and urea, was also a foliar application and showed good results, as did treatment K, with two foliar applications of the fungicide Bayer Antracol-Zn. Treatment L, containing Zn, Fe, I, and Se as a foliar application also showed positive results compared to the control. Finally, treatments N and O, with different concentrations of Mg (gypsum) applied to the soil, also showed positive and statistically similar effects (Tab. 1). Boêno *et al.* (2011) evaluated processing yield in red rice samples and found values ranging from 71.1% to 74.7%. Farinelli *et al.* (2004) evaluated processing yield of upland rice samples under no-till and nitrogen and potassium fertilization and found values ranging from 71.4% to 72.3%.

In general, samples had many broken grains, which can be the result of numerous factors occurring during polishing, namely: cracks before harvesting, immature and chalky grains, rapid drying, and uneven moisture distribution in the grains (Luz *et al.*, 2005).

The best PY result found in rice samples submitted to agronomic biofortification showed an improvement of approximately 3% in the number of polished grains (whole, ¾, ½, ¼ with broken grain) in relation to the weight of paddy rice, compared to the control.

For the GY quality, only two samples differed from the control. There was an increase in whole grain percentage in relation to processed grains (broken and whole), as illustrated in Table 1.

There was a significant effect of treatments and blocks at 1% probability according to the F test. Applying the Scott-Knott test at 5% probability showed that control samples differed from samples of the B treatment, which consisted of the application of $ZnSO_4 \cdot 7H_2O$ to the soil, and the K treatment, which consisted of the foliar application of Bayer Antracol-Zn; these samples were significantly different from each other and from the control. The B and K treatments showed higher GY with values of 56.67% and 47.08% (Tab. 1). The analysis of GY plays an important role for producers, because the greater the GY, the smaller the losses and, consequently, the greater the profit, since whole grains have higher market values. These same samples also showed good results when analyzing the PY. Boêno *et al.* (2011) evaluated the GY of red rice samples and found values ranging from 62.8% to 65.4%. Farinelli *et al.* (2004) evaluated the GY of upland rice samples under no-till and nitrogen and potassium fertilization and reported values ranging from 43.4% to 46.7%. When comparing the results of the present study with the literature data, in general, the values for GY in the present experiment were low, averaging 36.48%, except for treatments B and K.

In general, rice consumers prefer a uniform product, with reduced levels of damaged and broken grains. Therefore, producers and cereal manufacturers aim to achieve optimal performance during rice processing to obtain good yields of whole grains. The value of this product on the market varies according to the breakage index obtained during grain processing. This factor is also essential in determining the acceptance of new cultivars (Castro *et al.*, 1999).

The treatments used in agronomic biofortification did not affect the BRSMG Caravera rice variety in terms of the percentage of whole grains obtained from polished grains.

Classification of rice grains

According to the mentioned methodology for rice classification, the following results of sample classification were obtained: rice grains of treatment B, which consisted of the application of Zn to the soil as $ZnSO_4 \cdot 7H_2O$, were classified as type 4 and the other samples as out of type.

All rice grains intended for consumption must be classified into types, expressed numerically (type 1, 2, 3, 4, and 5). This classification is defined according to the total number of broken grains and grit and to the percentage of occurrence of defects, such as foreign matter, impurities, moldy, discolored, chopped, stained, chalky, green, streaked, and yellow grains.

The rice samples in this study showed high breakage during processing but did not present other defects considered for classification. Except for treatments B and K, all the other treatments showed a percentage of broken and grit greater than 45%, which classified them as out of type. Treatment B grains presented 33.47% of total broken and grits, with a maximum percentage of grit of 2.88, qualifying them as type 4. Treatment K grains presented 40.84% of total broken grains and grits, which would classify them as type 5, however they presented a maximum percentage of grit of 5.39, which also classifying them as out of type. The total percentage of broken grains for all samples can be seen in Table 2. Without the high breakage rate, these samples would have obtained a higher classification, as no other defects were observed.

TABLE 2. Total of broken grains and grain classification of biofortified BRSMG Caravera rice samples.

SWG		1/2 grains (g)	1/4 grains + grit (g)	SBG (g)	SPPG (g)	BGG (%)	WG (g)	GT (%)	Cl
T	W (g)								
A	532.66	66.89	428.74	495.64	1028.29	48.20	-	-	*
B	719.07	55.54	306.13	361.67	1080.73	33.47	31.21	2.89	**
C	563.16	66.53	454.60	521.13	1025.43	50.82	-	-	*
D	490.52	71.51	461.33	532.84	1023.36	52.07	-	-	*
E	535.89	79.66	451.30	530.96	1066.86	49.77	-	-	*
F	555.93	60.44	461.21	521.66	1077.58	48.41	-	-	*
G	501.02	80.78	472.72	553.51	1054.53	52.49	-	-	*
H	546.11	64.08	434.70	498.78	1044.89	47.74	-	-	*
I	573.56	77.43	419.32	496.75	1070.31	46.41	-	-	*
J	492.04	63.06	460.76	523.83	1015.86	51.56	-	-	*
K	629.15	61.00	373.31	434.31	1063.46	40.84	57.38	5.40	*
L	535.06	74.25	461.30	535.55	1070.61	50.02	-	-	*
M	533.43	54.73	434.23	488.96	1022.39	47.83	-	-	*
N	575.99	63.65	442.76	506.41	1082.41	46.79	-	-	*
O	571.66	71.64	426.18	497.82	1069.48	46.55	-	-	*
P	458.34	78.72	478.45	557.17	1015.51	54.87	-	-	*

SWG = sum of whole grains = whole grains (g) + 3/4 grains (g), SBG = sum of broken and grits (g) = 1/2 grains (g) + 1/4 grains + grit (g), SPPG = sum of processed and polished grains (g), BGG = broken grains and grits in relation to the total processed and polished (%), WG = weight of grits (g), GT = grits in relation to total processed and polished (%), Cl = classification, T = treatment, W = weight (g), * = out of type, ** = type 4.

Depending on their importance and effect on the rice grain product, defects are classified as general or severe. In products considered of good quality, the percentage of defects must be reduced as much as possible, especially severe defects, which result from contamination by foreign matter, moldy and discolored grains. Streaked, yellow, stained, chopped, and chalky grains refer to general defects. Rice samples that do not meet the requirements for commercial type classification are considered as substandard or out of type. The sale of substandard products is prohibited for both human and animal consumption, as they are in poor condition (Brazil, 2009). Products classified as out of type can be sold, provided this is clearly indicated on the packaging, or they can also be reprocessed, broken down and recomposed to fit into types (Brazil, 2009; Castro *et al.*, 1999).

Conclusions

Agronomic biofortification treatments can improve, in some cases, the technological quality parameters of rice such as processing yield and grain yield. Specific treatments show better results compared to the control treatment.

However, the rice samples did not present a good classification. According to the Ministry of Agriculture, Livestock and Food Supply of Brazil (MAPA), these rice grain samples must be classified as out of type, except for the sample of treatment B, which was classified as type 4. This classification is due to the large amount of broken grains, despite the absence of other defects, such as foreign matter, impurities, moldy, discolored, chopped, stained, chalky, green, streaked, and yellow grains. When considering all the parameters analyzed, the sample of treatment B, which consists of applying $ZnSO_4 \cdot 7H_2O$ to the soil, showed the best results.

Acknowledgments

The authors thank the following agencies and organizations of Brazil: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support, and the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG). The authors neither endorse nor disapprove of the use of the fertilizer formulations and/or chemical products mentioned in this article and have no affiliation with the fertilizer manufacturing companies or organizations involved in the treatments reviewed in the experiment.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

SMS: conceptualization, methodology, validation, formal analysis, research, resources, writing – original draft, writing – review & editing, visualization, supervision, project administration, and funding acquisition. LAA: conceptualization, research, resources, writing – review & editing, and visualization. JP: conceptualization, research, resource acquisition, supervision, visualization, writing – original draft, and writing – review & editing. All authors reviewed and approved the final version of the manuscript.

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Acknowledgments

When considered necessary, the authors may acknowledge the researchers or entities that contributed - conceptually, financially or practically - to the research: specialists, commercial organizations, governmental or private entities, and associations of professionals or technicians.

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AGRONOMIA COLOMBIANA

VOLUME XLII, No. 2 MAY-AUGUST 2024 ISSN (print): 0120-9965 / ISSN (online): 2357-3732

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