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Research Paper

Impact of ventilation system with sucrose doses and wavelength on biomass production and arbutin content in *Origanum majorana* L. plantlets

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A R T I C L E I N F O

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A B S T R A C T

The ventilation system using porous membranes allows for better plantlet growth and increased survival during the acclimatization phase. The objective was to investigate the effect of alternative membrane system and wavelength on the growth and accumulation of arbutin *in vitro* plantlets of *Origanum majorana*. Nodal segments containing a pair of leaves were cultivated *in vitro* using MS culture medium and an alternative membrane system consisting of four porous membranes. Sucrose was added at concentrations of 0, 7.5, 15, and 30 g. Another experiment was conducted using four porous membranes supplemented with 15 g L^{-1} of sucrose and under different light-emitting diodes (LEDs): white LED (W); blue (B); green (G); yellow (Y); red (R); a combination of blue and red (50%B:50%R; 30%B:70%R; 70%B:30%R). Marjoram (*Origanum majorana*) plantlets exhibit photomixotrophic growth, with a medium containing 15 g of sucrose, and an alternative membrane system with 4 porous membranes promoting increased growth. The sucrose concentration influenced the accumulation of arbutin. The wavelength, in conjunction with the use of a membrane system, affected the growth, concentration of photosynthetic pigments, and accumulation of arbutin in marjoram plantlets. The highest biomass accumulation and arbutin synthesis were induced by the 30%B:70%R spectrum combination, while lower biomass and accumulation were observed under the monochromatic blue spectrum. Improving factors such as ventilation, wavelength, and sucrose can lead to more efficient resource use, reducing costs and increasing the yield of arbutin *in vitro*.

1. Introduction

Plants cultured *in vitro* by the conventional method grow in a microenvironment with high humidity and low CO_2 concentration during the photoperiod (Nguyen et al., 2020; Xiao et al., 2011). Under these conditions, plants normally have less development, low gas exchange and photosynthetic rates, morphological disorders such as drastic reduction of the cuticle and formation of non-functional stomata, physiological and higher contamination rates, in addition to having lower survival during acclimatization (Kozai, 2010; Kozai and Kubota, 2001; Nguyen et al., 2020; Saldanha et al., 2012; Xiao et al., 2011). In order to overcome these issues, many studies seek alternatives through cultivation under photomixotrophic or photoautotrophic conditions (Iarema et al., 2012). To achieve this, the carbon source of the cultivation medium can be reduced or eliminated, the luminosity increased and gas diffusion between the internal and external environment of the flasks can be increased (Hoang et al., 2017; Nguyen et al., 2020; Xiao et al., 2011). To improve the ventilation of *in vitro* culture vessels, Saldanha et al. (2012) utilized a permeable membrane system. This system reduced the relative humidity inside the vessels and enhanced plant growth compared to a closed system without a gas-permeable membrane. Short et al. (1985) reported that optimum growth and *in vitro* hardening occurred when plantlets of cauliflower and chrysanthemum were cultured at 80% relative humidity. Thus, the plantlets could be transferred to the soil, without protection against humidity, and no wilting occurred. According to Chandra et al. (2010), the high relative humidity inside the culture vessel can lead to losses during acclimatization because it reduces the deposition of epicuticular

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waxes and the development of functional stomata. Saldanha et al. (2013) reported that daytime CO_2 concentrations exhibit variations. Also, according to Saldanha et al. (2013) a CO_2-enriched atmosphere improved *in vitro* growth of *Pfaffia glomerata*.

The low rates of photosynthetic activity *in vitro* plants can be overcome through the optimization of the culture medium and environment. Methods such as using containers that allow gas exchange, cultivation in a CO_2-enriched environment, and reducing or eliminating the sources of sucrose in the medium are employed to explore the photoautotrophic potential of *in vitro* plants (Yaseen et al., 2013). Cultivation under photoautotrophic conditions can have an effect on plant secondary metabolism, leading to an increase in the production of bioactive compounds of interest (Iarema et al., 2012; Saldanha et al., 2013, 2014).

The use of caps with gas-permeable membranes *in vitro* cultivation enhances gas exchange, promotes the renewal of the internal atmosphere within the containers, reduces humidity inside the containers, and facilitates the absorption of water, nutrients, and transpiration (Nguyen et al., 2020; Saldanha et al., 2012; Xiao et al., 2011). In addition to the increase in photosynthetic rates and greater biomass, the use of caps with gas-permeable membranes can also influence plant anatomy (Batista et al., 2016; Nguyen et al., 2020; Saldanha et al., 2013) and plant secondary metabolism (Xiao et al., 2011). Furthermore, the use of membranes enhances gas exchange and influences the growth and secondary metabolism of medicinal plants cultivated *in vitro*. Iarema et al. (2012) evaluated plantlets of Brazilian ginseng *Pfaffia glomerata* cultivated under conventional and natural ventilation systems with 1 and 2 membranes, observing greater growth in a photoautotrophic cultivation. Silva et al. (2017) observed that the use of a natural ventilation system with 1 and 2 membranes increases the dry weight of *Plectranthus amboinicus*, while the use of 4 membranes enhances the accumulation of carvacrol. Explants of *Aeollanthus suaveolens* were cultivated under conventional and alternative membrane systems and four porous membranes led to greater dry weight accumulation, increased production of photosynthetic pigments, and enhanced synthesis of (Z)-β-farnesene (Araújo et al., 2023). Also, Rocha et al. (2022a) reported that culture of *Lippia dulcis* plantlets under natural ventilation system with four membranes lead the highest growth, greatest accumulation of photosynthetic pigments, and the most anatomically organized tissues, in addition to the best antioxidant defense response. Others studies have shown positive responses on plantlets growth and content of secondary metabolites under natural ventilation systems *in vitro* as: *Lippia gracilis* Schauer (Verbenaceae) (Lazzarini et al., 2019); Aloysia *gratissima* ((Verbenaceae) (Coelho et al., 2022).

As a source of signal and energy, light is one of the most important environmental factors for the growth and development of plants. Light quality plays a complex and multifaceted role in the morphology and physiology of plants. Understanding how different colors and wavelengths of light affect plants is essential for optimizing plant cultivation in various environments and maximizing food and crop production (Wang et al., 2016). The use of wavelength *in vitro* culture has shown positive responses in terms of the growth and accumulation of secondary metabolites in some species. Araújo et al. (2023) reported that wavelength affected growth, photosynthetic pigments and volatile organic compound (VOC) accumulation in *Aeollanthus suaveolens* under *in vitro* culture. Also, Rocha et al. (2022b) evaluated the effects of light intensity and wavelength on the growth, antioxidant defense and production of volatile compounds of *Lippia dulcis*. They showed that plantlets cultivated under combinations of 50% Blue:50% Red and 30%B:70%R light provided greater accumulation of dry weight. Other studies also demonstrate the effect of wavelength on plantlet growth and secondary metabolites *in vitro: Achillea millefolium* L. (Alvarenga et al., 2015); *Plectranthus amboinicus* (Silva et al., 2017); *Lippia gracilis* (Lazzarini et al., 2018); *Lippia rotundifolia* (Hsie et al., 2019); *Urtica dioica* (Coelho et al., 2021); *Mentha arvensis* (Oliveira et al., 2021); *Origanum majorana* (Cossa et al., 2024).

Arbutin is a hydroquinone glycoside known for its inhibitory effect on tyrosinase activity and is commonly used as a skin lightening agent (Gallo et al., 2015; Kim et al., 2018). The biosynthesis pathway involves the conversion of shikimic acid to chorismic acid, which is then transformed into aromatic amino acids and eventually into hydroquinone, which is a key precursor for arbutin (Saeedi et al., 2021; Shen et al., 2017). Arbutin plays a crucial role in plants by serving as a defense mechanism, providing UV protection, and reguCoulating plant growth. Arbutin has antimicrobial properties that protect plants against pathogens such as bacteria and fungi (Martins et al., 2021). The compound can act as a deterrent to herbivores due to its potential toxicity when metabolized (Bernays et al., 2000). Also, can absorb UV radiation, helping to protect plant tissues from damage caused by excessive UV exposure (Shu et al., 2024). Furthermore, acts as an antioxidant, scavenging reactive oxygen species (ROS) and helping to maintain cellular homeostasis (Wu et al., 2014), which is vital for proper growth and development. Understanding where and how it is produced in plants, as well as its importance, not only highlights its role in plant physiology but also underscores its value in various industrial applications, particularly in cosmetics and pharmaceuticals.

So far, there are no studies reporting the effects of ventilation system on the *in vitro* cultivation of *Origanum majorana*. However, due to the fact that plants exhibit different growth responses and the production of secondary metabolites when exposed to different environment conditions *in vitro* culture, the objective was to evaluate the effect of alternative membrane system and wavelength on the growth and accumulation of arbutin *in vitro* plantlets of *Origanum majorana*.

2. Material and methods

The herbarium specimen of *Origanum majorana* L. was deposited in the PAMG herbarium of the Agricultural Research Company of Minas Gerais under registration number 58,898. The mother plants were cultivated in the Medicinal Garden of federal University of Lavras (UFLA). Establishment of the *in vitro* explant was in accordance with Cossa et al. (2024), which was carried out in the tissue culture laboratory of the Federal University of Lavras-UFLA (21°13′36″S; 44°58′23″W).

2.1. Alternative membrane system supplemented with different doses of sucrose

Preliminary experiment was carried out with alternative membrane system with control (no membrane - NMS), one (AMS1), two (AMS2), four (AMS4) porous membranes in the culture medium without addition of sucrose using nodal explants with one pair of leaves. However, this experiment was not successful as the explants did not grow due to the absence of a sucrose source. During this experiment, the explants cultured with caps containing four porous membranes showed growth potential, which led us to adjust different sucrose (≥99% (GC), Grade I, suitable for plant cell culture-Sigma Aldrich) doses to evaluate explant growth.

Plantlets already established *in vitro* were used to remove the nodal segment. Nodal segments (1 cm) containing a pair of leaves were inoculated into 200 mL capacity containers with 50 mL of MS culture medium (M5519, Sigma Chemical Company, St. Louis, MO) (Murashige and Skoog, 1962) and an alternative membrane system consisting of four porous membranes (AMS4), with the addition of sucrose at concentrations of 0; 7.5; 15; and 30 g, 6.0 g L^{-1} of agar (Himedia®, type I), and the pH was adjusted to 5.7 ± 0.1 (pHmeter PG2000-GEHAKA™). The containers with the culture media were autoclaved at 125 °C and 1.2 atm of pressure for 20 min. After inoculation, the containers were kept in a growth room with a 16-hour photoperiod, a temperature of 25±1 °C, and an irradiance of 42 μmol m^{-2} s^{-1} provided artificially by light-emitting diodes (LEDs) lamps (TECNAL© Piracicaba, Brazil). The light spectra were measured using a SPECTRA PEN Z850 manual spectrometer (Qubit Systems, Kingston, Ontario, USA) (Fig. 1). The experimental design was completely randomized (CRD) with four treatments,

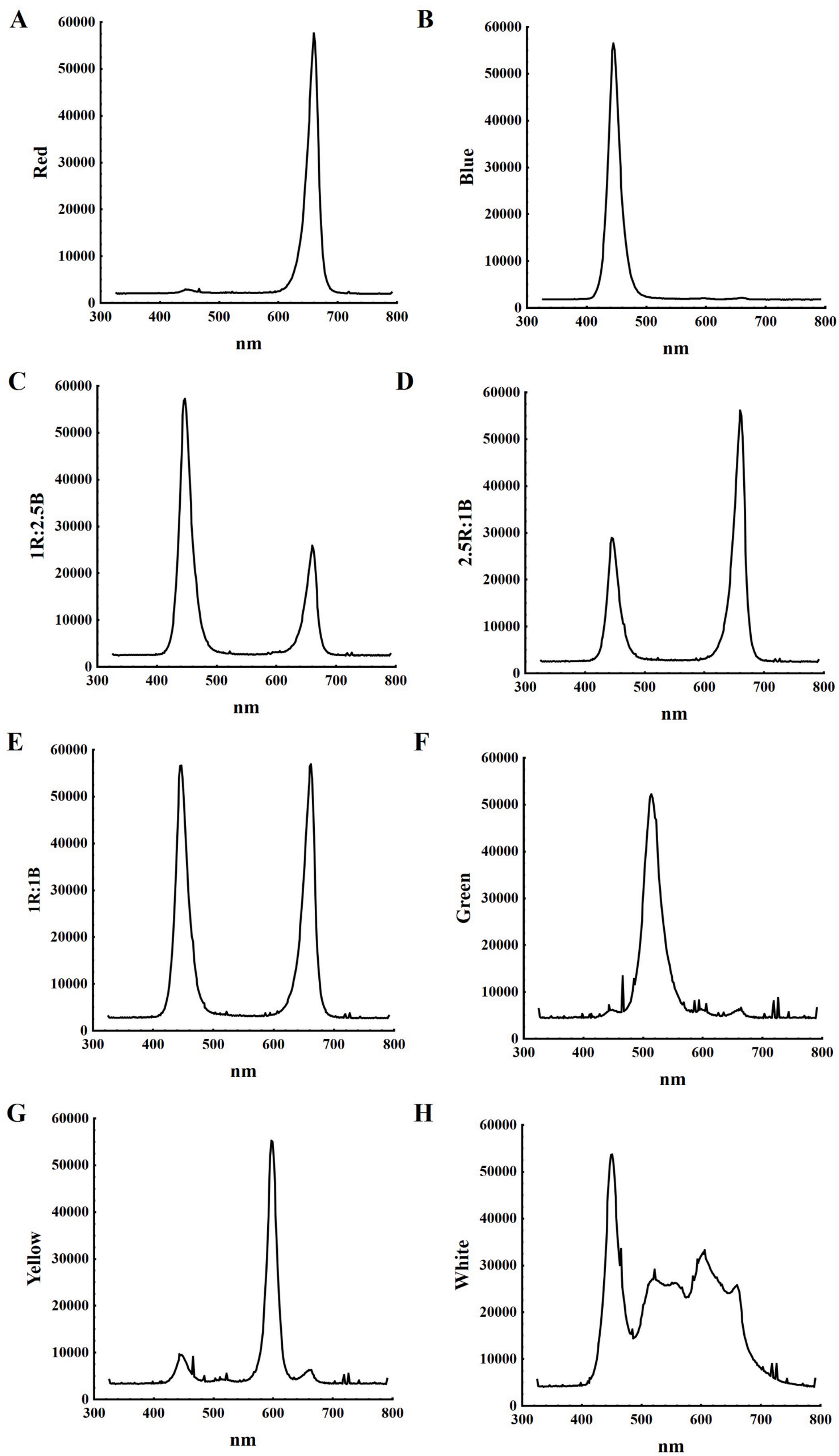

Fig. 1. Spectral profiles of the light sources used in the experiment. Red (A); blue (B); 1R:2.5B (C); 2.5R:1B (D); 1R:1B (E); green (F); yellow (G); white LED (H).

totaling 40 containers and 200 explants.

The porous membranes were manufactured according to the methodology proposed by Saldanha et al. (2012), using beige Cremer® microporous tape and polytetrafluoroethylene (PTFE) tape from Amanco®. The membrane was composed of four layers, with the first layer being microporous tape, the second layer PTFE, and the third layer microporous tape. The three layers were cut into 1 cm^2 squares and distributed separately on the adhesive side of the microporous tape, totaling four layers. Finally, the layers were uniformly cut into squares and distributed over the 1 cm diameter holes in the polypropylene caps of the culture containers.

2.2. Alternative membrane system supplemented with 15 g of sucrose

After the results of the experiment with sucrose doses, only the medium supplemented with 15 g of sucrose with different numbers of porous membranes was evaluated. Nodal segments (±1 cm) containing a pair of leaves were excised from *in vitro*-cultured plantlets and inoculated into 200 mL capacity containers with 50 mL of MS culture medium (Murashige and Skoog, 1962) and a natural ventilation system consisting of one (AMS1), two (AMS2), four (AMS4) porous membranes, and a control (no membrane - NMS), supplemented with 15 g of sucrose, 6.0 g L^{-1} of agar (Himedia®, type I), and pH adjusted to 5.7 ± 0.1(pHmeter PG2000-GEHAKA™). The experimental design was completely randomized (CRD) with four treatments, totaling 40 containers and 200 explants.

2.3. Alternative membrane system under the influence of wavelength

Nodal segments with one pair of leaves (±1 cm) were excised from *in vitro*-cultured plantlets and aseptically inoculated under a laminar flow hood into containers containing 50 mL of MS medium, an alternative membrane system consisting of four porous membranes (AMS4), supplemented with 15 g L^{-1} of sucrose, 6 g L^{-1} of agar (Himedia®, type I), and pH adjusted to 5.7 ± 0.1 (pHmeter PG2000-GEHAKA™). After inoculation, the explants were cultured under different light-emitting diodes (LEDs) (TECNAL© Piracicaba, Brazil): white LED (W); blue (B); green (G); yellow (Y); red (R); a combination of blue and red (50%B:50%R; 30%B:70%R; 70%B:30%R). The wavelength of each light is shown in Fig. 1. The experimental design was completely randomized (CRD) with eight treatments, totaling 96 containers and 480 explants. The LED light intensity was 42 μmol m^{-2} s^{-1}. The light spectra were measured using a SPECTRA PEN Z850 manual spectrometer (Qubit Systems, Kingston, Ontario, USA).

2.4. Growth analysis

At 40 days, the plantlets were assessed for the shoot number (SN), shoot length (SL), leaf dry weight (LDW), stem dry weight (SDW), and above-ground dry weight (ShDW=LDW+SDW). For the determination of dry weight in leaves and stems, the respective parts were placed in kraft paper bags and placed in a forced air circulation oven at 45 °C (Model 320E-FANEM™) until a constant weight was reached (approximately 72 h). Subsequently, the dried material was weighed on a precision balance. The roots of the explants were very small and could not be evaluated for dry weight.

2.5. Analysis of photosynthetic pigments

The methodology used was in accordance with Cossa et al. (2024), and is briefly described below. Fresh leaf of 50 mg from each treatment were collected in a dark room illuminated only with green light. Second pair of leaves from each plantlet was used and placed in Falcon-types tubes with 10 mL of dimethyl sulfoxide (DMSO) and wrapped in aluminum foil. Subsequently, the tubes were placed in an oven at 65 °C (24 h). After this period, aliquots of 3 mL of the content were collected and transferred to quartz cuvettes, and the absorbances were read at the wavelengths of 480 nm (carotenoids), 649 nm (chlorophyll a), and 665 nm (chlorophyll *b*). The TECAN INFINITY M200 PRO spectrophotometer, operated with the I-control® data processing system (version 3.37), was used for optical density readings.

The concentrations of chlorophyll and carotenoids were expressed as milligrams of pigment per gram of fresh leaf tissue (mg g^{-1}), calculated according to the following equations, following the methodology of Wellburn (1994): Chlorophyll a (Ca) = [(12.47 x A665) - (3.62 x A649)]; Chlorophyll *b* (Cb) = [(25.06 x A649) - (6.5 x A665)]; Carotenoids = (1000 x A480 - 1.29 x Ca - 53.78 x Cb) / 220. Where, A665= absorbance measured at 665 nm; A649= absorbance measured at 649 nm; A480= absorbance measured at 480.

2.6. Arbutin extraction, sample preparation

The methodology used was in accordance with Cossa et al. (2024), and is briefly described below. Aliquots of 50 mg of pulverized dry leaves of *O. majorana* were extracted by sonication for 3 cycles of 15 min, using 3 ml MeOH (50% H_2O + 50% MeOH) in each cycle. The volume of the 3 mL extract from each organic phase was collected, combined, and evaporated under vacuum using a rotary evaporator at a maximum temperature of 40 °C, and the residues were dissolved in 1, 000 μL of H_2O. Next, the sample was transferred to a microtube and subjected to sonication for 2 min, followed by centrifugation at 10,000 rpm for 10 min. The supernatant was automatically injected into the chromatograph. The chromatographic condition established for the quantification of arbutin in the extract of *O. majorana* leaves includes the use of an Eclipse XDB-C18 column (150 mm × 4.6 mm inner diameter, 5 μm particle size), column temperature of 25 °C, and a mobile phase consisting of Milli-Q water and HPLC-grade methanol.

2.7. Chromatographic conditions of HPLC–dad analysis

The methodology used was in accordance with Cossa et al. (2024). Two arbutin standard solutions were prepared. Initially, an aqueous solution was prepared at a concentration of 2 mg mL^{-1}. Then, by diluting the first, another solution was prepared at a concentration of 1 mg m^{-1}. Analyses by high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD) were performed in an Agilent 1200 liquid chromatography system (Agilent Technologies®, Waldbronn, Germany) equipped with a quaternary pump (G1311A) with a degassing system (G13222A), an ALS autosampler (G1322A) and a TCC heater set (G1316A). A variable-wavelength ultraviolet detector (G1315D) was used to obtain chromatograms at 220 nm. The equipment was controlled by OpenLAB software, version A07.04, build 04.07.28.

The separations were performed on a C18 reversed-phase analytical column (Eclipse XDB-C18, Agilent Technologies®, USA) with silica-based packing (150 mm × 4.6 mm di, 5 μm) and a precolumn (12.5 mm × 4.6 mm ID, 5 μm). A volume of 10 μL (50 mg mL^{-1} LDW) of the samples was injected into the column with the temperature maintained at 25 °C and a constant flow rate of 1.0 mL min^{-1}. Ultrapure water (A) and methanol (B) were used as eluents.

The elution program was as follows: 10% B in isocratic conditions for 0–5 min, followed by a linear gradient until 95% B for 5 min, remaining in isocratic conditions for another 5 min, returning to the initial condition in a linear gradient reversed for 5 min, totaling 20 min of analysis. Each run was followed by an equilibration period of 10 min.

2.8. Statistical analyzes

The data were subjected to analysis of variance and the means compared by the Scott-Knott test at 5% probability, using the SISVAR statistical software (Ferreira, 2019). Statistica, version 13.3 (StatSoft, Tulsa, OK, USA) was used for principal component analysis (PCA).

3. Results and discussion

3.1. Alternative membrane system (AMS4) supplemented with different doses of sucrose

Porous membranes can be used in micropropagation systems and for regulating the cultivation environment, while the sucrose dose is critical for the development and differentiation of plant tissues *in vitro* cultures.

The control of these factors can affect the success of high-quality plantlet production *in vitro* cultivation processes and the content of metabolite compounds. Therefore, the *in vitro* cultivation of *O. majorana* plantlets under different sucrose concentrations and an alternative membrane system with four porous membranes (AMS4) showed a significant effect. The explants cultured in a medium without sucrose and with four porous membranes (AMS4) did not develop (Fig. 2a). Therefore, the culture medium should be supplemented with sucrose, as it serves as an energy source to support photomixotrophic metabolism. Throughout the experiment, a decrease in the amount of culture medium was observed. This was attributed to increased evapotranspiration due to the presence of porous membranes (Fig. 2a), allowing for greater water loss from the culture medium (Saldanha et al., 2012).

Possibly, the treatment with four porous membranes, by enabling greater gas exchange, facilitated the increased growth of *O. majorana* plantlets *in vitro*. Similar results were described by Lazzarini et al. (2019) in the cultivation of *Lippia gracilis* using a ventilation system with different quantities of porous membranes on the lids, which showed greater shoot length and dry weight with the use of four membranes. The cultivation *in vitro* of *Aeollanthus suaveolens* under natural ventilation system with four membranes (AMS4) promoted better growth compared with conventional sealed system (Araújo et al., 2023). According to Silva et al. (2014), greater gas exchange *in vitro* cultivation leads to increased plantlet growth, expansion of leaf area, and photosynthetic rate.

Higher values of shoot length (3.9 cm) and dry weight were observed in a medium supplemented with 15 g of sucrose. Regarding the number of shoots, the highest value was observed in cultivation supplemented with 7.5 g of sucrose (3.7) (Fig. 3). However, Silva et al. (2017), in the cultivation of *Plectranthus amboinicus* using different ventilation systems and different types of explants, reported that the ventilation system with one membrane with apical explant and the ventilation system with two membranes with nodal segment provided higher biomass production. The use of porous membranes in a medium supplemented with sucrose showed differential growth in shoot regeneration and leaf expansion at different sucrose doses. Research reports indicate that the use and increase in the number of membranes in the lids allow for increased gas exchange and reduced humidity, promoting plant growth (Xiao et al., 2011).

The sucrose concentration significantly affected the plantlet dry weight (Fig. 3). Plantlets cultivated in a medium supplemented with 15 g L^{-1} accumulated higher dry weight in leaves, stems, and the shoot. The explants cultivated in the absence of sucrose did not exhibit growth (Fig. 2a). The explants cultivated in the medium supplemented with 15 g L^{-1} accumulated 76% and 37% more dry weight in the leaves compared to the medium with 7.5 g L^{-1} and 30 g L^{-1}, respectively. In the stem, the accumulation was 96% and 11%, respectively. These results are similar to those obtained in *Physalis angulata* (Solanaceae) cultivated in MS medium with different sucrose concentrations (0, 7.5, 15, 22.5, and 30 g L^{-1}), where greater growth occurred in a medium supplemented with 15 g of sucrose (Santos et al., 2020). Another study with similar results used

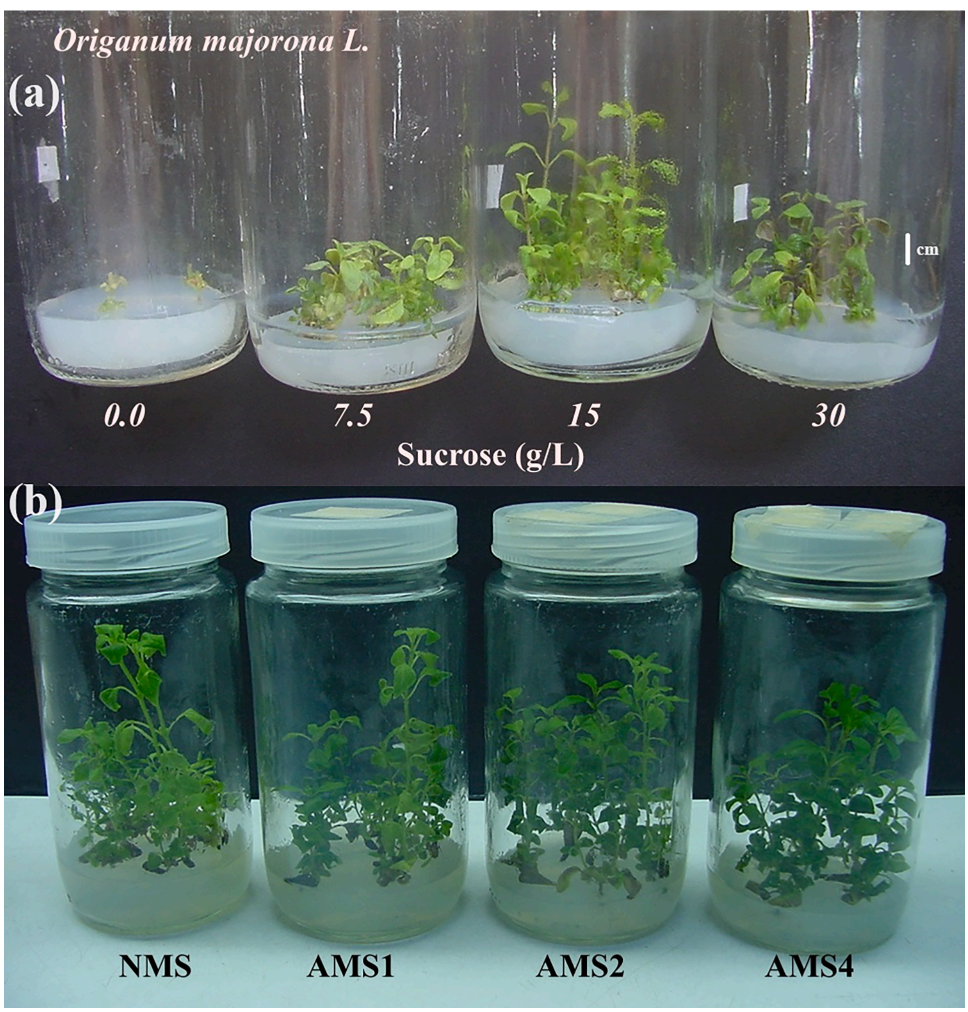

Fig. 2. *Origanum majorona* L. plantlets grown *in vitro* in MS medium supplemented with different concentrations of sucrose in combination with an alternative system with 4 porous membranes (AMS4) and plantlets grown *in vitro* in MS medium supplemented with 15 g of sucrose in combination with non-membrane system (NMS) and an alternative membrane system (1, 2 and 4 porous membranes), at 40 days.

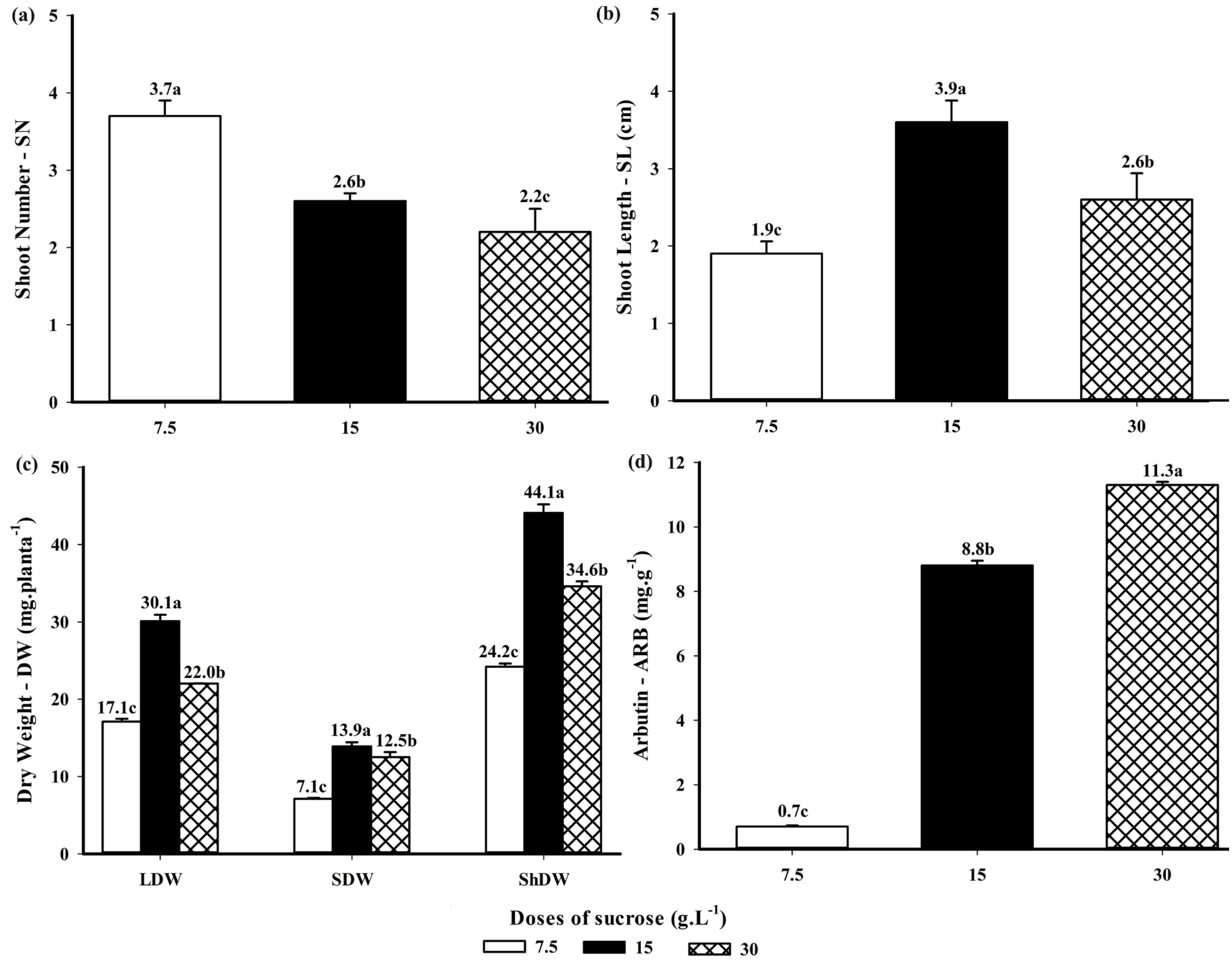

Fig. 3. *Origanum majorana* L. plantlets grown *in vitro* in MS medium supplemented with different doses of sucrose in combination with an alternative membrane system with 4 membranes (AMS4), A) shoot number (SN); B) shoot length (SL); C) leaf dry weight (LDW), stem (SDW), above-ground dry weight (ShDW=LDW+SDW); D) arbutin content in leaf (ARB mg g $^{-1}$), at 40 days.

the natural ventilation system with four porous membranes and 15 g L^{-1} sucrose in MS medium, which resulted in a higher dry weight production in *Lippia rotundifolia in vitro* (Hsie et al., 2022). However, Carvalho et al. (2018) reported that for better growth and multiplication of *Chenopodium ambrosioides* L., the explants should be inoculated in the half strength of MS salt concentration medium and 30 g L^{-1} sucrose. Nicoloso et al. (2003) evaluated the effect of sucrose and carbohydrate source and found increases in the number of shoots, length, and dry weight of the shoot with the increase of sucrose in the cultivation of *Pfaffia glomerata*, which supports the results observed in the present study. Therefore, the accumulation of dry weight *in vitro* cultivation results from the interaction between the plant species cultivated, the composition of the culture medium, carbohydrate metabolism, and the environment.

Photoautotrophic micropropagation (without added sugar) has been widely used to promote growth and photosynthesis, prevent morphological and physiological disorders and high survival in acclimatization (Nguyen et al., 2020). However, not all plant species respond positively to photoautotrophic *in vitro* cultivation, sometimes requiring the exogenous addition of sugar (photomixotrophic). Monfort et al. (2015) observed a negative effect on the cultivation of *Ocimum selloi* regarding the number of shoots, length, and dry weight in the absence of sucrose, which supports the results obtained in the present study. However, divergent results were obtained in the cultivation of *Ochroma pyramidate* with different sucrose concentrations (0, 15, and 20 g), showing better growth of the shoot and dry weight in a medium without the addition of sugar.

3.2. Quantification of arbutin (ARB) in the medium supplemented with doses of sucrose

The *in vitro* cultivation of *O. majorana* plantlets under different sucrose concentrations and a ventilation system with four porous membranes (AMS4) showed a significant effect on the arbutin content (Fig. 3). The arbutin content in leaves from a medium supplemented with 30 g of sucrose (11.3 mg g^{-1}) was higher than in the medium supplemented with 15 g (8.8 mg g^{-1}) and 7.5 g (0.7 mg g^{-1}), corresponding to 28% and 16 times, respectively. Therefore, the higher sugar availability in the culture medium was crucial for arbutin production in the leaves of *O. majorana*. It is possible to observe the quantitative difference in arbutin through the intensity of its chromatographic peak according to the sucrose availability (Fig. 4). Although a lower sucrose concentration (15 g) in the culture medium provided higher values in growth variables, it was not sufficient to promote an increase in the arbutin content in the leaves (Fig. 3). However, the arbutin yield was higher in the medium supplemented with 15 g of sucrose, due to the greater production of leaf dry weight. Similar results were described by Fortini et al. (2021), where they observed in the *in vitro* cultivation of *Vernonia condensata* that the addition of sucrose favored the increase of phenolic compounds and flavonoids, and that the increase in these compounds was higher when the plantlets were under a ventilation system.

The synthesis of arbutin involves the conversion of the hydroquinone glycoside with the assistance of an enzyme called β-glycosyltransferase.

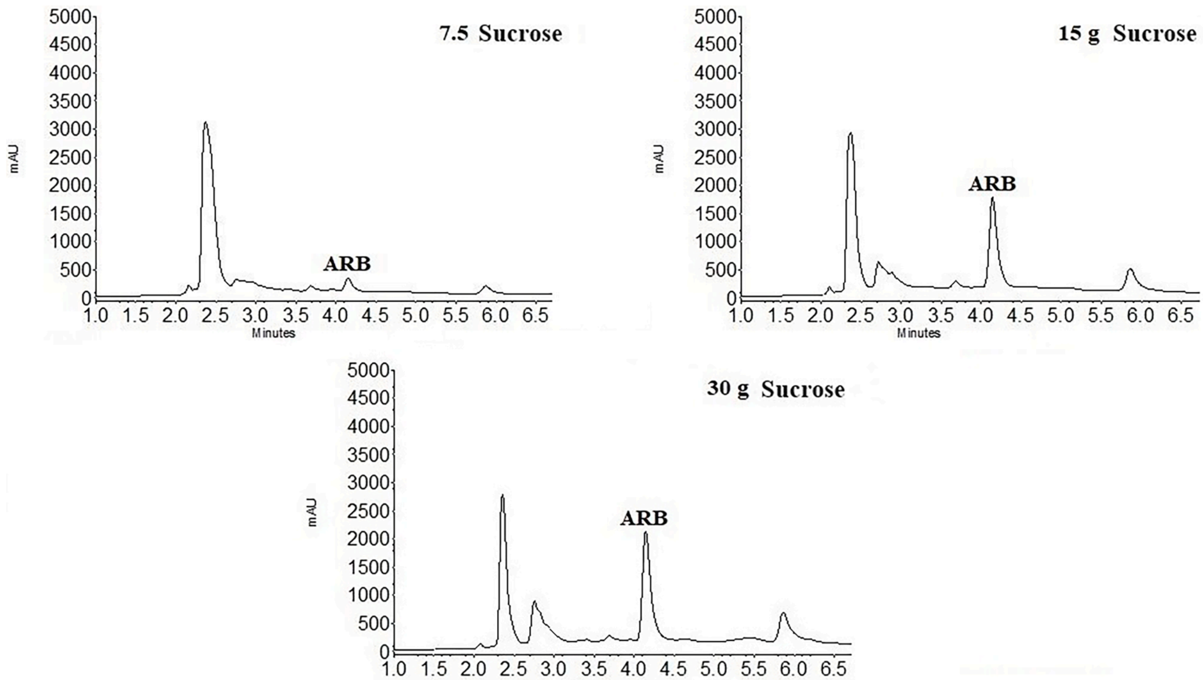

Fig. 4. Chromatographic profiles recorded by HPLC - DAD at 220 nm of *Origanum majorana* L. leaf extract in MS medium supplemented with different concentrations of sucrose in combination with an alternative membrane system with 4 porous membranes (AMS4), at 40 days. T1 (7.5 g), T2 (15 g), T3 (30 g).

In this process, the hydroquinone molecule is bound to a glucose molecule, forming arbutin. Sugar plays a crucial role in this synthesis process. Glucose is the molecule that combines with hydroquinone to form arbutin. Without the presence of sugar in the form of glucose, the synthesis of arbutin would not occur. Zhou et al. (2023) showed through metabolic engineering a higher production of α-arbutin from sucrose by biocatalysis. They reported that the rate of sucrose utilization was increased due to the attenuation of its hydrolysis and the assistance of intracellular enzymes that converted the secondary product (fructose) back into a substrate for the synthesis of α-arbutin. The whole cell biocatalysis of α-arbutin is performed by engineered microbial cells expressing glycosyltransferase from different sources (Zhu et al., 2018).

One potential mechanism that may have contributed to the accumulation of arbutin in marjoram is through sucrose, as sucrose plays a significant role in arbutin biosynthesis, particularly in its enzymatic production. In this process, sucrose acts as a glycosyl donor in transglycosylation reactions. Various enzymes such as sucrose phosphorylase and amylsucrase facilitate the transfer of glucose from sucrose to hydroquinone, forming arbutin. Biosynthesis involves the glycosylation of hydroquinone with glucose, catalyzed by the enzyme arbutin synthase (Xu et al., 2022; Zhu et al., 2018). The use of 20 mM sucrose and 5 mM hydroquinone as substrates provided higher efficiency in arbutin synthesis (Xu et al., 2022). Research using metabolic engineering techniques, including modification of sucrose utilization pathways, has demonstrated a significant increase in arbutin yield. By reducing competing pathways for sucrose metabolism, the efficiency of the biocatalytic process is enhanced (Zhu et al. 2018).

Sucrose is an important substance in disease resistance, as it not only provides the carbon skeleton for metabolite synthesis but also acts as a signal to induce plant defense. *In vitro* cultivation without supplementation of a carbon source can also alter the secondary metabolism of plants. Sucrose can function as a signaling molecule and control various metabolic processes. It is an important factor that affects the synthesis of secondary metabolites (Smeekens and Hellmann, 2014). Kim et al. (2020) observed an increase in flavonoids in *Melissa officinalis* plantlets cultivated *in vitro* with an increase in sucrose concentration (50 to 300 mM). They concluded that sucrose variation caused the accumulation of certain flavonoids as a defense mechanism against osmotic stress.

3.3. *Alternative membrane system supplemented with 15 g of sucrose*

The *in vitro* cultivation of *O. majorana* plantlets under different ventilation systems (without membrane, with one, two, and four porous membranes) showed a significant effect on dry weight production. Fig. 5 show *Origanum majorana* L. plantlets grown *in vitro* in MS medium supplemented with 15 g of sucrose in combination with non-membrane system (NMS) and an alternative membrane system (1, 2 and 4 porous membranes), at 40 days. There was no significant difference in the number of shoots and length of the aerial part (Table 1). When the alternative membrane system was used, it was visually observed that the plantlets exhibited larger leaf size and intense green color (Fig. 2b). Higher values of dry weight in leaves and the shoot were observed in cultivation with AMS4, 31.8 mg and 44.6 mg, respectively (Table 1). However, higher values of stem dry weight were observed in explants cultivated in the ventilation system without a membrane (NMS), as this system promoted greater formation of axillary shoots.

Possibly, the treatment with four porous membranes, by allowing greater gas exchange and facilitating increased nutrient absorption due to higher evapotranspiration from the culture medium, resulted in greater growth of *O. majorana* plantlets *in vitro* compared to the system without membranes (NMS). Studies have reported that increased gas exchange in the containers used for *in vitro* plant propagation promotes the growth and development of plants. According to Lazzarini et al. (2019) in the cultivation of *Lippia gracilis* using a natural ventilation system with different quantities of porous membranes on the lids, which showed greater shoot length and dry weight with the use of four membranes. Plantlets of *Aeollanthus suaveolens* also accumulated more dry weight with the use of four porous membranes (Araújo et al., 2023). In the study by Saldanha et al. (2012), it was observed that the natural ventilation system improved the *in vitro* growth of *Pfaffia glomerata* (Spreng.). These studies used the ventilation system without sucrose supplementation. However, preliminary experiment was carried out with alternative membrane system with control (no membrane - NMS), one (AMS1), two (AMS2), four (AMS4) porous membranes in the culture

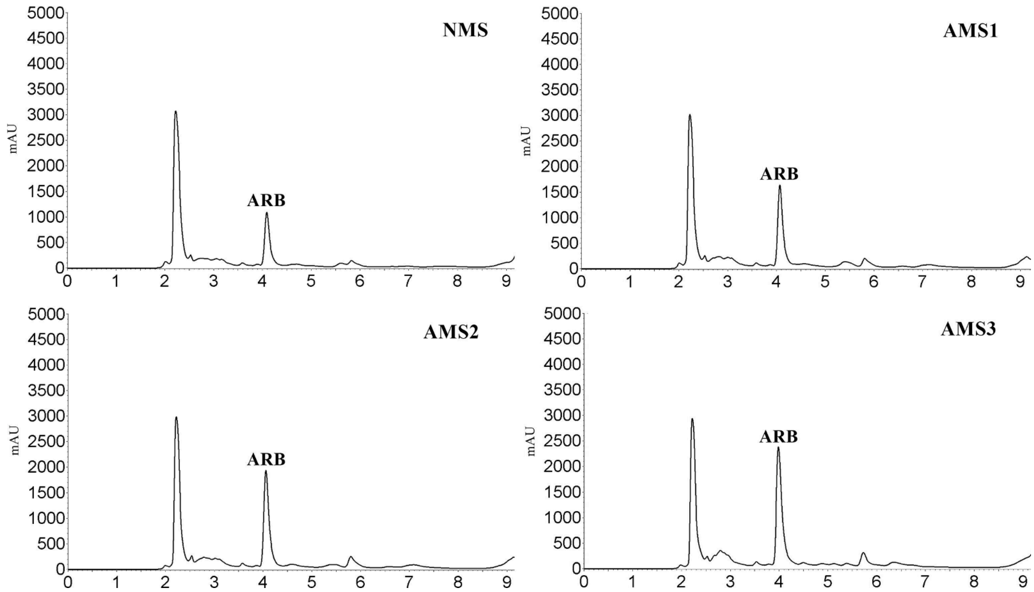

Fig. 5. Chromatographic profiles obtained by HPLC - DAD (quantification of arbutin in dry leaves). Retention time (RT) ARB = 4.2 min. Produced *in vitro* cultivation of *Origanum majorana* L. under different alternative membrane systems - one (AMS1), two (AMS2), four (AMS4) and non-membrane system (NMS), supplemented with 15 g of sucrose.

Table 1
Origanum majorana L. plantlets from nodal segments cultivated *in vitro* in MS medium supplemented with 15 g of sucrose in combination with an alternative membrane system - AMS (1, 2 and 4 porous membranes) and no membrane system (NMS), at 40 days. Shoots number (SN), shoot length (SL), leaf dry weight (LDW), stem (SDW), above-ground dry weight (ShDW=LDW+SDW), CV: coefficient variation.

Membrane system	SN	SL	LDW	SDW	ShDW
NMS	2.5a	5.0a	25.7d	16.1a	41.8b
AMS1	2.1a	4.5a	27.6c	12.2b	39.9b
AMS2	2.3a	4.5a	30.2b	13.0b	43.2a
AMS4	2.1a	4.3a	31.8a	12.8b	44.6a
CV	8.84	11.6	3.98	7.82	4.1

medium without supplementation of sucrose using nodal explants with one pair of leaves of *O. majorana*. However, this experiment was not successful as the explants did not grow due to the absence of a sucrose source.

3.4. Photosynthetic pigments under alternative membrane system supplemented with 15 g sucrose

The accumulation of photosynthetic pigments was significantly influenced by the ventilation system (Table 2). The leaves of *O. majorana* plantlets accumulated higher levels of chlorophyll *a, b*, and total chlorophyll under cultivation in the ventilation system with four porous membranes (AMS4). Carotenoids were also positively influenced with the higher numbers of porous membranes used in the culture flasks. The membrane system provided greater gas exchange led to an increase in plant photosynthetic pigment content compared to a closed system without a permeable membrane.

If the photosynthetic pigments are absent, damaged, or not functioning properly, the plant's ability to carry out photosynthesis and, consequently, to produce biomass will be compromised. Photosynthesis is essential for the growth and development of plants, and therefore, photosynthetic pigments can affect biomass in various ways. Factors such as the integrity of photosynthetic pigments, nutrient deficiencies, environmental stress, and physical damage can affect and consequently influence the growth and development of the plant. Cultivating plantlets using an alternative membrane system can minimize damage to the photosynthetic system. This, in turn, can directly influence the growth and biomass of the plant. Plantlets grown in flask without membrane porous may have anatomical and physiological abnormalities. These problems include abnormal stomatal opening and abnormal leaf parenchyma, consequently low photosynthetic activity, low chlorophyll content (Vahdati and Hassankhah, 2014). Alvarez et al. (2012) reported that anatomical and physiological changes can cause high plant mortality during acclimatization. Therefore, the use of an alternative membrane system presents great advantages for *in vitro* propagation.

Several studies reported increased photosynthetic activity with ventilation system for *Plectranthus amboinicus* (Lour.) (Silva et al., 2017); *Pfaffia glomerata* (Spreng.) (Saldanha et al., 2012); *Gevuina Avellana* (Alvarez et al., 2012); *Pfaffia glomerata* (Iarema et al., 2012). According to Saldanha et al. (2014), the increase in the concentration of CO_2 inside the flasks during *in vitro* growth leads to high photosynthetic rates in

Table 2
Concentration of photosynthetic pigments and arbutin content in *Origanum majorana* L. plantlets grown *in vitro* in MS medium supplemented with 15 g of sucrose in combination with an alternative membrane system - AMS (1, 2 and 4 porous membranes) and no-membrane system (NMS), at 40 days. FW: fresh weight. CV: coefficient variation.

Membrane system	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b*	Total Chlorophyll	Carotenoids	Arbutin content (mg g^{-1})
NMS	0.45d	0.14b	0.58d	0.12c	5.3d
AMS1	0.75c	0.24a	0.99c	0.19b	8.5c
AMS2	0.86b	0.24a	1.11b	0.22a	10.6b
AMS4	0.95a	0.25a	1.19a	0.20a	13.7a
CV	4.71	7.68	3.96	3.85	1.73

plants cultivated under photoautotrophic conditions. However, the photosynthetic activity of plantlets can vary according to genotype and cultivation conditions (light intensity, sucrose, etc.). *In vitro* culture can result in some species exhibiting weak photosynthetic activity, while others may display higher photosynthetic activity. Iarema et al. (2012) observed an increase in the concentration of photosynthetic pigments in the cultivation of *Pfaffia glomerata* under a ventilation system compared to cultivation without a ventilation system. Many studies also associate the higher availability of CO_2 within the flask with an increase in the levels of photosynthetic pigments, as observed in *Pfaffia glomerata* (Saldanha et al., 2012) and *Lippia alba* (Batista et al., 2016). However, contrary results were described by Lazzarini et al. (2019) in the *in vitro* cultivation of *Lippia gracilis* under different ventilation systems, where they observed a decrease in the content of photosynthetic pigments with an increase in the number of membranes in the caps. The authors justified the results by citing an increase in water evaporation due to the ventilation system, thereby inducing the formation of water stress, which can lead to a downregulation of photosynthesis.

3.5. Arbutin content under alternative membrane system supplemented with 15 g sucrose

Arbutin contents in plantlets were highly influenced by the number of porous membranes in the cap (Table 2). The increase in membranes in the caps (AMS4) resulted in an elevation of arbutin content of 2.6-fold in the marjoram leaves compared with no-membrane system (NMS). Quantification of arbutin in dry leaves is shown in Fig. 5 the chromatographic profiles obtained by HPLC – DAD of the ventilation system. Moreover, the increase in these compounds was higher when the plantlets were under a ventilation system. Other studies have demonstrated that the ventilation system has an effect on the production of secondary metabolites. Lazzarini et al. (2019) reported that the *in vitro* cultivation of *Lippia gracilis* under different ventilation systems showed that the system with four membranes promoted a higher accumulation of carvacrol and thymol. A higher number of porous membranes (AMS4) led to enhance synthesis of (Z)-β-farnesene (Araújo et al., 2023). Oliveira et al. (2021) reported that the membrane system promoted greater accumulation of menthol, menthone, and limonene synthesis in *Mentha arvensis* and menthol, trans-sabinene, linalool, and limonene in *M. viridis*.

Fig. 6. *Origanum majorana* L. plantlets grown *in vitro* in MS medium under different wavelengths, with an alternative membrane system with 4 porous membranes (AMS4), at 40 days.

3.6. Growth analysis under wavelength and alternative membrane system

The light quality and the alternative membrane system with four membranes (AMS4) significantly affected the *in vitro* cultivation of *O. majorana* (Fig. 6 and 7). In a visual analysis, plantlets cultivated under white light (W) and a combination of blue and red light (50%B:50%R, 30%B:70%R, 70%B:30%R) displayed larger leaves with a dark green color. Plantlets cultivated under blue and green light exhibited reduced growth, with smaller leaves and thinner stems. The light quality had a significant effect on the number of regenerated shoots and shoot length (Fig. 7a, 7b). Monochromatic yellow (5.1 cm) and red (5.2 cm) wavelengths induced the greatest shoot length, while lower values were

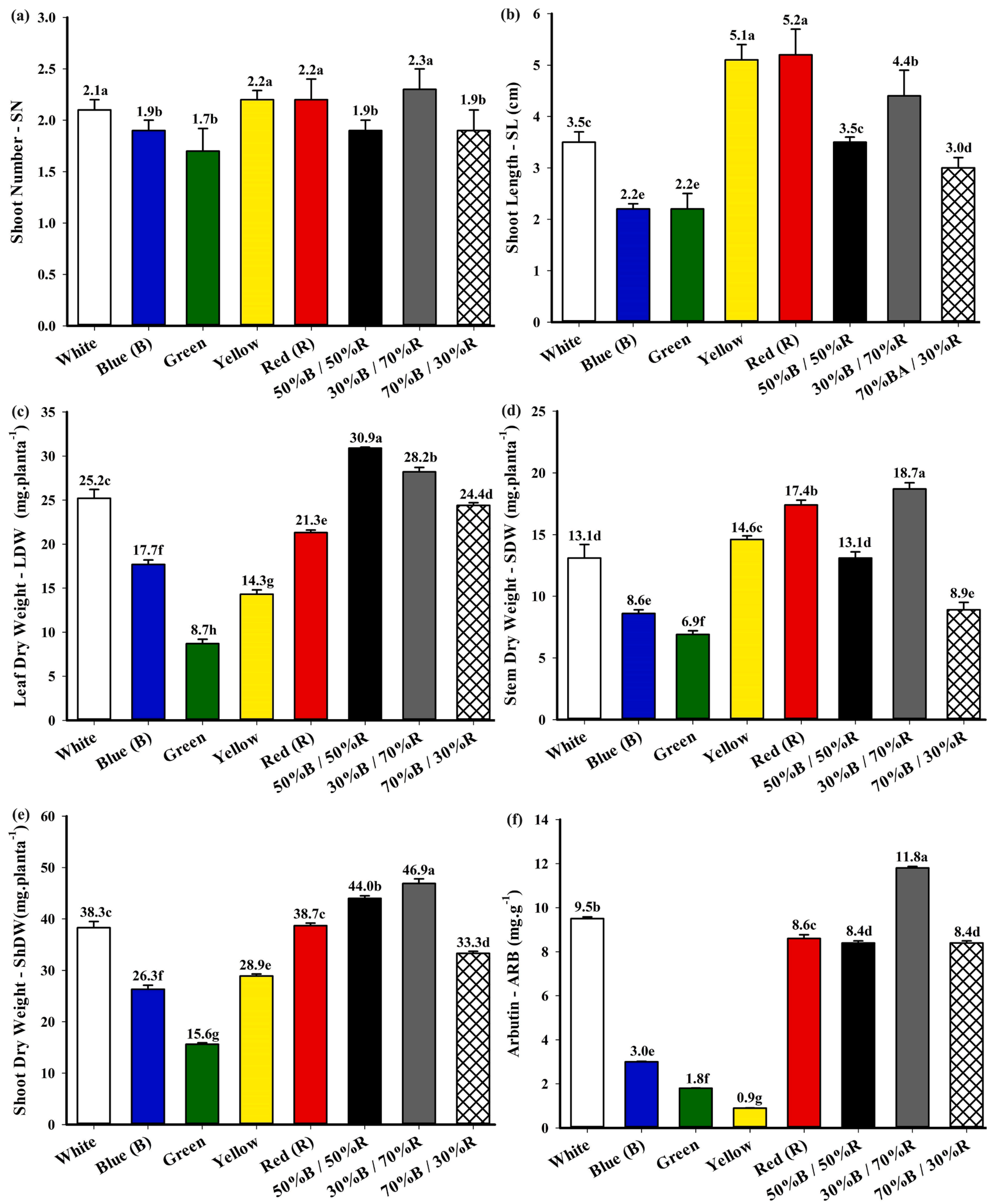

Fig. 7. Shoot number (SN), shoot length (SL), leaf dry weight (LDW), stem (SDW), above-ground dry weight (ShDW=LDW+SDW) and content of arbutin (ARB) (mg g-1) in leaves of *Origanum majorana* L. plantlets from nodal segments cultivated *in vitro* under different wavelength in an alternative membrane system with 4 porous membranes (AMS4), at 40 days.

observed under green (2.2 cm) and blue (2.2 cm) light. Cossa et al. (2024) worked with *O. majorana* using flask and without using the alternative membrane system (NMS) in the medium supplemented with 30 g L^{-1} sucrose observed higher shoot length under white light. The shoot size under different wavelengths can vary depending on the genotype. The green light provided the greatest growth of shoots of *Aeollanthus suaveolens* (Araújo et al., 2021). The wavelength had an influence on the vegetative growth of *Lippia dulcis* plantlets grown *in vitro*, where greater shoot length was under red, green, and yellow and shorter shoot length under blue and the 70% blue:30% red combination (Rocha et al., 2022b).

Cultivating plantlets under different light qualities and an alternative membrane system (AMS4) affected biomass accumulation. The highest accumulation of dry weight in the leaves occurred in cultivation under a combination of blue and red light (50%B:50%R), while in the stem (SDW) and above-ground parts (ShDW), it occurred under the combination 30%B:70%R. Similar results were described by Moon et al. (2006), where greater growth, fresh weight, and number of shoots of *Tripterospermum japonicum* were observed under a combination of blue and red light spectrum (30%B:70%R) in flasks with caps equipped with a ventilation system. Also, plantlets of *Lippia dulcis* cultivated under combinations of 30%B:70%R and 50%B:50%R light provided greater accumulation of dry weight (Rocha et al., 2022b). While, white LEDs light or fluorescent light appeared equally effective for growth and dry weight accumulation of *Aeollanthus suaveolens* (Araújo et al., 2021). According to Saldanha et al. (2012), plantlets of *Pfaffia glomerata* (Spreng.) cultivated in flasks with porous membranes showed a higher accumulation of dry weight in both above-ground parts and roots, indicating the importance of gas exchange *in vitro* morphogenesis. The ventilation system reduces relative humidity and ethylene within the flask, thereby promoting increased transpiration and mineral absorption by *in vitro* plants, resulting in greater growth. Plantlets grown under blue and green light accumulated less dry weight of LDW, SDW and ShDW (Fig. 7c, 7d, 7e). Cossa et al. (2024) worked with *O. majorana* using flask and without using the alternative membrane system (NMS) in the medium supplemented with 30 g L^{-1} sucrose observed similar results under the same lights.

3.7. Photosynthetic pigments under wavelength and alternative membrane system

The production of photosynthetic pigments was significantly influenced by different light spectra and the ventilation system with four membranes (Table 3). Chlorophyll, the pigment responsible for light absorption in photosynthesis, is more efficient in absorbing certain wavelengths. Plants primarily utilize visible light for photosynthesis, which includes wavelengths in the blue (400–500 nm) and red (600–700 nm) ranges. Blue light is absorbed by chlorophyll *a* and *b*, while red light is primarily absorbed by chlorophyll *a*. The spectrum of light that plants receive directly influences the rate of photosynthesis. If plants receive light in a wavelength range that is not ideal for chlorophyll absorption, photosynthetic efficiency may decrease. This can occur in artificial light conditions or in environments where sunlight is filtered by different means.

Table 3
Concentration of photosynthetic pigments in *Origanum majorana* L. plantlets grown *in vitro* in MS medium supplemented with 15 g of sucrose under different light spectrums and four-alternative membrane system (AMS4), at 40 days.

Wavelength	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b*	Total Chlorophyll	Carotenoids
White	0.20e	0.08e	0.25d	0.08c
Blue	0.07f	0.02f	0.10 g	0.02 g
Green	0.70b	0.19b	0.92b	0.17b
Yellow	0.50c	0.13c	0.58d	0.11e
Red	1.00a	0.20a	1.22a	0.22a
50%B:50%R	0.30d	0.13c	0.46e	0.12d
30%B:70%R	0.50c	0.19b	0.66c	0.15c
70%B:30%R	0.30d	0.09d	0.45e	0.11e

However, in the present study, the cultivation of *O. majorana* plantlets under blue and white light showed lower contents of chlorophyll *a, b*, total chlorophyll, and carotenoids (Table 3). Higher values of chlorophyll *a, b*, total chlorophyll, and carotenoids occurred in plantlets cultivated under red light. Contrasting results were described by Santos et al. (2020) in the photomixotrophic cultivation (15 g sucrose) of *Physalis angulata* with a ventilation system. In their study, higher values of chlorophyll *a, b*, total chlorophyll, and carotenoids were observed under cultivation in white light and a combination of blue and red light, while lower pigment values were observed under red light. However, these pigment content values did not show significant differences among the different light spectra, suggesting that photomixotrophic cultivation and various light spectra did not promote the growth of the species. *Aeollanthus suaveolens* is a succulent annual herb showed that highest content of chlorophyll *a*, total chlorophyll, and carotenoids were found in plants grown under yellow light and white LEDs light produced the highest concentration of chlorophyll *b* (Araújo et al., 2021). A similar result was found by Victório et al. (2007), wherein yellow light provided a higher concentration of chlorophyll *a* and total chlorophyll in plantlets of *Phyllanthus tenellus*.

3.8. Arbutin content under different wavelengths and alternative membrane system

The arbutin content was significantly affected by different light spectra in the *in vitro* cultivation of *O. majorana* (Fig. 7f). A higher arbutin content (11.8 mg g^{-1}) was produced under cultivation in the spectrum combination of blue and red light (30%B:70%R). The red light and combination of blue and red-light spectrum showed a positive effect on arbutin (ARB) production, as it exhibited higher levels compared to other light spectra. Cultivation under monochromatic blue (3 mg g^{-1}), green (1.8 mg g-1), and yellow (0.9 mg g^{-1}) light accumulated the lowest arbutin content in the leaves of *O. majorana* plantlets (Fig. 7f). A similar result was found by Cossa et al. (2024). The arbutin content in the 30%B:70%R combination was 3.9 times higher than that in monochromatic blue light. This result suggests that the presence of red light in the spectrum may aid in the synthesis of ARB in marjoram leaves *in vitro*. White LED light also has a peak in the red spectrum. Cossa et al. (2024) also observed that red light increases the arbutin content in MS medium supplemented with 30 g L^{-1} sucrose and no-membrane ventilation system was used. The spectra of blue and red light are the primary sources that affect secondary metabolism and biomass accumulation, stem elongation, leaf flattening, chloroplast development, stomatal opening (Dou et al., 2017).

The synthesis of chemical compounds, including arbutin, is typically regulated by specific metabolic pathways in plant cells. These metabolic pathways are influenced by a variety of factors such as nutrient availability, specific enzymes, and genetic regulators. However, it is important to note that the growth environment of plants, including the quality of light they receive, can influence the production of certain secondary compounds. In some cases, exposure to different wavelengths of light can modulate gene expression and, consequently, influence the synthesis of chemical compounds in plants. The increase in phenolic compounds in herbs can be caused by multiple responses to light quality, including the enhancement of activities of key metabolic enzymes, leading to improved synthesis of secondary metabolites. Therefore, it is possible that the application of supplemental red and/or blue light may increase the accumulation of phenolic compounds in some herb species, but the enhancing effect may depend on the species and specific compounds (Dou et al., 2017).

Another factor that may have contributed to arbutin accumulation is photosynthetic activity. Different wavelengths of light can have varying effects on photosynthesis. It is known that specific wavelengths, such as red and blue light, enhance photosynthesis more effectively than green light (Liu and Van Iersel, 2021). High photosynthetic activity can increase the production of primary metabolites, which serve as precursors to secondary metabolites such as arbutin (Kandar, 2021; Zhu et al., 2018). Furthermore, light wavelengths can influence the expression of genes involved in secondary metabolite biosynthesis pathways (Al Murad et al., 2021), including those of arbutin. For instance, specific wavelengths can positively regulate key enzymes responsible for arbutin synthesis. According to the literature, blue light induces the expression of arbutin synthesis genes in *Brassica rapa* L. ssp. (Zhang et al., 2023). Additionally, Chen et al. (2019) observed that a combination of red and blue light enhanced the activity of sucrose phosphate synthase in *Lactuca sativa*. This enzyme is responsible for the synthesis of hydroquinone, the main precursor of arbutin (Xu et al., 2022; Zhu et al., 2018).

The ventilation system also showed an effect on the production of secondary metabolites in other studies. Lazzarini et al. (2019), in the *in vitro* cultivation of *Lippia gracilis* under different ventilation systems, observed that the system with four membranes promoted a higher accumulation of carvacrol and thymol but a lower content of γ-terpinene and ρ-cymene. They emphasize that the increase or decrease in the content of compounds is specific and depends on the species. A higher number of porous membranes (AMS4) enhance synthesis of (Z)-β-farnesene in *Aeollanthus suaveolens* (Araújo et al., 2023). Plants respond to the environment in distinct ways, allocating their photoassimilates for the biosynthesis of elements necessary for their survival and adaptation to the environmental conditions provided by different light sources. Lobiuc et al. (2017), in their study on the effect of red, blue, and white LEDs on the production of phenolic compounds and flavonoids in basil (*Ocimum basilicum* L.), observed that the highest values were obtained with the combination of red and blue LEDs (30% B:70%R). This finding aligns with the results obtained in the current research.

3.9. Principal component analysis

The principal component analysis of 10 parameters of the *Origanum majorana in vitro* culture plantlets under different alternative membrane system (AMS) treatments was conducted. And the objective of the PCA was to assess the interactions between the variables and the type of alternative membrane system so that we could better explain our results. The results showed that the eigenvalues of the first principal components, were all greater than 1, and the cumulative contribution rate reached 97.07%, indicating that these principal factors could be used to explain 97.07% of the variation in our data (Table 1 and 2, Fig. 2b). The first principal component PC1 could explain 82.60% of the total variation and PC2 contributed 14.47% of the total variation. The graph of scores in Fig. 8 shows the separation of PC1 into two groups: alternative membrane system with 4 porous membrane (AMS4) and no-membrane system (NMS). These separations left each treatment in different quadrants of the graph, showing that there was a marked difference between treatments. The first group was the AMS4 (with 4 porous membrane) which was closely related to the original variables of LDW, ShDW, arbutin content, chlorophyll (a, b and total) and carotenoids. And second group (NMS) positively influenced the SDW, SN and SL. In general, the number of porous membranes on the lids provided the plant with favorable conditions for vegetative growth and production of arbutin.

3.10. Practical implications

Arbutin is valued for its skin-lightening properties and its use in treating hyperpigmentation. Understanding the optimal concentration of sucrose can help in formulating the best culture media. By identifying the right amount, researchers can promote better growth, development, and biomass production of *Origanum majorana*. In addition, identifying the optimal light wavelengths can enhance these processes, leading to improved growth and potentially higher yields of valuable compounds. By optimizing sucrose concentration, ventilation system and light wavelength, the *in vitro* cultivation of *O. majorana* can become a reliable source of arbutin for pharmaceutical and cosmetic industries. Understanding the precise requirements for *in vitro* cultivation can help in scaling up the production while ensuring consistent quality and yield of arbutin. This is particularly important for commercial applications where large quantities of plant material are needed. Optimized protocols can be easily transferred from laboratory-scale experiments to industrial-scale production.

In summary, the practical implications of studying sucrose, ventilation system and wavelength effects on the *in vitro* cultivation of *Origanum majorana* are significant. They can lead to optimized growth conditions, cost-effective production, improved secondary metabolite yields, and scalable cultivation practices. By integrating these findings into the cultivation practices of *Origanum majorana*, it is possible to develop a more effective and economically viable method for producing arbutin, which can benefit various industries relying on this compound. It is hoped that this study can contribute to improving arbutin production *in vitro*. The study raised some important points that must be evaluated in the future. Evaluate the effect of ChromatiNet (red nets) under field conditions on arbutin production.

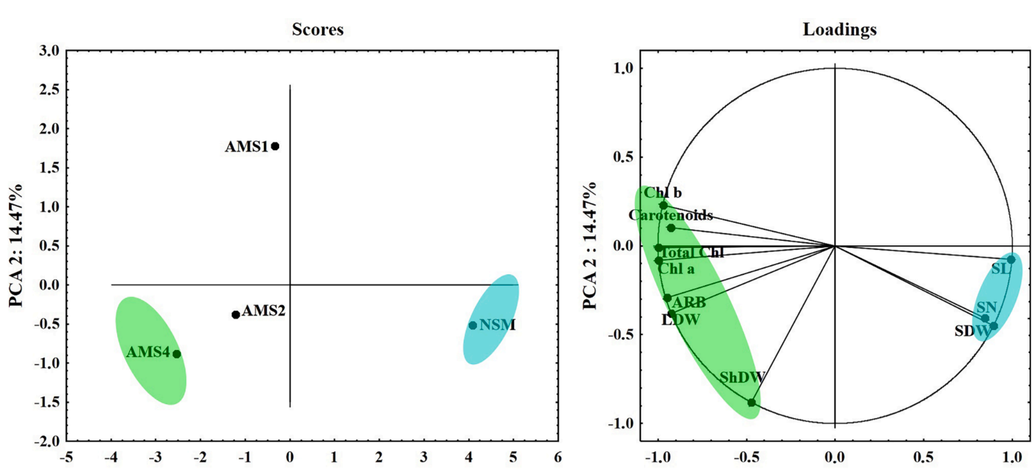

Fig. 8. Principal component analysis of the matrix correlation constructed using data from the alternative membrane system: LDW, SDW, ShDW, SN, SL, arbutin content (ARB), chlorophylls (*a, b* and total) and carotenoids of *Origanum majorana* L.

4. Conclusion

In vitro plantlets of *Origanum majorana* exhibit photomixotrophic cultivation, using a medium with 15 g of sucrose and an alternative membrane system with four membranes, which promoted greater growth. The sucrose concentration affected the *in vitro* accumulation of arbutin. The ventilation system and light spectra affected the growth, concentration of photosynthetic pigments, and arbutin accumulation in *Origanum majorana* plantlets. Arbutin accumulation was higher in the 30%B:70%R combination and lower under monochromatic blue spectrum.

CRediT authorship contribution statement

Melvis Celeste Vilanculos Cossa: Writing – original draft, Methodology, Investigation. **João Pedro Miranda Rocha:** Formal analysis. **Rafael Marlon Alves de Assis:** Software. **Jeremias José Ferreira Leite:** Investigation. **Flavia Dionisio Pereira:** Methodology. **Filipe Almendagna Rodrigues:** Investigation. **Suzan Kelly Vilela Bertolucci:** Writing – review & editing, Formal analysis. **Jose Eduardo Brasil Pereira Pinto:** Writing – review & editing, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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