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Colored shade nets and foliar ProLyks® application modulate plant growth and secondary metabolism in *Mikania laevigata* Sch. Bip. ex-Baker

Malhas coloridas e aplicação foliar de ProLyks® modulam o crescimento vegetal e o metabolismo secundário em *Mikania laevigata* Sch. Bip. ex-Baker

Mallas de sombreado de colores y aplicación foliar de ProLyks® modulan el crecimiento vegetal y el metabolismo secundario en *Mikania laevigata* Sch. Bip. ex-Baker

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Abstract

The efficacy and safety of phytotherapeutic medicines are intrinsically related to the quality of their plant-based raw materials, which is directly influenced by agronomic cultivation practices. The objective of this study was to evaluate the effects of colored shade nets and foliar application of ProLyks® on the growth and secondary metabolism of *Mikania laevigata* Sch. Bip. ex-Baker. The experiment followed a completely randomized 4 × 5 split-plot design, with three photoselective shade nets (red, blue, and black) compared to full sunlight (control) and five ProLyks® doses (0.00, 0.25, 0.50, 1.00, and 2.00 $mL \cdot L^{-1}$). Significant interactions between these factors were observed for plant height, number of leaves, leaf dry matter, and coumarin content. The red net promoted the highest biomass production and, when combined with the 1.5 $mL \cdot L^{-1}$ ProLyks® dose, also maximized coumarin accumulation in leaves, indicating a possible elicitor effect under these conditions. A considerable positive correlation between plant height and coumarin content suggests that taller plants may serve as a practical field indicator of superior pharmaceutical quality. Additionally, an inverse correlation between total chlorophyll and flavonoid content highlights a trade-off between photosynthetic pigment synthesis and secondary metabolism. These findings demonstrate that red shade nets combined with the optimal ProLyks® dosage can enhance both biomass yield and phytochemical production in *M. laevigata*, while also providing useful indicators for field selection of high-quality materials for the national pharmaceutical industry.

Keywords: Coumarin biosynthesis; Elicitor effect; Medicinal plants.

Resumo

A eficácia e a segurança dos medicamentos fitoterápicos estão intrinsecamente relacionadas à qualidade de suas matérias-primas de origem vegetal, as quais são diretamente influenciadas pelas práticas de cultivo agronômico. O objetivo deste estudo foi avaliar os efeitos de malhas coloridas e a aplicação foliar de ProLyks® sobre o crescimento e o metabolismo secundário de *Mikania laevigata* Sch. Bip. ex-Baker. O experimento foi conduzido em delineamento inteiramente casualizado, em esquema de parcelas subdivididas 4 × 5, com três malhas fotoseletivas (vermelha, azul e preta) comparadas ao pleno sol (controle) e cinco doses de ProLyks® (0,00; 0,25; 0,50; 1,00 e 2,00 mL·L⁻¹). Foram observadas interações significativas para altura de plantas, número de folhas, massa seca foliar e teor de cumarina. A malha vermelha promoveu a maior produção de biomassa e, quando combinada à dose de 1.5 mL·L⁻¹ de ProLyks®, também maximizou o acúmulo de cumarina nas folhas, indicando um possível efeito elicitor nessas condições. Uma correlação positiva entre altura de plantas e teor de cumarina sugere que plantas mais altas podem servir como um indicador prático, em campo, de qualidade farmacêutica superior. Além disso, a correlação inversa entre clorofila total e teor de flavonoides evidencia um balanço entre a síntese de pigmentos fotossintéticos e o metabolismo secundário. Esses resultados demonstram que a combinação da malha vermelha com a dose ótima de ProLyks® pode aumentar tanto a produtividade de biomassa quanto a produção de fitoquímicos em *M. laevigata*, além de fornecer indicadores úteis para a seleção prática e rápida de materiais de alta qualidade destinados à indústria nacional de fitoterápicos.

Palavras-chave: Biossíntese de cumarina; Efeito elicitor; Plantas medicinais.

Resumen

La eficacia y seguridad de los medicamentos fitoterapéuticos están intrínsecamente relacionadas con la calidad de sus materias primas de origen vegetal, la cual es directamente influenciada por las prácticas de cultivo agronómico. En este contexto, el objetivo de este estudio fue evaluar los efectos de las mallas de sombreado de colores y la aplicación foliar de ProLyks® sobre el crecimiento y el metabolismo secundario de *Mikania laevigata* Sch. Bip. ex-Baker. El experimento se realizó en un diseño completamente al azar, en un esquema de parcelas subdivididas 4 × 5, con tres mallas fotoselectivas (roja, azul y negra) comparadas con la luz solar directa (control) y cinco dosis de ProLyks® (0,00; 0,25; 0,50; 1,00 y 2,00 mL·L⁻¹). Se observaron interacciones significativas entre estos factores para la altura de la planta, número de hojas, materia seca foliar y contenido de cumarina. La malla roja promovió la mayor producción de biomasa y, cuando se combinó con la dosis de 1.5 mL·L⁻¹ de ProLyks®, también maximizó la acumulación de cumarina en las hojas, indicando un posible efecto elicitor en estas condiciones. Una correlación positiva entre la altura de la planta y el contenido de cumarina sugiere que las plantas más altas pueden servir como un indicador práctico de campo de una calidad farmacéutica superior. Además, la correlación inversa entre la clorofila total y el contenido de flavonoides evidência un equilibrio entre la síntesis de pigmentos fotosintéticos y el metabolismo secundario. Estos resultados demuestran que la combinación de la malla roja con la dosis óptima de ProLyks® puede aumentar tanto el rendimiento de biomasa como la producción de fitoquímicos en *M. laevigata*, además de proporcionar indicadores útiles para la selección práctica y rápida de materiales de alta calidad para la industria fitofarmacéutica nacional.

Palabras clave: Biosíntesis de cumarina; Efecto elicitor; Plantas medicinales.

1. Introduction

The increasing global demand for plant-derived therapeutic resources reflects an increasing confidence in traditional medicine, which plays a central role in both primary healthcare and pharmaceutical innovation (Singh et al., 2022). Notably, more than half of recently approved drugs are derived from medicinal plants or their constituents, underscoring their relevance to public health (Davis & Choisy, 2024). For instance, the World Health Organization (WHO) projects that the global herbal medicine market will reach USD 5 trillion by 2050 (Singh et al., 2022), reinforcing the urgency of adopting agronomic strategies that ensure efficient, standardized, and sustainable cultivation practices for pharmacological species. In this context, Brazil's remarkable biodiversity positions the country as a key player in developing value chains based on native species with bioactive potential. However, several structural and technical challenges persist in the Brazilian phytotherapeutic sector, including extractive harvesting methods, limited implementation of good agricultural practices, lack of production standardization, and insufficient institutional and policy support (Chen et al., 2016; Wang et al., 2020).

Among Brazil's native medicinal flora, *Mikania laevigata* Sch. Bip ex-Baker., commonly known as "Guaco", is extensively used in folk medicine and it is indicated as a medicinal plant in primary health care in the Unified Health System (SUS) (Gasparetto et al., 2010). This climbing herbaceous plant, a member of the Asteraceae family, naturally occurs in moderate shaded environments of tropical rainforests (Flor et al., 2022). Several bioactive compounds have been identified in

"Guaco" species, including coumarins, terpenes, flavonoids, tannins, kaurenoic acid, aldehydes, and organic esters. Nevertheless, these therapeutical properties, like antimicrobial, anti-inflammatory, bronchodilator and antiulcerogenic (Azevedo et al., 2018; Garcia et al., 2025), are predominantly attributed to the presence of coumarins (Ortiz & Sansinenea, 2023; Souza et al., 2022; Yatsuda et al., 2005). Moreover, beyond its established medicinal importance, the species of this botanical genus also harbor beneficial microorganisms that can promote plant development and enhance cash crop performance, as reported by Rosini et al. (2025).

Predominantly concentrated in the leaves, coumarins are secondary metabolites synthesized through the shikimate pathway. This biosynthetic route initiates with the condensation of phosphoenolpyruvate and erythrose-4-phosphate (Czelusniak et al., 2012), leading to the production of chorismic acid and subsequent synthesis of aromatic amino acids. Phenylalanine is converted by phenylalanine ammonia-lyase (PAL) into cinnamic acid, which is further transformed into o-coumaric acid. Through glycosylation, cis/trans isomerization, and lactonization, these intermediates give rise to coumarins (Alves & Deschamps, 2019). Like other pharmacological molecules, the production of this compound is modulated by genetic background, environmental variables, and cultivation practices (Punja et al., 2023; Thawonkit et al., 2025). In this regard, Souza et al. (2010) demonstrated that *M. laevigata* exhibits high metabolic plasticity, with secondary metabolite synthesis significantly influenced by environmental conditions, such as air temperature and solar radiation intensity.

Within these factors, light plays a central role in modulating photosynthesis, stress responses, and the expression of genes associated with specialized metabolism (Thakur & Kumar, 2021). Contin et al. (2021), for example, reported that light intensity directly affects the morphophysiological performance of *M. laevigata*, with enhanced photosynthetic efficiency, biometric parameters, and biomass accumulation observed under moderate shading. These findings emphasize the importance of managing light availability and understanding the environmental conditions of the medicinal species' origin to optimize both plant development and secondary metabolite production. Furthermore, a promising strategy for light manipulation involves the use of photoselective shade nets, such as ChromatiNET®, which selectively filter the solar radiation and consequently influence plant morphogenesis, resource allocation, and metabolic activity (Ahmed et al., 2016; Freire et al., 2024). For instance, according to the findings of Souza et al. (2011), *Mikania glomerata* Sprengel. plants cultivated under blue nets exhibited superior leaf expansion, dry matter production, and essential oil content compared to those under full sunlight, illustrating the potential of spectral manipulation to enhance biomass accumulation and phytochemical yield.

In parallel, recent studies have investigated the elicitor potential of monoterpenes such as D-limonene ($C_{10}H_{16}$), a compound naturally found in citrus essential oils. These molecules, categorized as elicitors of biotic or abiotic origin, stimulate plant defense responses and often induce the biosynthesis of specialized metabolites (Thakur et al., 2019). D-limonene has been shown to modulate physiological and metabolic processes related to plant development and secondary compound production (Chaimovitsh et al., 2017; De Souza et al., 2024). In this sense, Orsi et al. (2022) demonstrated that the foliar application of ProLyks® (a commercial formulation in which D-limonene is the main terpene component) promoted increased carotenoid accumulation during tomato ripening, suggesting its potential to stimulate specific metabolic pathways. Thus, given the increasing demand from the pharmaceutical industry for raw materials with standardized coumarin content, the present study aimed to evaluate the combined effects of foliar ProLyks® application and colored shade nets on the growth and secondary metabolism of *M. laevigata*. Specifically, our objectives were to investigate: (i) biometric parameters; (ii) the synthesis of photosynthetic pigments; and (iii) the accumulation of secondary metabolites: coumarins, flavonoids, and anthocyanins.

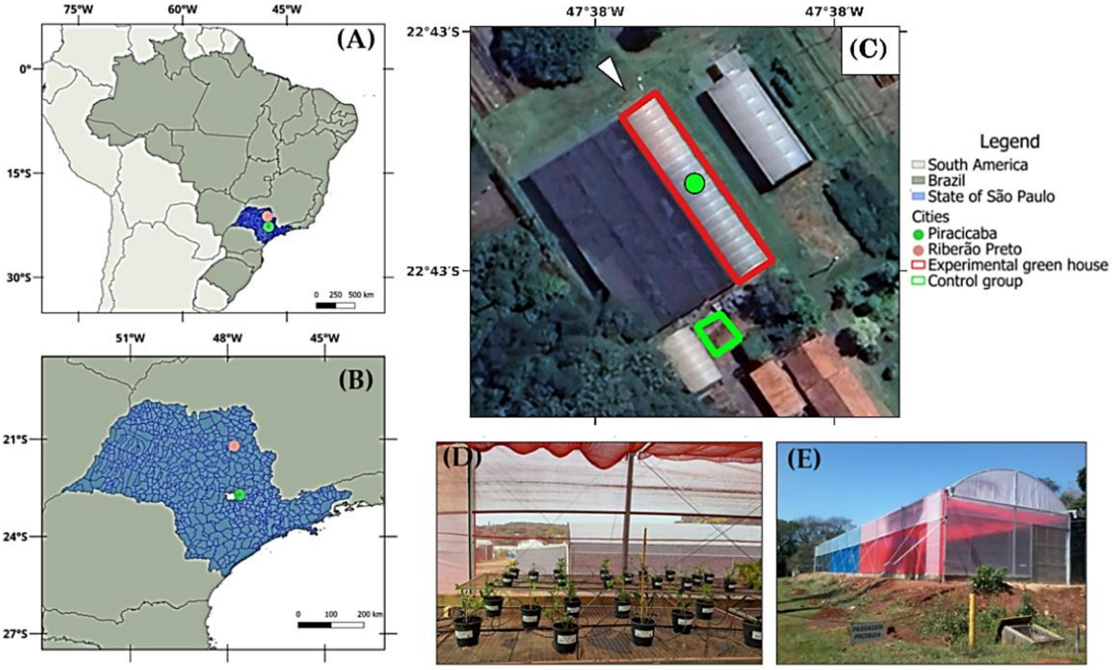
2. Materials and Methods

2.1 Experimental Conditions, Greenhouse Setup and Climatic Data

An experimental research was conducted in a field study of a quantitative nature, using simple descriptive statistics with the use of graphs and values of mean and standard deviations, and employing statistical analysis (Pereira et al., 2018; Vieira, 2021). Thus, the experiment was carried out from October 2 to November 30, 2024, under four light environments (three within a greenhouse and one in open-field conditions) at the experimental area of the Department of Crop Science (LPV) at the "Luiz de Queiroz" College of Agriculture, University of São Paulo (ESALQ/USP), located in Piracicaba-SP, Brazil (Lat. 22°42′31 S, Long. 47°37′44 W, 546 m altitude) (Figure 1A,B). According to the Köppen classification, the region presents a Cwa climate: a subtropical type with hot, wet summers and dry winters (Alvares et al., 2014).

The experimental units (*M. laevigata* seedlings) were installed in a single-span greenhouse (6.4 m wide x 36 m long) covered with a light-diffusing polyethylene film (nominal 100% light transmissivity). Three light environments were created using photoselective shade nets (ChromatiNET®, Ginegar; 5mm thickness, nominal 50% shading): red, blue, and black. The north-south oriented greenhouse was internally divided into three equal sections; each covered with one colored net. To prevent light interference during the early hours of the day, the west side of each section was also covered with a net of the same color as the respective treatment. Additionally, a fourth light condition, referred to as "external", was implemented in an adjacent open-field area under full sunlight, serving as the control group (Figure 1C,E).

Figure 1 - Locations of the experimental greenhouse in Piracicaba-SP and the laboratory in Ribeirão Preto-SP where coumarin analyses were conducted (A,B); aerial-satellite view of the greenhouse and location of the control treatment (C); internal view of the reticulated-shade net (10 days after transplanting) as an example for showing the arrangement of experimental units (D); and external ground-level view of the greenhouse to demonstrate the disposition of the colored shade nets (E).

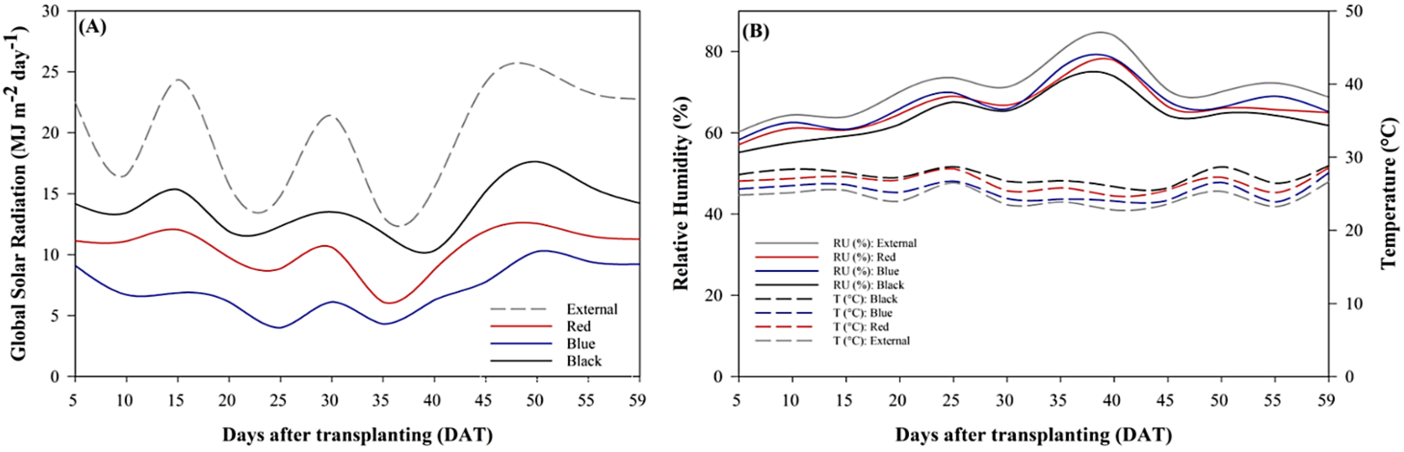


Source: Authors (2025).

Seedlings of *M. laevigata* were obtained from Flora Arco-Íris (Atibaia-SP, Brazil), a nursery specialized in ornamental and medicinal plants. The commercial origin of the material provides a reasonable assurance of species identity, minimizing the risk of confusion with other congeneric medicinal species. Uniformity in size (15-20 cm of height) and leaf number (approximately four pairs of leaves) were used as to ensure initial homogeneity. Thus, on October 2, 2024, the seedlings were transplanted into 1.8 L pots filled with commercial substrate (Basaplant Hortaliças®, Base Substratos), composed of pine bark, peat, charcoal, and vermiculite. At this moment, basal fertilization consisted of a single 15 g per pot application of a controlled-release fertilizer (Basacote® Plus 6M, COMPOEXPERT; 16-8-12 NPK). A drip irrigation system was used for all treatments, including the control group cultivated outdoors (Figure 1C). The system consisted of PVC main lines and polyethylene lateral lines equipped with 2.0 L.h^{-1} pressure-compensating drippers connected to microtubes and two drip sticks at each pot outlet. Plants received two daily irrigations of 2 minutes each, at 06:00 and 18:00 h. This irrigation protocol resulted in a total daily water supply of approximately 135 mL per plant. Additionally, 15 days after transplanting (DAT), plants were staked using bamboo rods and tied with twine to ensure upright growth and prevent mechanical damage (Figure 1D).

Daily meteorological conditions during the experimental period (October 2 to November 30, 2024) are presented in Figure 2. For external monitoring, data were obtained from the ESALQ/USP weather station, located approximately 300 m from the experimental site. In addition to temperature, relative humidity, and solar radiation, a total accumulated rainfall of 195 mm was recorded during the experimental period. Inside the greenhouse, microclimatic monitoring was performed independently in each light environment using three automated weather stations. Each station was equipped with a CR1000 data logger (Campbell Scientific®), an HMP45C sensor (Vaisala®) for temperature and relative humidity, a CS100 barometric pressure sensor, and an LI200X silicon photodiode pyranometer (LI-COR®) to measure global solar radiation levels. It is important to highlight that Pyranometer data were recorded in millivolts and converted to MJ.m^{-2}·day^{-1} using a calibration factor of 0.0075 V, following the validated methodology proposed by Freire et al., (2024). This method, based on the comparison with a reference Eppley pyranometer under clear sky, demonstrated strong linearity and accuracy: coefficients of determination of 0.98 to 0.99.

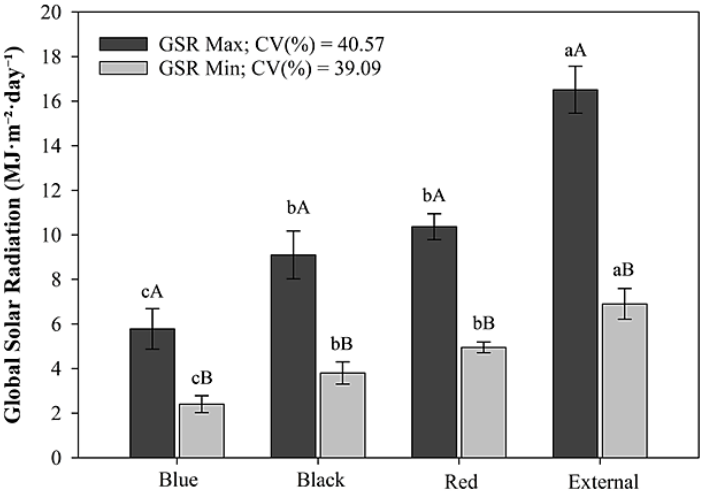
Figure 2 - Average values (5-day means) of global solar radiation (A), air temperature and relative humidity (B) recorded under different light environments (external condition and colored shade nets) throughout the experimental period (October 2 to November 30, 2024).



Source: Research data (2025).

Regarding light transmissivity, the photoselective shade nets promoted distinct levels of solar radiation reduction when compared to the external environment (control treatment). Based on global solar radiation data, the blue net reduced light transmissivity to 20.5% of the incident radiation, while the red and black nets allowed 38.9% and 32.3%, respectively. No significant difference was observed between the red and black nets (Figure 3). To distinguish the effects of shading intensity and light spectral quality on plant development, a Tukey's test was applied to the global solar radiation data. This statistical analysis was essential to the correct interpretation and results discussion of this study, given that colored shade nets simultaneously alter both the quantity and the spectral composition of incident light.

Figure 3 - Average maximum and minimum daily global solar radiation under the colored shade nets and full sunlight (external environment), recorded from October 2 to November 30, 2024. Values represented the mean ± standard error. Bars with the same lowercase letters (within each environment) and uppercase letters (between environments) do not differ by Tukey's test ($p \leq 0.05$). Coefficient of variation (CV).



Source: Research data (2025).

2.2 Experimental Design and Applied Treatments

This study was conducted using a completely randomized design arranged in a 4 × 5 split-plot scheme. The main plots corresponded to four light conditions: red, blue, and black ChromatiNET® shadenets, and full sunlight (open-field control / "external treatment"). The subplots consisted of five ProLyks® doses: 0.00, 0.25, 0.50, 1.00, and 2.00 $mL \cdot L^{-1}$. Each light environment contained 25 plants, with five plants allocated to each ProLyks® dose. Each plant represented a single experimental unit, totaling 100 plants across all treatments. Pots were arranged on central aluminum benches with approximately 40 cm spacing to ensure uniform exposure and adequate airflow (Figure 1D). To minimize the possibility of light diffusion between adjacent treatments, especially within the greenhouse compartments, plants were intentionally positioned at the center of each shade net section. This spatial arrangement aimed to ensure that the solar radiation reaching each group was primarily filtered through its corresponding photoselective net, thereby reducing potential interference from neighboring light environments.

ProLyks® (Hydroplan-EB®, São Paulo, Brazil) is a commercial formulation composed of 58% Citrus x sinensis (L.) Osbeck essential oil, along with surfactant, coadjuvant, and carrier. This product is rich in monoterpenes, particularly D-limonene. It is IBD-certified and approved for organic agriculture in accordance with international and national standards (NOP/USA, IBD/IFOAM, COR/Canada, and Brazilian Law 10.831/2003). Each solution was diluted in deionized water, which was also used for the subplot control treatment (0.00 $mL \cdot L^{-1}$). Applications were performed weekly by using a manual sprayer with a conical tip (Stihl SG 11®; v = 1.5 L; pressure = 3.0 bar; Q = 0.6 $L \cdot min^{-1}$), starting on October 7 and continuing until November 28, 2024. Initially, 100 mL of solution was applied per plant, with the volume gradually adjusted to ensure complete coverage of the expanding leaf surface, reaching up to 225 mL per plant by the end of the experimental period.

2.3 Biometric and Biomass Measurements

Biometric evaluations were performed at the end of the experimental period (59 DAT, November 30, 2024). Plant height was measured from the substrate surface to the apical meristem using a 10-meter fiberglass measuring tape. Plant diameter was recorded at the base of the main stem using a digital caliper (Digimess®, model 100.174BL). The number of leaves and secondary shoots was also counted. After these evaluations, all plants were harvested and separated into roots, stems, and leaves. The plant material was dried in a forced-air circulation oven at 65 ± 1 °C until reaching constant weight. Dry matter was then determined individually for each plant organ using an analytical balance (Shimadzu®, model AY220; precision 0.01 g).

2.4 Photosynthetic Pigments and Secondary Metabolites Analysis

For the analysis of photosynthetic pigments, a total of 10 g of fresh leaf tissue was collected on November 30, 2024. To ensure sample representativeness, eight fully expanded leaves were harvested from the apical, median, and basal regions of each plant. Therefore, chlorophyll *a*, chlorophyll *b*, and total chlorophyll were quantified using fresh macerated tissue, following the method described by Hiscox and Israelstam (1979). All pigments analyses were carried out in three independent replicates to ensure methodological consistency and analytical reliability. From each composite sample, 50 mg of fresh tissue was weighed using an analytical balance (Shimadzu®, model UX420H; precision 0.001 g), homogenized in 5 mL of dimethyl sulfoxide and incubated at 25 ± 5 °C for 48 hours in the dark. After extraction, the solution was used for spectrophotometric analysis (Bel, model V-M5) at wavelengths of 665 and 649 nm. Thus, pigment concentrations were calculated using the specific formulas (equations 1-3) proposed by Wellburn (1994) and the results were expressed in g·kg⁻¹ of fresh leaves biomass:

$$Chlorophyll\ a = \frac{[(12.19 \times A_{665}) - (3.45 \times A_{649})] \times \left(\frac{\text{dilution factor}}{1.1}\right)}{1000} \quad (1)$$

$$Chlorophyll\ b = \frac{[(21.99 \times A_{649}) - (5.32 \times A_{665})] \times \left(\frac{\text{dilution factor}}{1.1}\right)}{1000} \quad (2)$$

$$Total\ chlorophyll = Chlorophyll\ a + Chlorophyll\ b \quad (3)$$

For the analysis of secondary metabolites, leaf samples were also collected on November 30, 2024, from the apical, median, and basal regions of each plant. After collecting, approximately 10 g of fresh leaves per treatment were oven-dried in a forced-air circulation oven at 65 °C for 72 hours. The dried material was then ground using a Willey-type mill (Marconi®, model MA-048) equipped with a 0.5 mm sieve. Total Anthocyanins and flavonoids contents were determined by spectrophotometry using methods adapted from Lees & Francis (1972) and Siegelman and Hendricks (1958). Technical replicates (*n* = 3) were used for each biochemical determination to guarantee result accuracy. For each determination, 50 mg of dried and ground leaf material was weighed using an analytical balance and extracted in 10 mL of 1% HCl in 80% methanol. The samples were incubated in the dark at 4 °C for 12 hours. Thus, an aliquot of the extract was then collected, and absorbance was measured at 530 and 350 nm using a spectrophotometer. Results were expressed in g·kg⁻¹ of dry leaves using the appropriate formulas (equations 4-5):

$$Total\ anthocyanins = \frac{A_{530}\ \times \text{dilution factor}}{98.2} \tag{4}$$

$$Total\ flavonoids = \frac{A_{350}\ \times \text{dilution factor}}{76.5} \tag{5}$$

Coumarin content was analyzed at the Research Laboratory of Apis Flora in Ribeirão Preto-SP, Brazil (Figure 1A,B), using high-performance liquid chromatography (HPLC) as described by Medeiros and Kanis (2010). For extraction, dry leaf samples were prepared at a concentration of 50 mg·mL⁻¹ in a 1:1 (v/v) mixture of HPLC-grade methanol and ultrapure water, followed by ultrasonic treatment for 60 minutes. The extract was filtered through cotton into a volumetric flask, and the remaining material was subjected to a second extraction under identical conditions for 15 minutes. Subsequently, both extracts were combined, filtered, and passed through a 0.45 µm membrane filter prior to injection. Chromatographic separation was carried out on a Shimadzu HPLC system equipped with an SPD-M20A photodiode array detector and a Shim-Pack CLC-ODS column (4.6 × 250 mm, 5 µm, 100 Å). The mobile phase consisted of a methanol–ultrapure water gradient ranging from 35% to 95% over 24 minutes, at a constant flow rate of 1.0 mL·min⁻¹. The injection volume was 20 µL, with the column maintained at 40 °C. Detection was performed at 275 nm, and coumarin was identified by its retention time (~12.3 minutes). Quantification was based on a calibration curve constructed with standard coumarin (Sigma-Aldrich® solution) in methanol: ultrapure water (1:1, v/v) in the concentration of 800 µg·mL⁻¹. This solution was diluted in the same solvent in five different concentrations: 20, 40, 60, 80 and 100 µg·mL⁻¹. Ultimately, it is worth noting that while the experiment comprised 20 treatments with five replicates each (yielding 100 observations per variable) coumarin content was assessed using three biological replicates per treatment (n = 60), due to the higher cost and complexity of HPLC analysis compared to other measured variables.

2.5 Statistical Analysis

The original data were first tested for residue normality using the Shapiro-Wilk and Lilliefors tests, and for homogeneity of variances using Bartlett's test, all at the 5% significance level. When necessary, data were appropriately transformed to meet the assumptions of normal distribution and homoscedasticity. Subsequently, analysis of variance (ANOVA) was performed using the F-test ($p \leq 0.05$). For statistically significant main effects or interactions, qualitative factors (colored shade nets – SN) were compared using Tukey's and Scott-Knott tests, while quantitative factors (ProLyks® doses – PLD) were analysed by regression analysis. When a significant interaction was detected between main plots and subplots, regression analysis was conducted within each light condition. Additionally, Cohen's (1988) partial effect size (η^2_p) was calculated to support the interpretation of p-values and to demonstrate that, despite the relatively small number of observations (5 replicates per treatment), most variables in this study exhibited statistically meaningful effect sizes (supplementary material). All statistical procedures were performed using Assistat Software® (v. 7.7) (de Sousa & de Azevedo, 2016), while graphical representations and supplementary regression analysis were generated with SigmaPlot® (v. 16.0). Lastly, Pearson's correlation coefficient was calculated to assess relationships between the evaluated parameters (Mukaka, 2012).

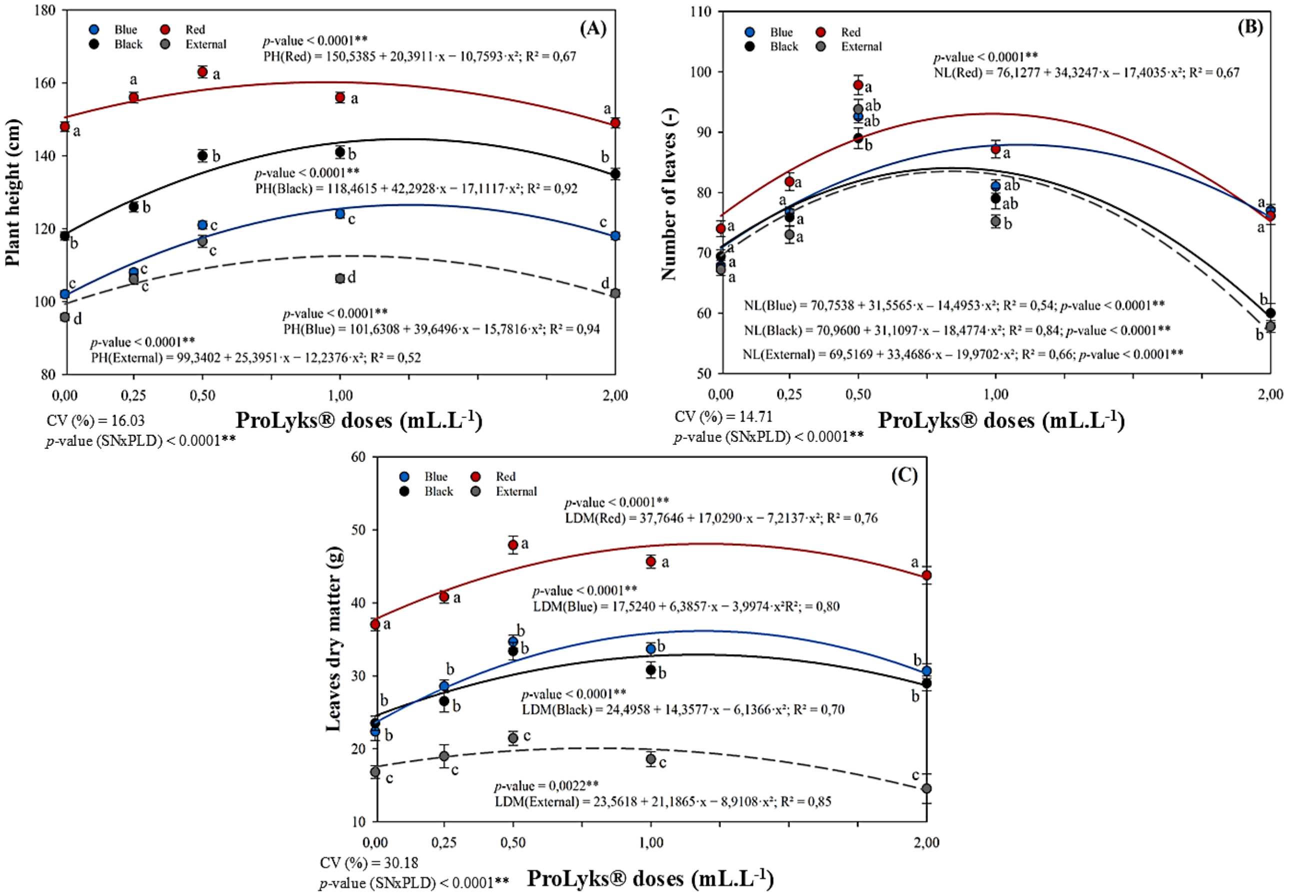
3. Results

3.1 Biometric and Biomass Measurements

The analysis of variance (ANOVA) revealed that plant height, number of leaves, leaf dry matter, shoot dry matter, root dry matter, and number of secondary shoots in *M. laevigata* were significantly influenced ($p < 0.01$) by the different colored shade nets (main plots). Likewise, ProLyks® doses (subplots) had a significant effect on all biometric traits evaluated. A significant interaction between these two factors ($p < 0.01$) was also observed for plant height, number of leaves, and leaf dry matter. Additionally, residuals from all biometric variables conformed to a normal distribution according to the Shapiro-Wilk test at the 5% significance level ($p > 0.05$), with two exceptions: plant height, which achieved normality only after $\log_{10}(x)$ transformation, and leaf dry matter, which met the normality assumption based on the Lilliefors test in the same threshold.

Plant height in *M. laevigata* responded significantly to ProLyks® doses across all shade nets, exhibiting a quadratic pattern (Figure 4A). Due to the statistical interaction between these factors, regression analysis was conducted within each shade net condition and was significant at the 1% level ($p < 0.01$) for all cases. The fitted regression models yielded coefficients of determination (R^2) ranging from 0.52 to 0.92, indicating a reasonable fit between the estimated curves and the observed data. The maximum plant height was observed under the red shade net, reaching 160.26 cm at an optimal ProLyks® dose of 0.95 $mL \cdot L^{-1}$, followed by the black net (144.70 cm at 1.24 $mL \cdot L^{-1}$), blue net (126.62 cm at 1.26 $mL \cdot L^{-1}$), and full sunlight (112.56 cm at 1.04 $mL \cdot L^{-1}$). This consistent ranking (red > black > blue > external) was statistically supported across all light environments. In general, plant height increased progressively with ProLyks® application up to intermediate doses (approximately 1.00-1.25 $mL \cdot L^{-1}$), followed by a reduction at the highest concentration tested (2.00 $mL \cdot L^{-1}$).

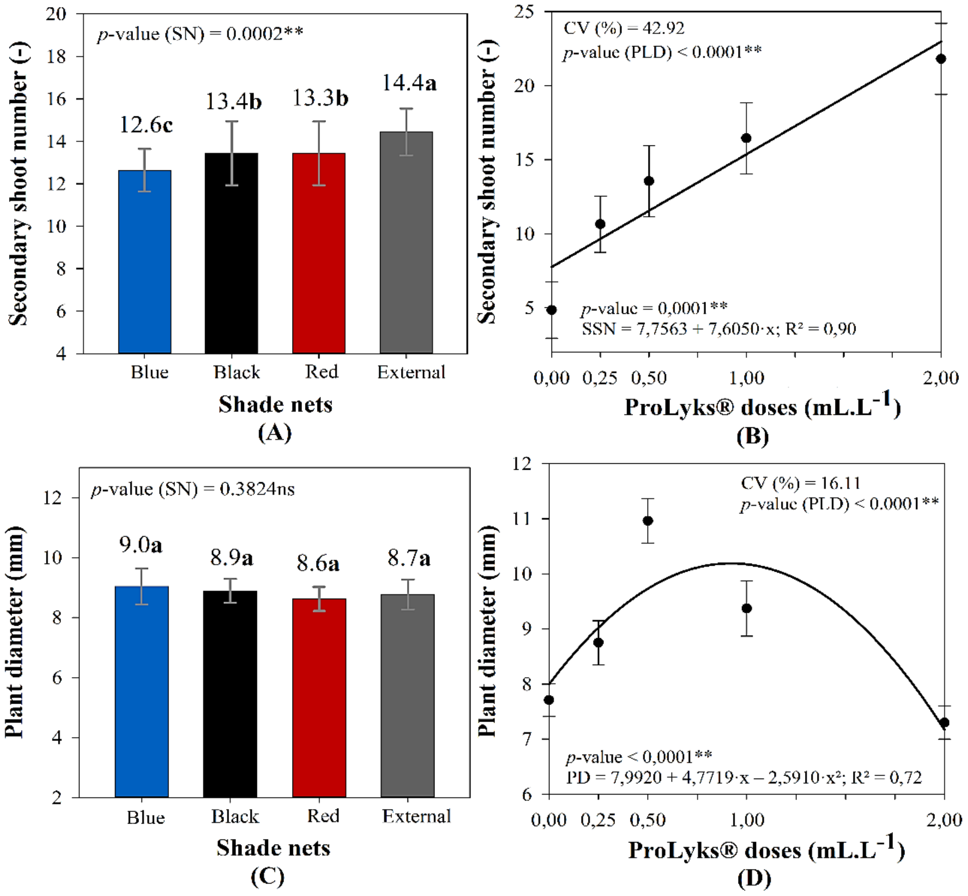
Figure 4 - Plant height (A), number of leaves (B), and leaves dry matter (C) in M. laevigata in response to ProLyks® application under different colored shade nets. Values represent means (n = 5) ± standard error. Different lowercase letters indicate significant differences among qualitative treatments according to Tukey's test ($p \leq 0.05$). CV (%) refers to coefficient of variation. p-value (SDxPLD) refers to the F-test for the interaction between shade nets (SD) and ProLyks® doses (PLD).



Source: Research data (2025).

For leaves dry matter (Figure 4C), regression analyses indicated that the highest estimated foliar biomass was obtained under the red shade net, reaching 47.81 g·plant⁻¹ at an optimal dose of 1.18 mL·L⁻¹. The blue and black nets yielded intermediate values, with estimated peaks of 36.16 g and 32.89 g, respectively, both occurring at concentrations approximating 1.18 mL·L⁻¹. While these two conditions did not statistically differ from each other, both produced significantly lower leaves biomass compared to the red net. The lowest performance at the optimal dose was observed under full sunlight, yielding a maximum estimated value of 20.07 g at 0.80 mL·L⁻¹. In this context, the number of leaves (Figure 4B) also followed a quadratic regression model, significant at the 1% probability level ($p < 0.01$), with R² values ranging from 0.54 to 0.88 depending on the light condition. The highest estimated value was recorded under the red shade net, reaching 93.10 leaves per plant at an optimal ProLyks® dose of 0.99 mL·L⁻¹. This trend was followed by the blue net (87.01 leaves at 1.09 mL·L⁻¹), the black net (84.04 leaves at 0.84 mL·L⁻¹), and full sunlight (83.56 leaves at 0.84 mL·L⁻¹). Thus, tukey's post-hoc test ($p < 0.05$) revealed that the red net differed significantly from all other light environments. Furthermore, the blue and external conditions were statistically equivalent and both superior to the black net.

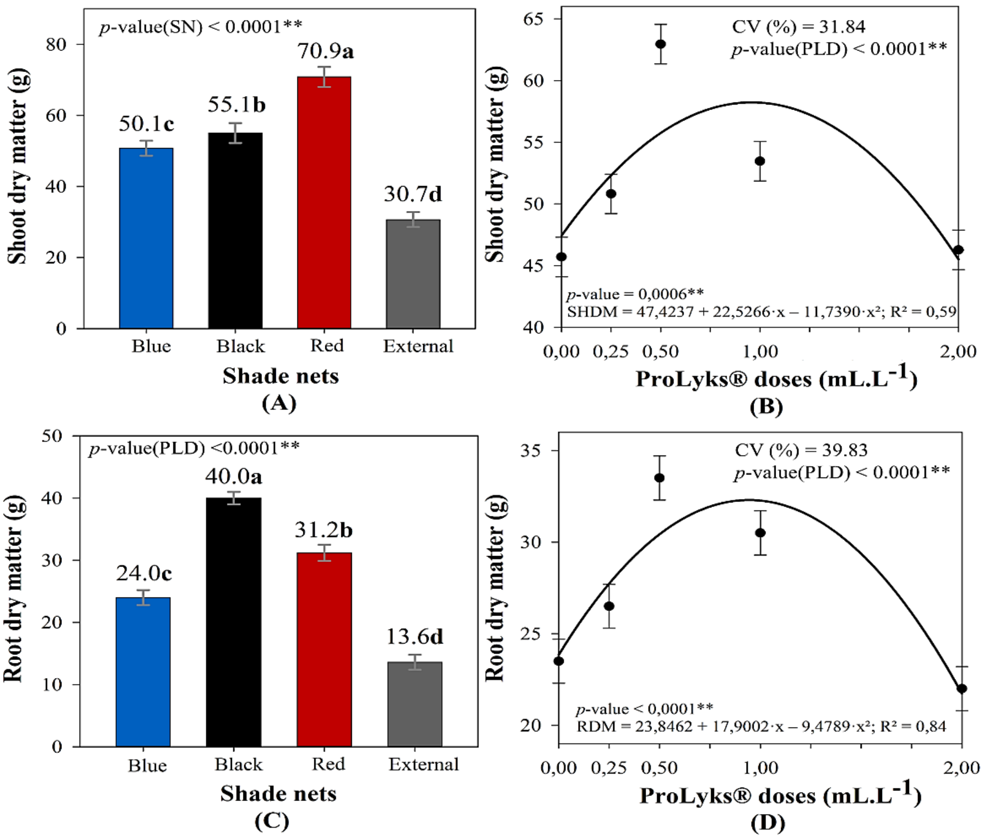
Figure 5 - Secondary shoot number (A, B) and plant diameter (C, D) in *M. laevigata* in response to ProLyks® application under different colored shade nets. Values represent means ($n = 5$) ± standard error. Different lowercase letters indicate significant differences among qualitative treatments (shade nets), as determined by Tukey's test for plant diameter (C) and the Scott–Knot (D) test for secondary shoot number.



Source: Research data (2025).

On the other hand, regarding plant diameter, only the ProLyks® dose had a statistically significant effect ($p < 0.01$), while no significant differences were observed for colored shade nets ($p = 0.38$) or for the interaction between main plots and subplots ($p = 0.94$) (Figure 5C,D). The regression model revealed a quadratic response to increasing ProLyks® concentrations, with an adjusted R^2 of 0.72, indicating a good fit. The optimal dose was estimated at 0.92 $mL \cdot L^{-1}$, corresponding to a maximum plant diameter of approximately 10.20 mm, after which higher concentrations resulted in a slight reduction in stem thickening. Thereafter, in terms of number of secondary shoots (Figure 5A,B), regression analysis revealed a significant linear effect ($p < 0.01$) of ProLyks® doses, with an adjusted $R^2 = 0.90$. The maximum value was recorded at the highest applied dose (2.00 $mL \cdot L^{-1}$), resulting in an average of 21.85 shoots per plant, which represents an increase of approximately 350.5% compared to the control treatment (4.85 shoots at 0.00 $mL \cdot L^{-1}$). Regarding the effect of shade nets, plants grown under full sunlight exhibited the highest average number of secondary shoots, significantly differing from all other treatments. The black and red shade nets yielded intermediate values and did not significantly differ from each other. In contrast, the blue net presented the lowest average, which was statistically inferior to the external condition.

Figure 6 - Root dry matter (A, B) and shoot dry matter (C,D) in M. laevigata in response to ProLyks® application under different colored shade nets. Values represent means ($n = 5$) ± standard error. Different lowercase letters indicate significant differences among qualitative treatments (shade nets), as determined by Tukey's test.



Source: Research data (2025).

Within biomass production, independent regression analyses for each variable revealed a significant quadratic response ($p < 0.01$). The models demonstrated good statistical fit, with $R^2 = 0.59$ for shoot dry mass and $R^2 = 0.84$ for root dry mass. In terms of shoot dry mass (Figures 6A, B), the maximum estimated value was 58.25 g per plant, achieved at an optimal ProLyks® dose of 0.96 $mL \cdot L^{-1}$. This corresponded to an increase of approximately 27.4% compared to the control group (45.71 g at 0.00 $mL \cdot L^{-1}$). Regarding the light conditions, the red shade net consistently promoted the highest shoot biomass, significantly outperforming both the black and blue nets, which did not differ statistically from each other. The full sunlight condition ("external") yielded the lowest value, representing a 59.9% reduction relative to the red net. Moreover, for root dry mass (Figure 6C, D), the quadratic regression model indicated a peak of 32.28 g per plant at a ProLyks® dose of 0.94 $mL \cdot L^{-1}$, representing an increase of approximately 37.4% compared to the control treatment (23.50 g at 0.00 $mL \cdot L^{-1}$). Regarding the effect of colored shade nets, the black net promoted the greatest root biomass accumulation, followed by the red and blue nets, which formed a statistically intermediate group. Once again, the lowest value was recorded under full sunlight ("external environment").

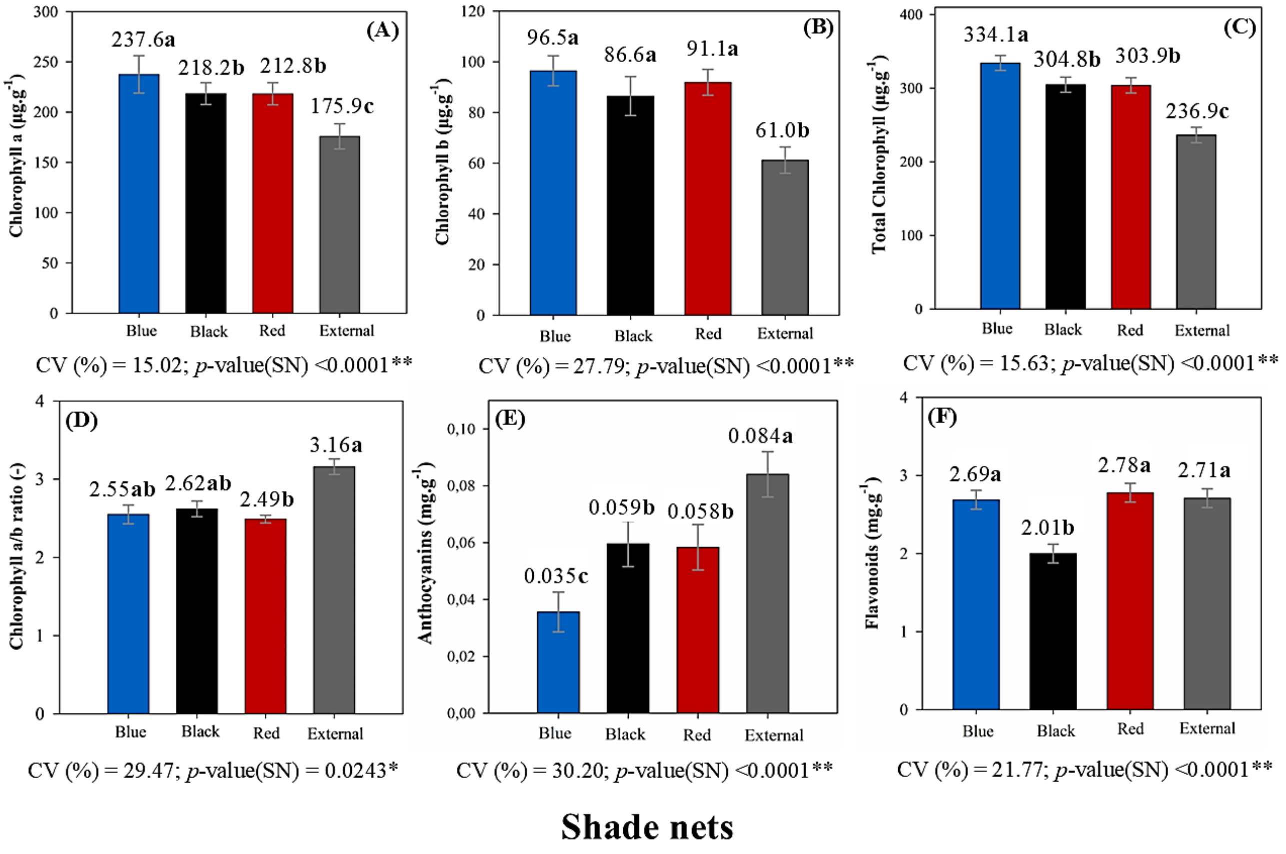
3.2 Photosynthetic Pigments and Secondary Metabolites Analysis

The analysis of variance (ANOVA) revealed that colored shade nets had a significant effect ($p < 0.05$ to $p < 0.01$) on all photosynthetic pigments evaluated: chlorophyll *a*, chlorophyll *b*, total chlorophyll, and the chlorophyll *a/b* ratio. In addition, the concentrations of anthocyanins, flavonoids, and coumarins were also significantly influenced by the shade nets ($p < 0.01$).

While ProLyks® doses and their interaction with the main plots exhibited no significant effects for most variables, a notable exception was observed for coumarin content, which was significantly affected both by terpene doses and by its interaction with shade nets ($p < 0.05$). All pigment related data conformed to normality according to the Shapiro-Wilk test, except for the chlorophyll *a/b* ratio, which required a $\log_{10}(x)$ transformation.

Regarding secondary metabolites, flavonoid and coumarin data passed through the Shapiro-Wilk normality test, while anthocyanin data met normality assumptions only after $\log_{10}(x + 0.5)$ transformation, as verified by the Lilliefors test. In relation to chlorophyll *a* content (Figure 7A), plants grown under the blue shade net exhibited the highest concentration, which significantly surpassed all other light environments. The red and black nets yielded intermediate values, demonstrating no statistical difference between them. Full sunlight exposure resulted in the lowest value, representing a substantial decrease of approximately 35% when compared to blue net conditions. A similar pattern was observed for chlorophyll *b* (Figure 7B). The blue net again yielded the highest concentrations, although it was not statistically different from the red and black nets. In contrast, full sunlight”) exposition resulted in the lowest mean.

Figure 7 - Content of photosynthetic pigments and bioactive compounds in leaves of M. laevigata under different colored shade nets. Bars represent the mean (*n*=5) ± standard error. Different lowercase letters indicate significant differences among light conditions according to Tukey’s test (p < 0.05). (A) Chlorophyll a content, (B) Chlorophyll b content, (C) Total chlorophyll content, (D) Chlorophyll a/b ratio, (E) Anthocyanin content, (F) Flavonoid content.

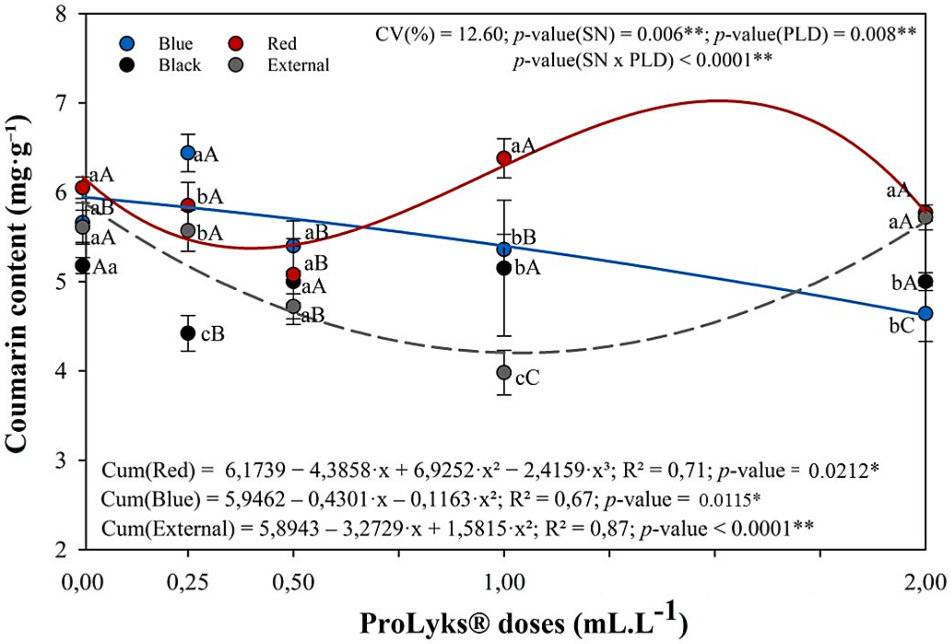


Source: Research data (2025).

Total chlorophyll content (Figure 7C) followed a similar trend, with the blue net promoting the highest accumulation, significantly higher than all other treatments. The red and black nets formed a statistically indistinguishable group, whereas full sunlight yielded the lowest pigment concentration. The chlorophyll *a/b* ratio (Figure 7D) displayed an inverse behavior. The highest ratio was recorded under full sunlight, significantly greater than values observed under the black and red nets. The blue net yielded the lowest ratio, which was statistically similar to the red and black treatments but significantly lower than that under full sunlight. Anthocyanin content (Figure 7E) was highest under full sunlight, significantly surpassing all other light environments. The lowest value was recorded under the blue net, representing a reduction of over 57% relative to full sunlight. Moreover, both the red and black nets formed a statistical group with intermediate anthocyanin levels. For flavonoids (Figure 7F), the red, blue and external environments exhibited the highest and statistically equal concentrations. In contrast, the black net resulted in the lowest value, which was significantly different from the other light treatments. This represented a 27% decrease in flavonoid content compared to the red net.

Regarding coumarin content (Figure 8), due to the significant interaction between factors, regression analysis was performed within each light environment. Under the red shade net, coumarin content exhibited a significant cubic response to ProLyks® doses ($p = 0.0212$; $R^2 = 0.71$), with the highest accumulation observed with the dose of 1.5 mL.L^{-1}. In the blue net and external condition, the data followed a significant quadratic model ($p = 0.00115$ and $p < 0.0001$, respectively), although with distinct patterns: under the blue net, coumarin content declined gradually with increasing doses. In contrast, under external conditions, the response followed an inverted quadratic trend, with the lowest coumarin content observed at approximately 1.0 mL·L^{-1}. No significant regression was observed under the black net, indicating a limited or inconsistent influence of ProLyks® application on coumarin accumulation in this environment.

Figure 8 - Content of Coumarin (mg.g^{-1}) in leaves of M. laevigata as a function of ProLyks® doses under different colored shade nets. Values represent means ± standard error ($n = 5$). Different lowercase letters indicate significant differences among ProLyks® doses within the same light condition; uppercase letters indicate differences among light conditions for the same dose, according to Tukey's test ($p \leq 0.05$). Regression equation and R^2 values are presented for each light environment.



Source: Research data (2025).

3.3 Correlation Analysis Among Experimental Variables

According to the classification proposed by Mukaka et al. (2012), only correlation coefficients equal to or above 0.50 were considered relevant for interpretation, as lower values are categorized as negligible. Based on this criterion, plant height exhibited a positive correlation with coumarin content ($r = 0.61$; $p \leq 0.01$), suggesting a link between shoot elongation and coumarin content in leaves. Plant diameter correlated positively with the number of secondary shoots (r = 0.77; p ≤ 0.01), number of leaves (r = 0.60; p ≤ 0.01), and root dry matter (r = 0.68; p ≤ 0.01). A significant correlation was also observed between plant height and shoot dry matter (r = 0.61; p ≤ 0.01). Additionally, a moderate negative correlation was found between total chlorophyll content and flavonoid concentration in foliar tissues (r = - 0.57; p ≤ 0.01).

Table 1 - Pearson's correlation coefficients among biometric and biochemical variables of *M. laevigata* grown under four light environments (colored shade nets and full sunlight) and subjected to the foliar application of five ProLyks® doses.

Parameter	PH	PD	NL	SSN	LDM	RDM	SDM	Chl*a*	Chl*b*	Chl*a*/Chl*b*	ChlT	Flav	Anth	Cum
PH	---	0.25**	**0.60****	0.17ns	**0.88****	**0.71****	**0.87****	0.30**	0.31**	- 0.15ns	0.36**	-0.25**	-0.11ns	**0.61****
PD		---	**0.77****	**0.60****	0.28**	0.20*	0.27**	-0.11ns	-0.04ns	-0.02ns	-0.05ns	-0.17ns	-0.02ns	-0.19*
NL			---	0.35**	**0.68****	0.37**	**0.63****	-0.02ns	0.13ns	-0.06ns	0.05ns	-0.25**	0.05ns	0.07ns
SSN				---	0.14ns	-0.04ns	0.05ns	-0.14ns	-0.002ns	-0.04ns	-0.10ns	0.07ns	-0.08ns	0.02ns
LDM					---	**0.56****	**0.93****	0.28**	0.39**	-0.24*	0.40**	-0.46**	0.09ns	0.39**
RDM						---	0.68**	0.30**	0.31**	-0.20*	0.36**	-0.36**	-0.43**	0.49**
SDM							---	0.32**	0.38**	-0.21*	0.41**	-0.49**	-0.01ns	0.40**
Chl*a*								---	0.40**	0.03ns	**0.88****	-0.45**	-0.03ns	0.05ns
Chl*b*									---	**-0.83****	**0.78****	**-0.51****	-0.08ns	-0.01ns
Chl*a*/Chl*b*										---	-0.40**	0.35**	0.12ns	0.03ns
ChlT											---	**-0.57****	-0.06ns	0.03ns
Flav												---	0.02ns	0.43**
Anth													---	-0.20*
Cum														---

Significant correlations at 5% (*) and 1% (**) probability levels according to the *t*-test ($p \leq 0.05$; $p \leq 0.01$). Bold values indicate a considerable correlation coefficient ($r > 0.50$) according to Mukaka et al. (2012). PH: plant height; PD: plant diameter; NL: number of leaves; SSN: number of secondary shoots; LDM: leaves dry matter; RDM: root dry matter; SDM: shoot dry matter; Chl*a*: chlorophyll a; Chl*b*: chlorophyll b; Chl*a*/Chl*b*: chlorophyll a/b ratio; ChlT: total chlorophyll content; Flav: total flavonoid content; Anth: anthocyanin content; Cum: coumarin content.

4. Discussion

4.1 Biometric and Biomass Measurements

Among microclimatic variables, global solar radiation exhibited the most pronounced variation across treatments (Figures 2 and 3), confirming the effect of colored shade nets in modifying both the intensity and spectral composition of incident light. These results are consistent with Honorato et al. (2023), who also reported lower solar radiation transmittance under blue nets and intermediate levels under red and black nets. Thus, although ChromatiNET® shade nets are advertised as providing 50% shading efficiency, the transmissivity values observed in this study were substantially lower, revealing a discrepancy between manufacturer specifications and actual field performance. Similar deviations have been noted in other studies, which also highlight that colored nets influence not only light quality but broader environmental factors such as air temperature, humidity, airflow, and overall radiation levels (Oliveira et al., 2024; Vuković et al., 2022; Zare et al., 2019).

Despite these microclimatic shifts, other environmental parameters remained relatively stable across treatments (Figure 2). This outcome suggests that light intensity and its spectral composition were the primary drivers of the observed plant responses in this study (Kabir et al., 2024; Sharma et al., 2019; Tittonell & Giller, 2013), as supported by the elevated partial effect sizes values ($\eta^2_p > 0.14$) observed for the colored shade nets treatments (supplementary material). In this context, the reduced plant development and biomass production under full sunlight conditions likely resulted from photoinhibition due to excessive light availability, whereas the limited light under the blue net probably led to insufficient photosynthetic stimulation (Figures 4 and 6). This interpretation is further corroborated by the pattern of anthocyanin accumulation (Figure 7E), which was highest under full sunlight and lowest under the blue net, thereby indicating varying degrees of light-induced stress (Li & Ahammed, 2023; Song et al., 2022). Thus, these findings align with those of Contin et al. (2021), who reported increased growth of *M. laevigata* cultivated under moderate shading (25-50%).

Additionally, the significantly greater plant height observed under the red net, likely due to its higher red-to-far-red (R/FR) ratio, and the reduced height under the blue net, associated with cryptochrome activation, suggest that the light spectrum exerted a pronounced influence on shoot development in *M. laevigata* (Figure 4A and Figure 6A). In this manner, Han et al. (2024) demonstrated that shifts in R/FR ratios modulate phytochrome-mediated signaling pathways, promoting gibberellin biosynthesis and internode elongation (Dubois & Brutnell, 2011; Song et al., 2022). These findings are consistent with those reported by Harish et al. (2022) in *Curcuma longa* L. and Zhang et al. (2022) in *Camellia sinensis* L. Kuntze, both of which observed increased vertical growth in response to red netting. Conversely, blue light has been shown to strongly inhibit plant elongation through the activation of cryptochrome and phototropin signaling pathways, which regulate hormonal homeostasis and suppress cell expansion in stem tissues (Costa et al., 2010; Santos et al., 2023).

In contrast, plant diameter did not differ significantly among light conditions (Figure 5C), reflecting similar findings by Souza et al. (2010) and Souza et al. (2011), who also reported no changes in this parameter for *M. glomerata* cultivated under colored shade nets. These results suggest that, in shade-adapted species, plant thickening is less sensitive to variations in light intensity and spectral quality, whereas shoot elongation appears more responsive to such environmental cues, likely due to etiolation processes. This interpretation is supported by the low Pearson's correlation coefficient ($r = 0.25$) found between plant height and plant diameter (Table 1), indicating a weak association between vertical and radial growth under the tested conditions. Similar responses have been observed in *Physalis peruviana* L. (Santos et al., 2023) and *Ocimum selloi* Benth. (Costa et al., 2010), reinforcing the hypothesis that, under shaded environments, morphological plasticity is more pronounced in plant height than in diameter. Subsequently, the results regarding leaf number per plant (Figure 4B) also appear to align with Harish et al. (2022), who observed enhanced vegetative performance under red and blue shade nets when compared to full sunlight. According to Ilić et al. (2017), the greater leaf production under the red net may be attributed to a higher photosynthetic photon flux density (PPFD), particularly in the red and far-red regions, which could stimulate cell division and meristematic development (Costa et al., 2012). From this perspective, blue nets might also influence leaf formation by modulating photomorphogenic pathways through the activation of cryptochromes and phototropins (de Oliveira et al., 2023). In contrast, the limited spectral selectivity of the black net, coupled with potential stress under full sunlight, likely restricted overall vegetative growth and suppressed cell division and, subsequently, leaf emergence (Contin et al., 2021; Harish et al., 2022; Honorato et al., 2023).

Contrary to our initial expectations, the number of secondary shoots varied significantly across the colored shade nets (Figure 5A). The distribution of this morphologic variable is closely related to the gradient of solar radiation among light conditions (Figure 3), suggesting that solar radiation intensity likely played a central role in regulating lateral shoot formation. One possible explanation for this phenomenon involves the dynamic behavior of auxin, a hormone that maintains apical dominance by suppressing axillary bud growth (Taiz et al., 2015). Under high solar irradiance, auxins are more susceptible to photodegradation, which can reduce their inhibitory effect and thereby promote increased lateral branching (Thakur et al., 2019; Zhou et al., 2024).

Beyond morphological traits, biomass accumulation provided additional insights into the effects of colored shade nets on *M. laevigata* growth. In this study, shoot dry matter closely followed the trend observed for leaf dry matter ($r = 0.93$), with the highest values recorded under the red net (Figure 6A). These results contrast with those of Souza et al. (2010) and Ribeiro et al. (2024), who reported greater aboveground biomass under blue netting for *M. glomerata* and *Lippia dulcis* Trevir., respectively. Conversely, the present findings are consistent with Ribeiro et al. (2018), who observed a similar pattern in *Pogostemon cablin* (Blanco) Benth., with the red net promoting the highest yields, followed by the black net, blue net, and full sunlight. Lastly, Pearson's correlation analysis further revealed that the highest *M. laevigata* plants exhibited significantly greater shoot ($r = 0.87$) and root dry matters ($r = 0.71$), highlighting a positive association between vertical growth and biomass accumulation. These results suggest that light-induced plant elongation under red netting is not merely morphological plasticity but reflects a broader physiological response that favors overall plant growth (Taiz et al., 2015).

Conversely, root dry matter was highest under the black net (Figure 6C), which contrasts with previous studies, including Souza et al. (2011), who reported increased root biomass of *M. glomerata* under blue and red nets, as well as similar trends in other medicinal species (Harish et al., 2022; Honorato et al., 2023; Souza et al., 2010; Vargas-Hernandez et al., 2017). Although the black and red nets transmitted comparable levels of solar radiation (Figure 3), only the red net significantly altered the spectral composition by enriching red and far-red wavelengths, which are associated with phytochrome-mediated responses that promote shoot elongation and often suppress root development (Costa et al., 2010; Ribeiro et al., 2018). In contrast, the black net, lacking such spectral enrichment, may have reduced stimulation of shoot growth and consequently favoured carbon allocation to roots. According to Taiz et al. (2015), under conditions that limit shoot expansion or apical dominance, plants tend to reallocate photoassimilates to belowground organs as an adaptive strategy to enhance resource acquisition (water and nutrients). This physiological mechanism could explain the greater root biomass observed under the black net, despite its similar light transmittance compared to the red one.

In addition to the morphological responses promoted by the colored shade nets (main plots), this study revealed that *M. laevigata* responded significantly to ProLyks® foliar application in a dose-dependent manner (Figures 4, 5 and 6). Most biometric traits exhibited a similar quadratic pattern, characterized by increases up to approximately 1.20 $mL \cdot L^{-1}$, followed by reductions at higher concentrations, indicative of a typical hormetic response (Pannacci et al., 2006, 2022). In contrast, the number of secondary shoots increased linearly with dosage (Figure 5B). This behavior, in which low concentrations stimulate plant development and higher suppress it, has been widely documented for various bioactive compounds, including bioherbicides, allelochemicals and other terpenoids (Belz & Duke, 2014; Lin et al., 2024). Extending on this, a plausible explanation for the observed growth response is proposed by Lin et al. (2024), who reported that D-limonene (the principal component of ProLyks® formulation) can influence key metabolic pathways related to energy production (glycolysis, Krebs cycle, and oxidative phosphorylation), potentially increasing ATP availability and supporting cell expansion, division and biomass accumulation.

Furthermore, Ninkuu et al. (2021) demonstrate that monoterpenes, such as D-limonene, can interact with hormonal pathways and intracellular signaling mechanisms, modulating plant development in a concentration-dependent manner. These combined mechanisms may help explain the biomass and morphological development gains observed at lower concentrations. Similarly, Hasan et al. (2016) and Pannacci et al. (2022) reported hormetic effects, with increased root and shoot biomass at low concentrations and inhibition at higher levels, following the foliar application of other bioactive compounds present in other commercial products.

In contrast, the inhibitory effects observed at higher ProLyks® concentrations suggest a shift from physiological stimulation to phytotoxicity (Gettys et al., 2021; Sieniawska et al., 2015), as evidenced by the decline in biomass accumulation and morphological development from the 1.20 $mL \cdot L^{-1}$ dose onward. This trend is consistent with findings by Gettys et al. (2021), who reported biomass reductions exceeding 90% in *Pistia stratiotes* L. treated with 20-30% of D-limonene. Similarly, Sbaghi and el Aalaoui (2025) observed severe tissue degradation and turgor loss in plants exposed to high concentrations of this terpenoid. Furthermore, according to Lin et al. (2024), D-limonene compromises the integrity of cell membranes and walls by increasing permeability and disturbing ion homeostasis (Díaz-Perez & St John, 2019; Han et al., 2020), which may also underline the physiological stress and growth suppression observed in *M. laevigata* treated with a higher dosage.

In this regard, the linear increase in the number of secondary shoots (Figure 5B) may reflect a stress-induced developmental response resulting from the degradation of cell membranes caused by D-limonene (Díaz-Perez & St John, 2019; Han et al., 2020; Ninkuu et al., 2021), considering that meristematic tissues are particularly sensitive to structural disruptions (Taiz et al., 2015). Moreover, such damage may interfere with cell division and hormonal regulation, potentially leading to altered shoot initiation as a compensatory mechanism under stress conditions (Sbaghi & el Aalaoui, 2025). Unlike other biometric variables that exhibited a biphasic response, the continuous increase in the secondary shoot number suggests a distinct adaptive strategy rather than a typical hormetic curve. This linear pattern may reflect the plant's attempt to preserve morphogenic stability by promoting lateral shoot formation as apical dominance is progressively weakened under increasing doses of D-limonene (Taiz et al., 2015). Such behavior aligns with stress-adaptive mechanisms observed in hormesis-related studies, where moderate, non-lethal stress triggers a shift in developmental priorities to maintain growth plasticity and ensure survival (Sbaghi & el Aalaoui, 2025; Taiz et al., 2015). To some extent, this observation is also consistent with the hormetic effect described for the other biometric variables, as it suggests a shift in developmental patterns in response to increasing doses of ProLyks®, reinforcing the dose-dependent duality between stimulation and inhibition.

4.2 Photosynthetic Pigments and Secondary Metabolites

Photosynthetic pigment levels are widely recognized as reliable indicators of photosynthetic performance, given their central role in light absorption and energy conversion, which directly influence plant development and adaptation to environmental conditions (Ilić et al., 2017; Taiz et al., 2015). In the present research, the absence of significant changes in chlorophyll content following foliar Prolyks® application suggests that D-limonene does not exert its effects through direct modulation of the photosynthetic apparatus of *M. laevigata*. This finding is consistent with the previously proposed hypothesis that the biostimulatory responses observed for this terpenoid are likely mediated by post-photosynthetic metabolic pathways, potentially involving enhanced efficiency in photoassimilate utilization and the regulation of hormonal signalling (Hasan et al., 2016; Ninkuu et al., 2021). However, a limitation of our study is that the partial effect sizes for pigment-related variables were relatively low ($\eta^2_p < 0.14$), indicating limited statistical power (supplementary material). As such, the experimental design may not have been sufficiently sensitive to detect subtle effects of Prolyks® on chlorophyll content, and these results should therefore be interpreted with caution.

In this manner, despite the absence of a response to D-limonene, variations in pigment content were observed across the photoselective shade nets with elevated partial effect sizes ($\eta^2_p > 0.14$). In this experiment, total chlorophyll and chlorophyll *a* exhibited a distribution pattern aligned with global solar radiation levels, with higher concentrations under the blue net and lower values under full sunlight (Figure 7A,C). This outcome suggests a predominant role of light intensity in their accumulation (Ahmed et al., 2016; Kabir et al., 2024; Oliveira et al., 2024; Vuković et al., 2022; Zare et al., 2019). Thus, according to Honorato et al. (2023), increased pigment content under shading likely reflects an adaptive response to enhance the capture of diffuse radiation, thereby improving carbohydrate production and supporting plant growth under conditions of reduced irradiance. Similar results were reported by Ilić et al. (2017) and Harish et al. (2022), who also found higher chlorophyll levels in leaves under colored nets compared to external environments, reinforcing the influence of light intensity on pigment accumulation.

In this context, the greater accumulation of total chlorophyll and chlorophyll *a* under the blue shade net aligns with the findings of Souza et al. (2011), that reported higher levels of these pigments under blue nets and the lowest under full sunlight for *M. glomerata*. Consistent with this, Contin et al. (2021) observed reduced chlorophyll content in the same species cultivated under full sunlight compared to shaded conditions. However, the present experiment found no significant differences in chlorophyll *b* among the colored nets (Figure 7B). This may be attributed to the saturation of this specific chlorophyll synthesis under moderate shading, beyond which additional changes in light quantity do not affect its accumulation (de Oliveira et al., 2023; Taiz et al., 2015). A similar result was reported by Díaz-Perez and St John (2019), who also observed uniform chlorophyll *b* levels across colored shade nets in *Capsicum annuum* L.

Furthermore, higher chlorophyll a/b ratios were observed under the external environment, while lower values occurred under shaded conditions, particularly beneath the red net (Figure 7D). This pattern likely reflects a photomorphogenic adjustment, in which plants exposed to high irradiance reduce chlorophyll *b* content to mitigate excessive light absorption, whereas those under lower irradiance increase their proportion to enhance light capture through antenna complexes (Honorato et al., 2023; Taiz et al., 2015). Thus, although the red and black nets provided comparable irradiance levels, the red net resulted in lower a/b ratios, possibly due to its distinct red to far-red light ratio, a spectral feature known to regulate pigment synthesis and allocation (Han et al., 2024; Harish et al., 2022; Taiz et al., 2015). Nevertheless, this outcome contrasts with the findings of Souza et al. (2011), who reported no significant differences in the a/b ratio of *M. laevigata* cultivated under different colored shade nets.

Similarly, a positive correlation was observed between light quantity and anthocyanin synthesis (Figure 7E), suggesting an adaptive role of these pigments as part of a response to potential light-induced stress (Li & Ahammed, 2023; Song et al., 2022). Given the well-recognized photoprotective function of anthocyanins in safeguarding the photosynthetic apparatus, their higher accumulation in leaves under full sunlight conditions may be associated with increased radiation exposure, which could lead to greater production of reactive oxygen species and, consequently, induce antioxidant responses (Costa et al., 2012; Taiz et al., 2015). This hypothesis is supported by Zoratti et al. (2015), who reported greater anthocyanin accumulation in *Vaccinium corymbosum* L. under high irradiance. Furthermore, Wolske et al. (2021) observed significant reductions in this compound in *Ribes nigrum* L. subjected to artificial shading made with black ChromatiNET®.

Conversely, our results for flavonoid content in *M. laevigata* leaves (Figure 7F) contrast with findings reported by several authors, who observed significant reductions in these compounds under shading compared to full sunlight (Jiménez-

Viveros et al., 2023; Lima et al., 2019; Pereira Feitosa et al., 2021; Wang et al., 2012; Ye et al., 2021). Although flavonoids are well known for their photoprotective and antioxidant functions (Ilić et al., 2017), it is likely that their distribution across light environments was not solely influenced by irradiance, but also by the spectral quality of light. In support of this interpretation, Fu et al. (2016) demonstrated that light spectra enriched in blue and far-red wavelengths can enhance flavonoid biosynthesis, even under low light intensity, through the activation of photoreceptors and the upregulation of specific genes.

Expanding on this, Ye et al. (2021) also reported that spectral modulation can influence the phenylpropanoid pathway in *Camellia sinensis* (L.) Kuntze leaves, even under shaded conditions. Accordingly, the elevated flavonoid levels observed under the blue net in the present study may be associated with the enrichment of blue wavelengths, which are known to stimulate this metabolic route (Fu et al., 2016; Li & Ahammed, 2023; Song et al., 2022). In contrast, the accumulation of flavonoids under full sunlight is probably related to increased photoprotective demand in response to high irradiance. The lower concentrations detected under the black net, by comparison, may reflect the combined effects of reduced light intensity and the absence of spectrally active wavelengths necessary to induce this biosynthetic response (Taiz et al., 2015). Moreover, the negative correlation observed between total chlorophyll and flavonoid content ($r = -0.57$) supports this pattern (Table 1), suggesting that increased investment in photosynthetic pigments is accompanied by reduced production of this compound. This outcome reflects a metabolic adjustment, where secondary metabolism is favoured under stress conditions and downregulated when photosynthetic performance is optimized (Taiz et al., 2015).

Unlike the findings of Souza et al. (2011), who found no significant effect of colored nets on coumarin content in *M. glomerata*, the present study observed higher concentrations of this compound in *M. laevigata* under red and blue nets. These results align with de Lazzari Almeida et al. (2017), who reported increased coumarin levels in *M. laevigata* under 50% shading, while *M. glomerata* showed little response to light variation. Similarly, Bertolucci et al. (2013) also observed higher coumarin content under 80% shading for *M. laevigata*, suggesting that this species responds favourably to reduced irradiance. Thus, as reported by de Lazzari Almeida et al. (2017), this outcome may reflect the species' adaptation to the Atlantic Forest, a biome marked by high humidity, frequent rainfall, and filtered light due to dense canopy cover (Gasparetto et al., 2010). Accordingly, the red and blue shade nets may have reduced excess light and mimicked the native environment of *M. laevigata*, thereby minimizing photodegradation and promoting coumarin accumulation.

Interestingly, the concentration of this molecule in “Guaco” leaves was influenced not only by the type of shade net but also by its interaction with the foliar application of ProLyks®. Although black and red nets transmitted similar levels of solar radiation (Figure 3), only the latter increased coumarin content in response to the subplot’s treatment. This outcome suggests that red light might have activated phytochrome-mediated signaling, thereby priming the phenylpropanoid pathway and potentially enhancing the plant’s responsiveness to the apparent elicitor effect of D-limonene (Alves & Deschamps, 2019; Czelusniak et al., 2012). The present hypothesis is consistent with the finding of Han et al. (2024), who observed that red wavelengths could stimulate phenolic compound production by modulating light-responsive regulatory genes (Dubois & Brutnell, 2011). Thus, the peak at 1.5 $mL \cdot L^{-1}$ could reflect a balanced interaction, where red light maintained metabolic readiness while the D-limonene present in ProLyks® formulation triggered a moderate, non-inhibitory defense response.

On the other hand, within the blue shade, coumarin levels declined progressively with increasing ProLyks® doses, suggesting that this environment may have constrained the plant’s ability to respond to the apparent elicitor effect. According to Zou et al. (2023), exposure to abiotic stress can reduce PAL activity and disrupt phenylpropanoid metabolism, ultimately leading to lower coumarin accumulation. Therefore, it is plausible that the spectral properties of blue light, when combined with increasing concentrations of D-limonene, created metabolically adverse conditions that impaired enzymatic activity. Conversely, under full sunlight, coumarin content followed a non-linear pattern: declining up to the 1.0 $mL \cdot L^{-1}$ dose and rising at higher levels. As reported by Wang et al. (2025), excessive irradiance may initially suppress secondary metabolism due to oxidative stress; however, higher ProLyks® doses could induce compensatory antioxidant mechanisms, partially restoring coumarin biosynthesis.

Another relevant aspect regarding this pharmacologically important compound is the significant and positive correlation with the plant height of *Mikania laevigata* ($r = 0.61$; $p \leq 0.01$). This finding is consistent with Castro et al. (2006), who suggested that the higher concentration of coumarin in the upper third leaves of *M. glomerata* is likely related to a physiological role of this molecule in plant growth. Similarly, Castro et al. (2006) reported that the greater accumulation of this compound in leaves and stems from the upper part of *M. glomerata* is due to its predominant synthesis in the plastids of apical meristem cells, followed by translocation to other plant regions. For the national pharmaceutical industry, this result is particularly noteworthy, as it indicates that taller *M. laevigata* plants may serve as a reliable field indicator of higher coumarin content. Such a correlation could facilitate the selection of superior plants, leading to more effective phytotherapeutic products and reduced production costs.

Ultimately, this study offers valuable insights into the effects of ProLyks® application under different colored shade nets on the growth, biomass accumulation, and secondary metabolism of *Mikania laevigata*, yet some limitations must be considered. The short evaluation period and limited number of replications may have contributed to the low ($\eta^2_p < 0.14$) partial effect sizes observed for photosynthetic pigment accumulation. Moreover, the physiological and biochemical mechanisms behind the potential hormetic effect on biomass and growth, as well as the apparent elicitor-like increase in coumarin content following foliar ProLyks® application, remain unclear. Thus, the interpretation of these results must be realized with caution by other researchers and medicinal plants growers due to the interaction of multiple experimental factors, including light intensity combined with spectrum (colored shade nets) and other additives present in the commercial D-limonene-based formulation.

Nevertheless, the combination of a red shading net with 1.5 $mL \cdot L^{-1}$ ProLyks® foliar application emerged as the most promising treatment, with potential economic benefits for *M. laevigata* cultivation by enhancing coumarin content and biomass production under controlled conditions. This improvement could enhance raw material yield and quality for the phytopharmaceutical industry, potentially increasing product value and reducing costs if reproducible at commercial scale and compatible with current practices. Despite that, studies on the biosynthetic pathways and signalling mechanisms involved in plant responses to ProLylyks® and D-limonene remain extremely necessary.

5. Conclusions

This study demonstrates that the use of a red shading net in combination with foliar ProLyks® application at 1.5 $mL \cdot L^{-1}$ is a promising strategy for enhancing plant growth and increasing the accumulation of coumarin in leaves of *Mikania laevigata*. This approach shows strong potential for integration into organic agriculture or in combination with other agronomic practices, contributing to more sustainable production systems. Our findings revealed positive morphophysiological responses and a characteristic hormetic effect of ProLyks® application, underscoring its biostimulant potential in medicinal plant cultivation. The strong positive correlation between plant height and coumarin content suggests that taller plants may serve as a simple field indicator of higher pharmaceutical quality, while the significant association between total chlorophyll and flavonoid content highlights the link between photosynthetic pigment accumulation and secondary metabolism. Pigment synthesis and the production of pharmacologically relevant compounds were predominantly influenced by the light environment rather than by D-limonene, emphasizing the role of light quality in regulating key metabolic pathways. Further research should investigate the physiological, hormonal, and genetic mechanisms involved in plant responses to ProLyks® and

D-limonene, as well as assess their effects across different phenological stages and under field conditions, to broaden the understanding of their functionality and practical applications.

Supplementary Material

The file containing the supplementary statistical output, including effect size values (η^2_p), as well as the figures related to the high-performance liquid chromatography (HPLC) process for coumarin quantification, can be accessed at the following Google Drive link: https://drive.google.com/drive/folders/1GvrejXP6TykFIuo-B2lngS9dettZyp50?usp=sharing

Author Contributions

Conceptualization, P.H.V.R., M.E.A.S. and V.O.D.; methodology, P.H.V.R. and M.E.A.S.; formal analysis, M.E.A.S. and V.O.D.; resources, P.H.V.R., M.E.A.S. and V.O.D.; data curation, M.E.A.S., V.O.D., and J.C.A-J; writing—original draft preparation, M.E.A.S., V.O.D., and J.C.A-J.; writing—review and editing P.H.V.R., A.A.B., L.G.Z. and J.A.L.; supervision, P.H.V.R.; project administration, P.H.V.R. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

L.G.Z., J.A.L and A.A.B. are employed by Apis Flora® Industrial and Commercial Ltda. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Ahmed, H. A., Al-Faraj, A. A., & Abdel-Ghany, A. M. (2016). Shading greenhouses to improve the microclimate, energy and water saving in hot regions: A review. *Scientia Horticulturae*, *201*, 36–45. https://doi.org/10.1016/j.scienta.2016.01.030

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M., & Sparovek, G. (2014). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, *22*(6), 711–728. https://doi.org/10.1127/0941-2948/2013/0507

Alves, L., & Deschamps, C. (2019). Radiation levels of UV-A and UV-B on growth parameters and coumarin content in guaco. *Ciência Rural*, *49*(10), e20190042. https://doi.org/10.1590/0103-8478cr20190042

Azevedo, S. G. D., Oliveira, L. P. H., Manzali, S. I., & Car, S. A. (2018). *Fitoterapia contemporânea: Tradição e ciência na prática clínica* (2nd ed.). Guanabara Koogan.

Belz, R. G., & Duke, S. O. (2014). Herbicides and plant hormesis. *Pest Management Science*, *70*(5), 698–707. https://doi.org/10.1002/ps.3726

Bertolucci, S. K. V., Pereira, A. B. D., Pinto, J. E. B. P., Oliveira, A. B., & Braga, F. C. (2013). Seasonal variation on the contents of coumarin and kaurane-type diterpenes in *Mikania laevigata* and *Mikania glomerata* leaves under different shade levels. *Chemistry & Biodiversity*, *10*(2), 288–295. https://doi.org/10.1002/cbdv.201200166

Castro, E. M., Pinto, J. E. B. P., Bertolucci, S. K. V., & Malta, M. R. (2006). Coumarin contents in young *Mikania glomerata* plants (Guaco) under different radiation levels and photoperiod. *Acta Farmaceutica Bonaerense*, *25*(3), 387-392.

Chaimovitsh, D., Abu-Abied, M., Rubin, B., & Sadot, E. (2017). Herbicidal activity of monoterpenes is associated with disruption of microtubule functionality and membrane integrity. *Weed Science*, *65*(1), 19–30. https://doi.org/10.1614/WS-D-16-00044.1

Chen, S. L., Yu, H., Luo, H. M., Wu, Q., Li, C. F., & Steinmetz, A. (2016). Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese Medicine*, *11*(1), 37. https://doi.org/10.1186/s13020-016-0108-7

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Lawrence Erlbaum Associates.

Contin, D. R., Habermann, E., Alves, V. M., & Martinez, C. A. (2021). Morpho-physiological performance of *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip ex Baker plants under different light conditions. *Hoehnea*, *48*, e742020. https://doi.org/10.1590/2236-8906-74/2020

Costa, A. G., Chagas, J. H., Pinto, J. E. B. P., & Bertolucci, S. K. V. (2012). Crescimento vegetativo e produção de óleo essencial de hortelã-pimenta cultivada sob malhas. *Pesquisa Agropecuária Brasileira*, *47*(4), 534–540. https://doi.org/10.1590/S0100-204X2012000400009

Costa, L. C. B., Pinto, J. E. B. P., Castro, E. M., Alves, E., Bertolucci, S. K. V., Rosal, L. F., & Costa, L. (2010). Shading affecting growth and leaf structure of *Ocimum selloi*: Effects of coloured shade netting on the vegetative development and leaf structure of *Ocimum selloi*. *Bragantia*, *69*(2), 349–359. https://doi.org/10.1590/S0006-87052010000200012

Czelusniak, K. E., Brocco, A., Pereira, D. F., & Freitas, G. B. L. (2012). Pharmacobotany, phytochemistry, and pharmacology of guaco: a review considering *Mikania glomerata* Sprengel and *Mikania laevigata* Schultz Bip. ex Baker. *Revista Brasileira de Plantas Medicinais*, *14*, 400–409. https://doi.org/10.1590/S1516-05722012000200022

Davis, C. C., & Choisy, P. (2024). Medicinal plants meet modern biodiversity science. *Current Biology*, *34*(4), R158–R173. https://doi.org/10.1016/j.cub.2023.12.038

de Lazzari Almeida, C., Xavier, R. M., Borghi, A. A., dos Santos, V. F., & Sawaya, A. C. H. F. (2017). Effect of seasonality and growth conditions on the content of coumarin, chlorogenic acid and dicaffeoylquinic acids in *Mikania laevigata* Schultz and *Mikania glomerata* Sprengel (Asteraceae) by UHPLC–MS/MS. *International Journal of Mass Spectrometry*, *418*, 162–172. https://doi.org/10.1016/j.ijms.2016.09.016

de Oliveira, R. C., Blank, A. F., de Oliveira, R. C., Luz, J. M. Q., & dos Santos, A. P. (2023). Optimizing in vitro growth of basil using LED lights. *Ciência Rural*, *53*(5). https://doi.org/10.1590/0103-8478cr20220030

De Souza, T., Ferreira, J. V., Damaso, D. C., Vasconcelos, P. S., Lima, A. M., & Perfeito, J. P. S. (2024). Uso de cascas de laranja para extração de óleo essencial e avaliação de suas atividades biológicas. *Revista Ifes Ciência*, *10*(1), 1–23. https://doi.org/10.36524/ric.v10i1.2419

de Sousa, F. A. S., & de Azevedo, C. A. V. (2016). The Assistat software version 7.7 and its use in the analysis of experimental data. *African Journal of Agricultural Research*, *11*(39), 3733-3740. https://doi.org/10.5897/AJAR2016.11522

Díaz-Perez, J. C., & St John, K. (2019). Bell pepper (*Capsicum annuum* L.) under colored shade nets: Plant growth and physiological responses. *HortScience*, *54*(10), 1795–1801. https://doi.org/10.21273/HORTSCI14233-19

Dubois, P. G., & Brutnell, T. P. (2011). Topology of a maize field: Distinguishing the influence of end-of-day far-red light and shade avoidance syndrome on plant height. *Plant Signaling & Behavior*, *6*(4), 467–470. https://doi.org/10.4161/psb.6.4.14305

Flor, I. C., Rodrigues, A. R., Silva, S. A., Proença, B., & Maia, V. C. (2022). Insect galls on Asteraceae in Brazil: Richness, geographic distribution, associated fauna, endemism and economic importance. *Biota Neotropica*, *22*, e20211234. https://doi.org/10.1590/1676-0611-bn-2021-1234

Freire, M. M., Herc, P., Rodrigues, V., Duarte, S. N., Beserra, G., & Marques, A. (2024). Influence of colored shade nets and salinity on the development of roselle plants. *Agronomy*, *14*(10), 2252. https://doi.org/10.3390/agronomy14102252

Fu, B., Ji, X., Zhao, M., He, F., Wang, X., Wang, Y., Liu, P., & Niu, L. (2016). The influence of light quality on the accumulation of flavonoids in tobacco (*Nicotiana tabacum* L.) leaves. *Journal of Photochemistry and Photobiology B: Biology*, *162*, 544–549. https://doi.org/10.1016/j.jphotobiol.2016.07.016

Garcia, T. P., Gorski, D., Cobre, A. F., Lazo, R. E. L., Bertol, G., Ferreira, L. M., & Pontarolo, R. (2025). Biological activities of *Mikania glomerata* and *Mikania laevigata*: a scoping review and evidence gap mapping. *Pharmaceuticals*, *18*(4), 552. https://doi.org/10.3390/ph18040552

Gasparetto, J. C., Campos, F. R., Budel, J. M., & Pontarolo, R. (2010). *Mikania glomerata* Spreng. and *M. laevigata* Sch. Bip. ex Baker (Asteraceae): Agronomic, genetic, morpho-anatomical, chemical, pharmacological, toxicological studies and their use in Brazilian phytotherapy programs. *Revista Brasileira de Farmacognosia*, *20*(4), 627–640. https://doi.org/10.1590/S0102-695X2010000400025

Gettys, L. A., Thayer, K. L., & Sigmon, J. W. (2021). Evaluating the effects of acetic acid and d-limonene on four aquatic plants. *HortTechnology*, *31*(2), 225–233. https://doi.org/10.21273/HORTTECH04769-20

Han, S., Liu, Y., Bao, A., Jiao, T., Zeng, H., Yue, W., et al. (2024). A characterization of the functions of OsCSN1 in the control of sheath elongation and height in rice plants under red light. *Agronomy*, *14*(3), 572. https://doi.org/10.3390/agronomy14030572

Han, Y., Sun, Z., & Chen, W. (2020). Antimicrobial susceptibility and antibacterial mechanism of limonene against *Listeria monocytogenes*. *Molecules*, *25*(1), 33. https://doi.org/10.3390/molecules25010033

Harish, B. S., Umesha, K., Venugopalan, R., & Prasad, B. N. M. (2022). Photo-selective nets influence physiology, growth, yield and quality of turmeric (*Curcuma longa* L.). *Industrial Crops and Products*, *186*, 115202. https://doi.org/10.1016/j.indcrop.2022.115202

Hasan, S. A., Irfan, M., Masrahi, Y. S., Khalaf, M. A., & Hayat, S. (2016). Growth, photosynthesis, and antioxidant responses of *Vigna unguiculata* L. treated with hydrogen peroxide. *Cogent Food & Agriculture*, *2*(1), 1155331. https://doi.org/10.1080/23311932.2016.1155331

Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, *57*(12), 1332-1334. https://doi.org/10.1139/b79-163

Honorato, A. C., Nohara, G. A., Assis, R. M. A., Maciel, J. F. A., Carvalho, A. A., Pinto, J. E. B. P., & Bertolucci, S. K. V. (2023). Colored shade nets and different harvest times alter the growth, antioxidant status, and quantitative attributes of glandular trichomes and essential oil of *Thymus vulgaris* L. *Journal of Applied Research on Medicinal and Aromatic Plants*, *35*, 100474. https://doi.org/10.1016/j.jarmap.2023.100474

Ilić, Z. S., Milenković, L., Šunić, L., Barać, S., Mastilović, J., Kevrešan, Ž., & Fallik, E. (2017). Effect of shading by coloured nets on yield and fruit quality of sweet pepper. *Zemdirbyste-Agriculture*, *104*(1), 53–62. https://doi.org/10.13080/z-a.2017.104.008

Jiménez-Viveros, Y., Núñez-Palenius, H. G., Fierros-Romero, G., & Valiente-Banuet, J. I. (2023). Modification of light characteristics affect the phytochemical profile of peppers. *Horticulturae*, *9*(1), 72. https://doi.org/10.3390/horticulturae9010072

Kabir, M. Y., Nambeesan, S. U., & Díaz-Pérez, J. C. (2024). Shade nets improve vegetable performance. *Scientia Horticulturae*, *334*, 113326. https://doi.org/10.1016/j.scienta.2024.113326

Lees, D. H., & Francis, F. J. (1972). Standardization of pigment analysis in cranberries. *HortScience*, *7*(1), 83-84. https://doi.org/10.21273/HORTSCI.7.1.83

Li, Z., & Ahammed, G. J. (2023). Plant stress response and adaptation via anthocyanins: A review. *Plant Stress*, *10*, 100230. https://doi.org/10.1016/j.stress.2023.100230

Lima, R. H. S., Mazzafera, P., Rachwal, M. F. G., & Ballarin, A. W. (2019). Growth, biomass and secondary metabolite production of *Achyrocline satureioides* under colored shading nets. *Industrial Crops and Products*, *141*, 111734. https://doi.org/10.1016/j.indcrop.2019.111734

Lin, H., Li, Z., Sun, Y., Zhang, Y., Wang, S., Zhang, Q., et al. (2024). D-limonene: Promising and sustainable natural bioactive compound. *Applied Sciences*, *14*(11), 4605. https://doi.org/10.3390/app14114605

Medeiros, J., & Kanis, L. A. (2010). Avaliação do efeito de polietilenoglicóis no perfil de extratos de *Mikania glomerata* Spreng. e *Passiflora edulis* Sims. *Revista Brasileira de Farmacognosia*, *20*, 796-802. https://doi.org/10.1590/S0102-695X2010005000001

Mukaka, M. M. (2012). Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Medical Journal*, *24*(3), 69–71.

Ninkuu, V., Zhang, L., Yan, J., Fu, Z., Yang, T., & Zeng, H. (2021). Biochemistry of terpenes and recent advances in plant protection. *International Journal of Molecular Sciences*, *22*(11), 5710. https://doi.org/10.3390/ijms22115710

Orsi, B., Demétrio, C. A., Jacob, J. F. O., & Rodrigues, P. H. V. (2022). Effect of terpene treatment on tomato fruit. *Bragantia*, *81*, e0322. https://doi.org/10.1590/1678-4499.20210134

Ortiz, A., & Sansinenea, E. (2023). Phenylpropanoid derivatives and their role in plants' health and as antimicrobials. *Current Microbiology*, *80*, 380. https://doi.org/10.1007/s00284-023-03502-x

Pannacci, E., Baratta, S., Falcinelli, B., Farneselli, M., & Tei, F. (2022). Mugwort (*Artemisia vulgaris* L.) aqueous extract: Hormesis and biostimulant activity for seed germination and seedling growth in vegetable crops. *Agriculture*, *12*(9), 1329. https://doi.org/10.3390/agriculture12091329

Pannacci, E., Onofri, A., & Covarelli, G. (2006). Biological activity, availability and duration of phytotoxicity for imazamox in four different soils of central Italy. *Weed Research*, *46*(3), 243–250. https://doi.org/10.1111/j.1365-3180.2006.00503.x

Pereira, A. S. et al. (2018). Metodologia da pesquisa científica. [free ebook]. Santa Maria: Editora da UFSM.

Pereira Feitosa, L. G., Monge, M., Lopes, N. P., & Rodrigues de Oliveira, D. C. (2021). Distribution of flavonoids and other phenolics in *Mikania* species (Compositae) of Brazil. *Biochemical Systematics and Ecology*, *97*, 104273. https://doi.org/10.1016/j.bse.2021.104273

Punja, Z. K., Sutton, D. B., & Kim, T. (2023). Glandular trichome development, morphology, and maturation are influenced by plant age and genotype in high THC-containing *Cannabis sativa* L. inflorescences. *Journal of Cannabis Research*, *5*(1), 12. https://doi.org/10.1186/s42238-023-00190-w

Ribeiro, A. S., Ribeiro, M. S., Bertolucci, S. K. V., Bittencourt, W. J. M., de Carvalho, A. A., Tostes, W. N., Alves, E., & Pinto, J. E. B. P. (2018). Colored shade nets induced changes in growth, anatomy and essential oil of *Pogostemon cablin*. *Anais da Academia Brasileira de Ciências*, *90*, 1823–1835. https://doi.org/10.1590/0001-3765201820170299

Ribeiro, F. N. S., de Assis, R. M. A., Leite, J. J. F., Miranda, T. F., Alves, E., Bertolucci, S. K. V., & Pinto, J. E. B. P. (2024). The cultivation of *Lippia dulcis* under ChromatiNet induces changes in vegetative growth, anatomy and essential oil chemical composition. *South African Journal of Botany*, *174*, 393–404. https://doi.org/10.1016/j.sajb.2024.09.003

Rosini, B., Bulla, A. M., Polonio, J. C., Polli, A. D., da Silva, A. A., Schoffen, R. P., de Oliveira-Junior, V. A., Santos, S. S., Golias, H. C., Azevedo, J. L., & Pamphile, J. A. (2025). Isolation, identification, and bioprospection of endophytic bacteria from medicinal plant *Mikania glomerata* (Spreng.) and the consortium of *Pseudomonas* as plant growth promoters. *Biocatalysis and Agricultural Biotechnology*, *64*, 103530. https://doi.org/10.1016/j.bcab.2025.103530

Santos, L. D. G., Lima, C. S. M., Bonome, L. T. S., & Rosa, G. G. (2023). Colors of shadowing meshes on phenology, biometrics and physicochemical characteristics of *Physalis peruviana* L. in organic production system. *Revista de Ciências Agroveterinárias*, *22*, 285–294. https://doi.org/10.5965/223811712222023285

Sbaghi, M., & el Aalaoui, M. (2025). Evaluating natural product-based herbicides for effective control of invasive water lettuce (*Pistia stratiotes* L.). *Advances in Weed Science*, *43*, 00001. https://doi.org/10.51694/AdvWeedSci/2025;43:00001

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., & Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules*, *24*(13), 2452. https://doi.org/10.3390/molecules24132452

Siegelman, H. W., & Hendricks, S. B. (1958). Photocontrol of anthocyanin synthesis in apple skin. *Plant Physiology*, *33*(3), 185-190. https://doi.org/10.1104/pp.33.3.185

Sieniawska, E., Swatko-Ossor, M., Sawicki, R., & Ginalska, G. (2015). Morphological changes in the overall *Mycobacterium tuberculosis* H37Ra cell shape and cytoplasm homogeneity due to *Mutellina purpurea* L. essential oil and its main constituents. *Medical Principles and Practice*, *24*(6), 527–532. https://doi.org/10.1159/000438479

Singh, P. A., Bajwa, N., Chinnam, S., Chandan, A., & Baldi, A. (2022). An overview of some important deliberations to promote medicinal plants cultivation. *Journal of Applied Research on Medicinal and Aromatic Plants*, *29*, 100400. https://doi.org/10.1016/j.jarmap.2022.100400

Song, S., He, A., Zhao, T., Yin, Q., Mu, Y., Wang, Y., Liu, H., Nie, L., & Peng, S. (2022). Effects of shading at different growth stages with various shading intensities on the grain yield and anthocyanin content of colored rice (*Oryza sativa* L.). *Field Crops Research*, *283*, 108555. https://doi.org/10.1016/j.fcr.2022.108555

Souza, G. S., Castro, E. M., Soares, A. M., & Pinto, J. E. B. P. (2010). Biometric and physiological aspects of young plants of *Mikania glomerata* Sprengel and *Mikania laevigata* Schultz Bip. ex Baker under colored nets. *Revista Brasileira de Biociências*, *8*(4), 330–335.

Souza, G. S., Castro, E. M., Soares, A. M., Pinto, J. E. B. P., Resende, M. G., & Bertolucci, S. K. V. (2011). Crescimento, teor de óleo essencial e conteúdo de cumarina de plantas jovens de guaco (*Mikania glomerata* Sprengel) cultivadas sob malhas coloridas. *Biotemas*, *24*(3), 1–11. https://doi.org/10.5007/2175-7925.2011v24n3p1

Souza, J. O., Oliveira, E. F., Santos, M. E. S., & Kirsten, C. N. (2022). *Mikania glomerata* Spreng (Asteraceae): its therapeutic use and potential in the COVID-19 pandemic. *Revista Fitos*, *16*, 270–276. https://doi.org/10.32712/2446-4775.2022.1292

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development* (6th ed.). Sinauer Associates, Inc.

Thakur, M., & Kumar, R. (2021). Microclimatic buffering on medicinal and aromatic plants: A review. *Industrial Crops and Products*, *160*, 113144. https://doi.org/10.1016/j.indcrop.2020.113144

Thakur, M., Bhattacharya, S., Khosla, P. K., & Puri, S. (2019). Improving production of plant secondary metabolites through biotic and abiotic elicitation. *Journal of Applied Research on Medicinal and Aromatic Plants*, *12*, 1–12. https://doi.org/10.1016/j.jarmap.2018.11.004

Thawonkit, T., Insalud, N., Dangtungee, R., & Bhuyar, P. (2025). Integrating sustainable cultivation practices and advanced extraction methods for improved cannabis yield and cannabinoid production. *International Journal of Plant Biology*, *16*(2), 38. https://doi.org/10.3390/ijpb16020038

Tittonell, P., & Giller, K. E. (2013). When yield gaps are poverty traps: The paradigm of ecological intensification in African smallholder agriculture. *Field Crops Research*, *143*, 76–90. https://doi.org/10.1016/j.fcr.2012.10.007

Vargas-Hernandez, M., Macias-Bobadilla, I., Guevara-Gonzalez, R. G., Romero-Gomez, S. J., Rico-Garcia, E., Ocampo-Velazquez, R. V., et al. (2017). Plant hormesis management with biostimulants of biotic origin in agriculture. *Frontiers in Plant Science*, *8*, 1762. https://doi.org/10.3389/fpls.2017.01762

Vuković, M., Jurić, S., Maslov Bandić, L., Levaj, B., Fu, D. Q., & Jemrić, T. (2022). Sustainable food production: Innovative netting concepts and their mode of action on fruit crops. *Sustainability*, *14*(15), 9264. https://doi.org/10.3390/su14159264

Vieira, S. (2021). Introdução à bioestatística. Editora GEN/Guanabara Koogan.

Wang, W., Xu, J., Fang, H., Li, Z., & Li, M. (2020). Advances and challenges in medicinal plant breeding. *Plant Science*, *298*, 110573. https://doi.org/10.1016/j.plantsci.2020.110573

Wang, Y., Guan, T., Yue, X., Yang, J., Zhao, X., Chang, A., Yang, C., Fan, Z., Liu, K., & Li, Y. (2025). The biosynthetic pathway of coumarin and its genetic regulation in response to biotic and abiotic stresses. *Frontiers in Plant Science*, *16*, 1599591. https://doi.org/10.3389/fpls.2025.1599591

Wang, Y. S., Gao, L. P., Shan, Y., Liu, Y. J., Tian, Y. W., & Xia, T. (2012). Influence of shade on flavonoid biosynthesis in tea (*Camellia sinensis* (L.) O. Kuntze). *Scientia Horticulturae*, *141*, 7–16. https://doi.org/10.1016/j.scienta.2012.04.013

Wellburn, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, *144*(3), 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2

Wolske, E., Chatham, L., Juvik, J., & Branham, B. (2021). Berry quality and anthocyanin content of 'Consort' black currants grown under artificial shade. *Plants*, *10*(4), 766. https://doi.org/10.3390/plants10040766

Yatsuda, R., Rosalen, P. L., Cury, J. A., Murata, R. M., Rehder, V. L. G., Melo, L. V., & Koo, H. (2005). Effects of *Mikania* genus plants on growth and cell adherence of mutans streptococci. *Journal of Ethnopharmacology*, *97*(2), 183–189. https://doi.org/10.1016/j.jep.2004.09.042

Ye, J. H., Lv, Y. Q., Liu, S. R., Jin, J., Wang, Y. F., Wei, C. L., & Zhao, S. Q. (2021). Effects of light intensity and spectral composition on the transcriptome profiles of leaves in shade grown tea plants (*Camellia sinensis* L.) and regulatory network of flavonoid biosynthesis. *Molecules*, *26*(19), 5836. https://doi.org/10.3390/molecules26195836

Zare, S., Naderi, R., & Amini Dehaghi, M. (2019). Effect of colored shade nets on growth, photosynthesis and yield of ornamental plants. *Acta Horticulturae*, *1266*, 341–348. https://doi.org/10.17660/ActaHortic.2019.1266.47

Zhang, Q., Bi, G., Li, T., Wang, Q., Xing, Z., LeCompte, J., & Harkess, R. L. (2022). Color shade nets affect plant growth and seasonal leaf quality of *Camellia sinensis* grown in Mississippi, the United States. *Frontiers in Nutrition*, *9*, 786421. https://doi.org/10.3389/fnut.2022.786421

Zhou, T., Chang, F., Li, X., Yang, W., Huang, X., Yan, J., Wu, Q., Wen, F., Pei, J., Ma, Y., & Xu, B. (2024). Role of auxin and gibberellin under low light in enhancing saffron corm starch degradation during sprouting. *International Journal of Biological Macromolecules*, *279*, 135234. https://doi.org/10.1016/j.ijbiomac.2024.135234

Zoratti, L., Jaakola, L., Häggman, H., & Giongo, L. (2015). Modification of sunlight radiation through colored photo-selective nets affects anthocyanin profile in *Vaccinium* spp. berries. *PLoS ONE*, *10*(8), e0135935. https://doi.org/10.1371/journal.pone.0135935

Zou, J., Gong, Z., Liu, Z., Ren, J., & Feng, H. (2023). Investigation of the key genes associated with anthocyanin accumulation during inner leaf reddening in ornamental kale (*Brassica oleracea* L. var. *acephala*). *International Journal of Mlecular Sciences*, *24*(3), 2837. https://doi.org/10.3390/ijms24032837