Leaf pigment modeling using ClorofiLOG®: a non-destructive methodological approach for *Mikania laevigata* Sch. Bip. Ex-Baker

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Abstract

Unlike conventional crops, medicinal plants require continuous physiological monitoring, since their pharmacological compounds are synthesized through stress-sensitive secondary metabolism. In this context, accurate and non-destructive methods for determining chlorophyll content could be valuable tools for assessing the physiological status of these species. Thus, *Mikania laevigata* Sch. Bip. ex-Baker, widely used in herbal medicine, can benefit from chlorophyll monitoring to enhance its cultivation. This work was developed in order to evaluate the potential of the ClorofiLOG® index as a non-destructive predictor of leaf pigment concentrations in *M. laevigata* during the vegetative stage. Plants were cultivated for 60 days in greenhouse conditions, and both destructive (spectrophotometric) and non-destructive pigment measurements were collected from medium leaves. Linear regression equations were adjusted between the index and the concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids. The adjusted models yielded high coefficients of determination for total chlorophyll and chlorophyll *b*, with a moderate value for carotenoids. Predictive performance of the adjustments, assessed by the ratio of performance to deviation, was classified as "very good" for total chlorophyll (2.91) and chlorophyll *b* (2.67), and "weak" for chlorophyll *a* (1.79) and carotenoids (1.43). Therefore, models based on the ClorofiLOG® index are suitable for non-destructive estimation of total chlorophyll and chlorophyll *b* in *M. laevigata*, supporting its application as a practical tool for the physiological monitoring of this species. This approach may assist in the early detection of plant stress, guide nutrient management decisions, and contribute to optimizing cultivation strategies for this medicinal species under both experimental and production-scale conditions.

Keywords: Medicinal plants. Plant physiology. Primary metabolism.

Introduction

Despite the increasing interest in the pharmacological potential of the Brazil's native flora, the scientific literature still indicates a scarcity of national studies in crop science aimed at improving cultivation practices for medicinal species (SINGH *et al*., 2022; ALVES, DESCHAMPS, 2025). Unlike conventional crops, which are managed to maximize biomass through net photosynthesis, medicinal plants require continuous physiological monitoring because their therapeutic compounds derive mainly from secondary metabolism, a process highly sensitive to environmental conditions and stress factors. Drought, light intensity, temperature fluctuations, nutrient availability, and pathogen pressure, for instance, are key factors influencing the synthesis of pharmacological compounds in plants (WANG *et al*., 2025; TAIZ *et al*., 2017; VISHWAKARMA *et al*., 2022).

In this manner, *Mikania laevigata* Sch. Bip. ex-Baker, commonly known as "Guaco," is a native Brazilian medicinal plant widely employed in traditional medicine and officially recognized by the Brazilian Unified Health System (SUS) as a recommended phytotherapeutic species (GASPARETTO *et al*., 2010). Naturally found in shaded tropical forests, this species has high ecological plasticity, particularly due to its adaptability to elevated temperatures and humid conditions (FLOR *et al*., 2022). Its pharmacological properties are primarily associated with coumarins and flavonoids, which are responsible for anti-inflammatory, bronchodilatory, and antimicrobial activities. These and other characteristics make it one of the most extensively used medicinal plants by the Brazilian pharmaceutical industry (AZEVEDO *et al*., 2018; SOUZA *et al*., 2022). Expanding on its therapeutic properties, the symbiotic microorganisms of *Mikania* genus play a key role in regulating plant development, offering functions that transcend medicinal use, such as increasing biomass and enhancing phosphorus uptake efficiency through bacterial consortia (ROSINI *et al*., 2025).

Among the physiological indicators used to assess plant performance, leaf chlorophyll content (LCC) stands out for its strong correlation with primary metabolic processes and its responsiveness to abiotic stressors such as nitrogen availability, drought, salinity, and disease pressure (ZHANG *et al*., 2022; TAIZ *et al*., 2017). In medicinal species, this parameter could be useful as an indirect tool to monitor secondary metabolism. For example, in *Mikania glomerata*, Alves and Deschamps (2025) observed a hormetic response in coumarin production under different doses of ammonium nitrate. Likewise, Souza *et al*. (2025a) reported a significant inverse relationship between total chlorophyll content and flavonoid concentration in *M. laevigata*, reinforcing the notion that alterations in photosynthetic physiology can reflect changes in the synthesis of bioactive secondary metabolites.

Nevertheless, chlorophyll quantification has traditionally relied on destructive methods based on solvent extraction followed by spectrophotometric analysis, which are time-consuming, costly, and dependent on laboratory infrastructure (SHIBAEVA *et al*., 2020; ZHANG *et al*., 2022). In contrast, portable non-destructive devices, such as SPAD-based meters (Soil Plant Analysis Development), have been widely adopted in agronomy to estimate chlorophyll content by measuring leaf optical transmittance at specific wavelengths (VILLA e VILA *et al*., 2022). However, because these readings are dimensionless and species-specific, they require prior calibration for accurate interpretation (HAWKINS *et al*., 2009; WENNECK *et al*., 2021). Furthermore, specific calibration models for *M. laevigata* using accessible Brazilian technology, such as the ClorofiLOG® sensor, are still absent in the scientific literature.(HAWKINS *et al*., 2009; WENNECK *et al*., 2021).

Therefore, we hypothesize that the ClorofiLOG® index could be significantly correlated with absolute chlorophyll content in *M. laevigata* leaves and may serve as a practical, non-destructive predictor of its physiological status . Thus, this work was developed in order to evaluate the performance of the ClorofiLOG® device in estimating pigment concentrations (during the vegetative stage of *M. laevigata*, aiming to contribute to the development of more efficient agronomic cultivation practices for Brazilian medicinal species.

Material and methods

Plants of *M. laevigata* were cultivated from October 2 to November 30, 2024, in an experimental greenhouse located at the Department of Crop Science (LPV) of the “Luiz de Queiroz” College of Agriculture (ESALQ), University of São Paulo (USP), in Piracicaba, São Paulo, Brazil (22°42’31” S, 47°37’44” W;

546 m). According to the Köppen climate classification, the region is categorized as Cwa, which is defined as subtropical with hot and humid summers followed by dry winters (ALVARES *et al*., 2014). The greenhouse structure, measuring 6.4 meters in width and 36 meters in length, was aligned along a north-south axis and was covered with a polyethylene film for diffuse light (Suncover Diff, Ginegar®), which offers a nominal 100 % light transmittance. During the experimental period, the average daily climatic conditions recorded by an internal weather station were as follows: air temperature of 24.3 °C, relative humidity of 57.5 %, and global solar radiation of 6.45 MJ m^{-2} day^{-1}.

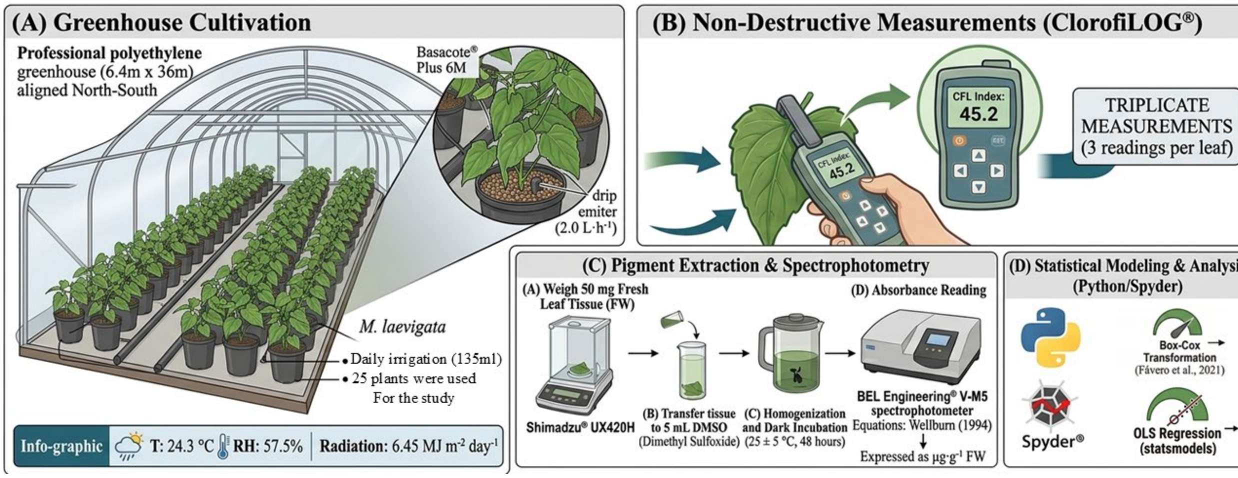
The seedlings of *M. laevigata* were obtained from Flora Arco-Íris (Atibaia, São Paulo, Brazil), a nursery specializing in medicinal plants, which ensured the botanical accuracy of the material. On October 2nd, 2024, a total of 25 uniform seedlings, each approximately 15 cm tall and bearing six pairs of leaves, were transplanted into 1.8 L pots filled with a commercial substrate (Basaplant Hortaliças®). Basal fertilization was carried out by applying 15 g per pot of a controlled-release fertilizer (Basacote® Plus 6 M, 16-8-12 NPK). Irrigation was managed using a drip system composed of PVC main lines and polyethylene lateral lines equipped with pressure-compensating emitters delivering 2.0 L h^{-1}. Plants received irrigation twice daily, at 06:00 and 18:00 hours, with each event lasting two minutes. This irrigation schedule provided approximately 135 mL of water per plant per day. The experiment followed a completely randomized design comprising 100 experimental units (25 plants x 4 leaves each), all maintained under uniform environmental, nutritional, and irrigation conditions.

At 60 days after transplanting (60 DAT), when plants were still in the vegetative stage, indirect estimation of chlorophyll content was performed using the ClorofiLOG® CFL2026 device (Falker®, Brazil), which operates based on leaf optical reflectance. Prior to data collection, the device was carefully calibrated according to the manufacturer's instructions to ensure accurate measurements. For each plant, four fully expanded leaves from the upper-middle portion of the canopy were selected. From each leaf, three readings were taken following the protocols of Villa e Vila *et al*. (2022) and Wenneck *et al*. (2021). The arithmetic mean of these triplicate measurements was calculated to represent each leaf in the subsequent statistical analyses (ordinary least squares regression). Additionally, leaves were selected across a spectrum of visual green coloration to account for intra-plant pigment variability.

Subsequently, these same leaves were used for the direct quantification of photosynthetic pigments. Each sample consisted of 50 mg of fresh leaf tissue, which was weighed using an analytical balance (Shimadzu®, UX420 H model). The tissue was then homogenized in 5 mL of dimethyl sulfoxide (DMSO) and incubated in darkness at 25 ± 5 °C for 48 hours, following the protocol proposed by Hiscox and Israelstam (1979). After the extraction period (Figure 1), absorbance readings were obtained using a spectrophotometer (V-M5, BEL Engineering®) at wavelengths of 665 nm and 649 nm. Based on these absorbance values, concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a* + *b*), and carotenoids were calculated using the equations described by Wellburn (1994). The results were then expressed in micrograms per gram of fresh weight ($\mu g \cdot g^{-1}$ FW).

To validate the regression models linking the ClorofiLOG® index values to foliar pigment concentrations, the dataset was first subjected to diagnostic tests to assess the assumptions of the ordinary least squares (OLS) method. Residual normality was evaluated using the Shapiro-Francia test at a 5 % significance

Figure 1. Methodological workflow of the research conducted in Piracicaba (SP), University of São Paulo (ESALQ/USP), 2024: (A) Greenhouse cultivation and climatic conditions; (B) Non-destructive chlorophyll estimation using ClorofiLOG®; (C) Destructive pigment extraction in DMSO and spectrophotometric quantification ; and (D) Data processing and statistical validation (OLS/Box-Cox) using Python.



Source: authors (2024).

level (selected due to its superior stringency in detecting deviations in the distribution tails for datasets with $n > 30$). Simultaneously, the homogeneity of variances was verified by the Breusch-Pagan test ($p > 0.05$). To satisfy the assumption of normality, the dependent variables (pigment concentrations) underwent Box-Cox transformation, following the procedures described by Fávero *et al*. (2021).

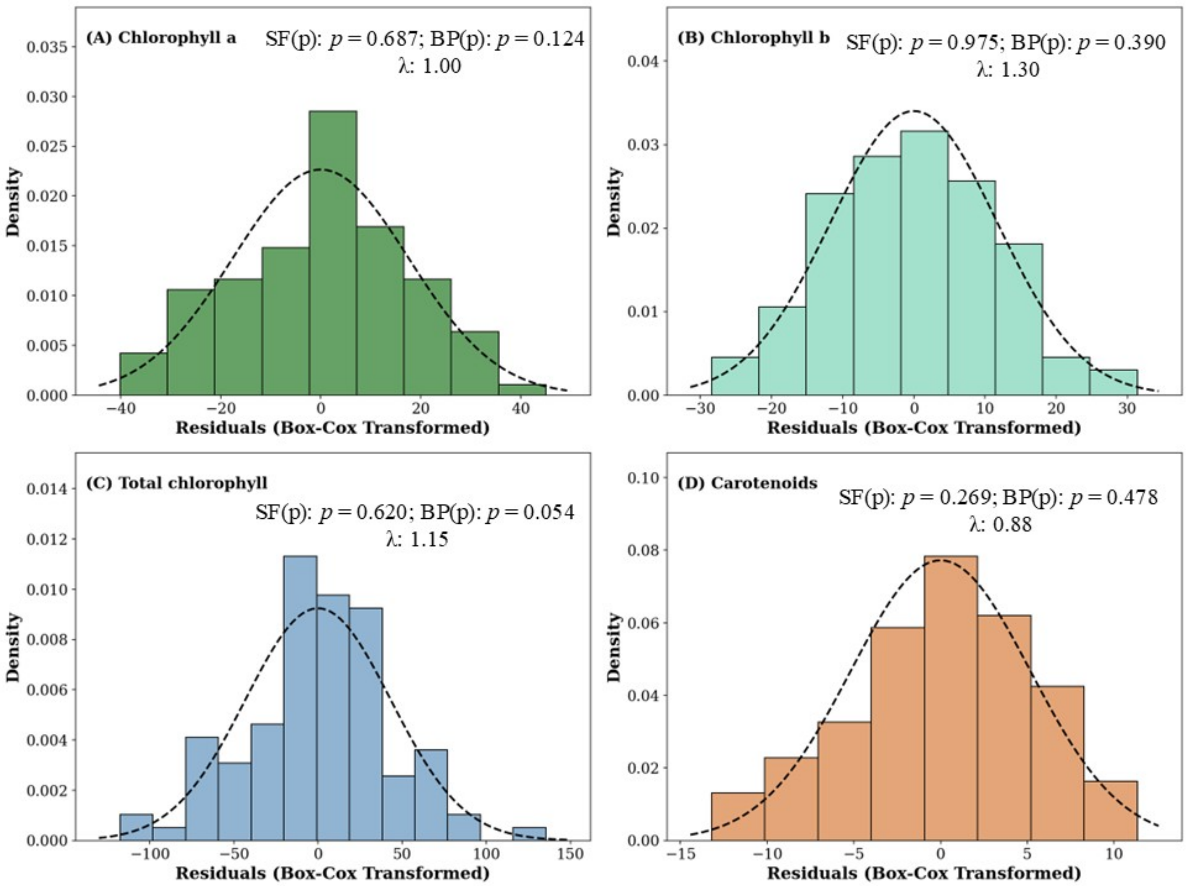
Once these assumptions were satisfied, linear and polynomial regression models were fitted, and the best-performing equation for each pigment was selected based on the highest adjusted coefficient of determination (R^2) and the significance of the F-test ($p < 0.05$). Pearson's correlation coefficients (r) were also computed to examine the strength and direction of the linear relationships, with interpretations based on Mukaka (2012). Additionally, the predictive ability of the models was quantified using the Ratio of Performance to Deviation (RPD), as classified by Williams and Sobering (1993). All statistical analyses, model fitting, and data visualizations were conducted in the Python programming environment using Spyder® software (version 6.0.7), primarily utilizing the statsmodels, scipy.stats, and pandas packages.

Results

Diagnostic evaluation of statistical assumptions, performed prior to model fitting, confirmed the compliance of the data with Ordinary Least Squares (OLS) requirements. Given the sample size ($n=100$), the Shapiro-Francia test was employed to assess residual normality. Because preliminary assessments detected slight deviations from normality, the Box-Cox transformation was applied to the dependent variables, as described by Fávero *et al*. (2021). Following this transformation, the residuals for all foliar pigment concentrations conformed to a normal distribution, with p-values ranging from 0.27 to 0.97. This normality pattern is further corroborated by the frequency distributions presented in the histograms (Figure 2).

Furthermore, homoscedasticity of the residuals was verified via the Breusch-Pagan test, as p-values remained consistently above 0.05 across all variables, satisfying the assumption of

Figure 2. Density histograms of residuals following Box-Cox transformation for leaf pigment concentrations in *Mikania laevigata* in Piracicaba (SP), University of São Paulo (ESALQ/USP), 2024: (A) Chlorophyll a; (B) Chlorophyll b; (C) Total chlorophyll; and (D) Carotenoids. Dashed lines represent the theoretical normal distribution. Individual λ values and p-values for Shapiro-Francia (SW - normality) and Breusch-Pagan (BP- homoscedasticity) tests are displayed within each panel.



Source: authors (2024).

constant error variance across the ClorofiLOG® index range. The calculated λ values were close to unity for chlorophyll *a* (λ=1.041), chlorophyll *b* (λ=1.031), and total chlorophyll (λ=1.153), which characterizes an inherently linear relationship between the optical index and foliar pigment concentrations (FÁVERO *et al*., 2021).

On the other hand, in terms of descriptive analysis, variation in leaf pigment concentrations of *M. laevigata* was observed at 60 DAT (Table 1). The highest mean concentration, expressed in micrograms per gram of fresh weight ($\mu g \cdot g^{-1}$ FW), occurred for total chlorophyll, followed by chlorophyll *a*, chlorophyll *b*, and carotenoids. Coefficients of variation were relatively low for chlorophyll *a* (15.0 %) and the ClorofiLOG® index (12.9 %), reflecting greater data homogeneity, while higher data dispersion was found for chlorophyll *b* (32.3 %) and carotenoids (22.6 %) (Table 1).

Positive and significant associations ($p<0.01$) were identified between the ClorofiLOG® index and the evaluated foliar pigments (Table 2). The strongest correlations occurred for total chlorophyll (r=0.93) and chlorophyll *b* (r=0.92), followed by chlorophyll *a* (r=0.82) and carotenoids (r=0.71). These coefficients confirm that increases in the non-destructive ClorofiLOG® index correspond

Table 1. Descriptive analysis of ClorofiLOG® Index (before Box-Cox transformation), chlorophyll, and carotenoid values ($n = 100$) in medium leaves of *Mikania laevigata* at 60 DAT (vegetative stage) under greenhouse conditions at Piracicaba (SP), University of São Paulo (ESALQ/USP), 2024.

Parameters	ClorofiLOG(1)	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids
Average	35.11	207.02	85.04	292.06	53.46
Maximum	44.57	274.00	145.05	405.08	86.00
Minimum	23.81	132.60	10.31	151.28	26.40
Standard deviation	4.54	30.98	27.45	54.46	12.07
CV (%)	12.90	15.00	32.30	18.60	22.60

(1)ClorofiLOG® Index; CV(%): Coefficient of variation in percentage values.

Source: authors (2024).

consistently to higher concentrations of these compounds. According to the interpretative scale proposed by Mukaka (2012), the associations between the ClorofiLOG® index and chlorophyll pigments are categorized within the "very high" correlation range ($r > 0.90$), whereas the association with carotenoids is classified as "high" ($0.70 < r < 0.90$). Strong internal correlations were also verified among the chlorophyll pigments, particularly between total chlorophyll and chlorophyll *a* ($r = 0.94$), a pattern related to the shared spectral absorption bands in their spectrophotometric quantification

Regarding the regression analysis, the linear model provided the best fit and high statistical significance ($p < 0.001$) for all evaluated pigments. The adjusted coefficients of determination (R^2) were notably high for total chlorophyll (0.88), chlorophyll *b* (0.86), and chlorophyll *a* (0.68), indicating that the ClorofiLOG® index accounts for a substantial portion of the variability in these pigment concentrations. For carotenoids, the R^2 was relatively lower (0.51), suggesting a weaker, yet still highly significant, relationship. These results, in turn, were supported by the Box-Cox transformation, which confirmed the suitability of the linear approach by meeting all residual normality and homoscedasticity assumptions.

Thus, model predictive capacity was further evaluated using the Ratio of Performance to Deviation (RPD) (Figure 4). The models

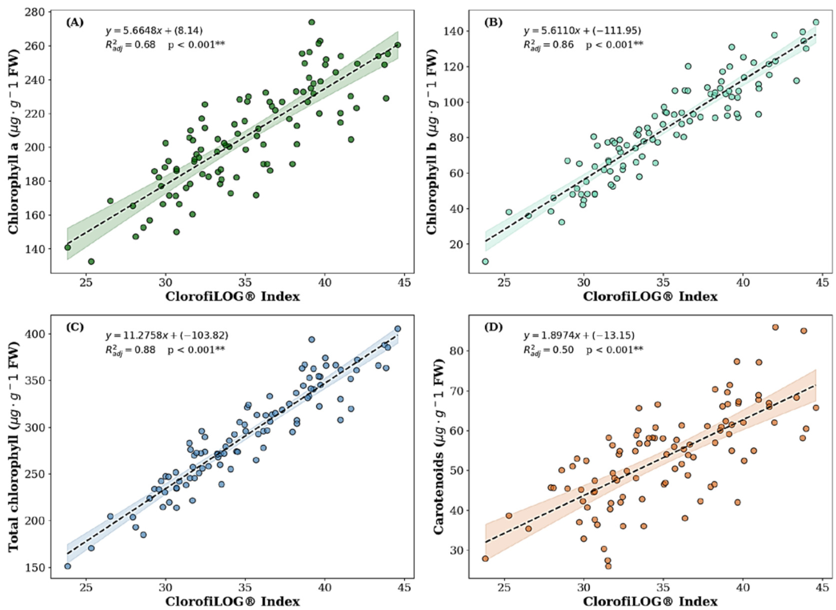
Table 2. Pearson's correlation matrix among ClorofiLOG® Index (before Box-Cox transformation), chlorophylls, and carotenoids (n = 100) in medium leaves of *Mikania laevigata* at 60 DAT (vegetative stage) under greenhouse conditions at Piracicaba (SP), University of São Paulo (ESALQ/USP), 2024.

	ClorofiLOG(1)	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids
ClorofiLOG(1)	1.00	--	--	--	--
Chlorophyll *a*	0.82**	1.00	--	--	--
Chlorophyll *b*	0.92**	0.73**	1.00	--	--
Total chlorophyll	0.93**	0.94**	0.92**	1.00	--
Carotenoids	0.71**	0.63**	0.62**	0.66**	1.00

(1)ClorofiLOG® Index; $p < 0.05$; ** $p < 0.01$; NS = not significant.

Source: authors (2024).

Figure 3. Linear regressions between the ClorofiLOG® index and leaf pigment contents (n = 100) in medium leaves of *Mikania laevigata* in Piracicaba (SP), University of São Paulo (ESALQ/USP), 2024 : (A) Chlorophyll *a*; (B) Chlorophyll *b*; (C) Total chlorophyll ; and (D) Carotenoids. Dashed lines represent the fitted models with 95 % confidence bands around the predictions.



Source: authors (2024).

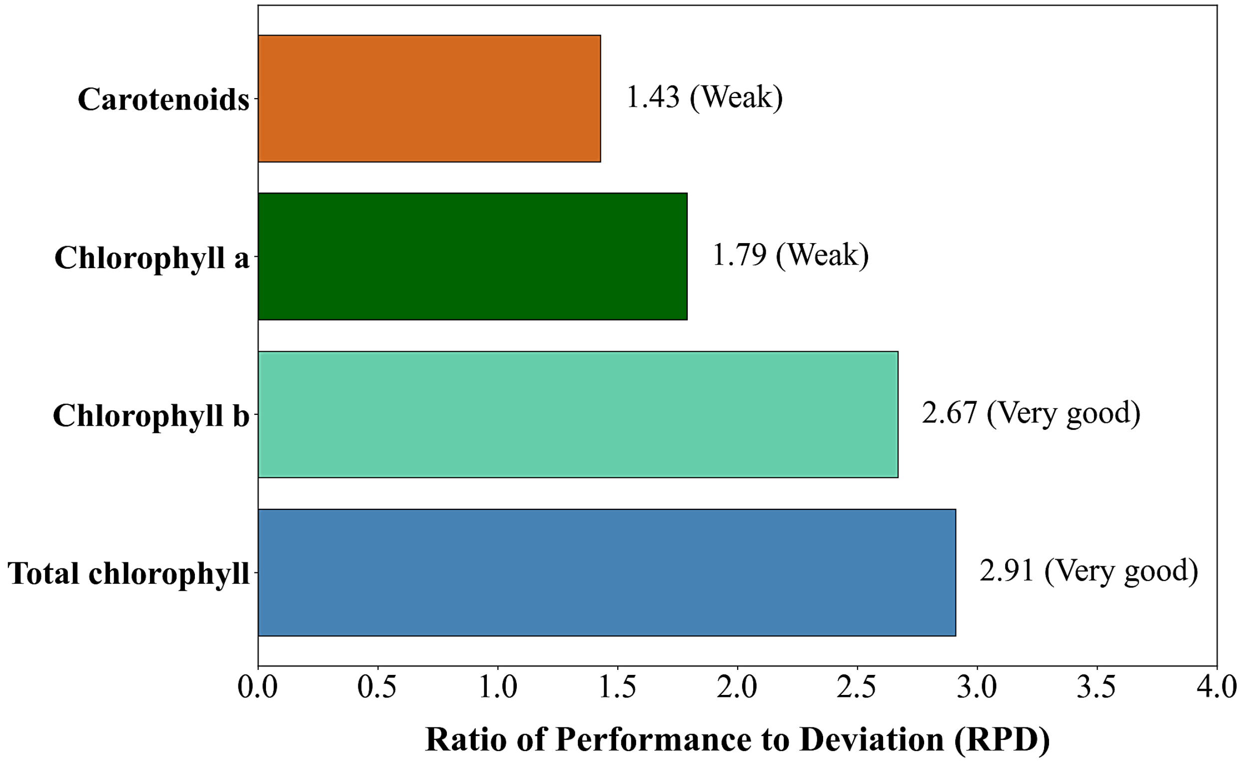
achieved a "very good" predictive classification for total chlorophyll (2.91) and chlorophyll *b* (2.67), based on the criteria of Williams and Sobering (1993), confirming their suitability for non-destructive estimation of these pigments. Conversely, lower RPD values were obtained for chloropyll *a* (1.79) and carorenoids (1.43), indicating limited predictive performance for these variables under the evaluated conditions. These parameters validate the applicability of the ClorofiLOG® index as a non-invasive tool, particularly for estimating total foliar chlorophyll.

Discussion

In addition to the absence of an algorithm capable of directly converting ClorofiLOG® index values into actual chlorophyll concentrations, the use of this equipment is further constrained by plant-specific anatomical and physiological characteristics. Factors such as leaf internal structure, water content, and the distribution of foliar pigments Factors such as leaf internal structure, water content, and the heterogeneous distribution of foliar pigments can significantly limit the extrapolation of results to different species and environmental conditions. This phenomenon is often attributed to the "sieve effect", where light passes through areas of the mesophyll with low chlorophyll density, and the "detour effect", caused by light scattering within the leaf tissue, which underscores the necessity of preliminary calibration to ensure measurement accuracy for specific species (PARRY *et al*., 2014; VILLA e VILA *et al*., 2022).

In this context, the interpretation of the present findings may be shaped by both the spectral characteristics of the evaluated pigments and the underlying operating principles of the ClorofiLOG® sensor. This device is presumed to operate based on the differential transmittance of light at two wavelengths: red light (660 nm), which is absorbed by chlorophyll pigments, and near-infrared light (940 nm), which is not absorbed by chlorophylls and thus functions as a reference signal (WELLBURN, 1994; ZHANG

Figure 4. Relationship between the ClorofiLOG® Index and the predictive performance of fitted models for foliar pigments in *Mikania laevigata* at 60 DAT, expressed by the Ratio of Performance to Deviation (RPD), in Piracicaba (SP), University of São Paulo (ESALQ/USP), 2024.



Classification according to Williams and Sobering (1993): RPD < 1.0 = unacceptable; 1.0 ≤ RPD < 1.4 = very weak; 1.4 ≤ RPD < 1.8 = weak; 1.8 ≤ RPD < 2.0 = acceptable; 2.0 ≤ RPD < 2.5 = good; 2.5 ≤ RPD < 3.0 = very good; RPD ≥ 3.0 = excellent.

Source: authors (2024).

et al., 2022). Although chlorophyll *a* is the main pigment directly involved in photochemical processes, chlorophyll *b*, located primarily in the light-harvesting complexes of thylakoid membranes, may exhibit proportionally greater absorption in the 640 – 660 nm spectral range. This theory could indicate a stronger contribution of chlorophyll *b* to the optical response captured by the sensor, thereby helping to explain the higher coefficient of determination (R^2) observed between this pigment and the observed ClorofiLOG® readings (Figure 3) (TAIZ *et al*., 2017).

Moreover, this spectral pattern may partially account for the stronger associations observed between the ClorofiLOG® index and the concentrations of chlorophyll *b* and total chlorophyll, when compared to the other evaluated pigments (Table 2). This difference is likely related to the fact that carotenoids absorb light predominantly in the blue spectral region (400–500 nm), which lies outside the detection range of the ClorofiLOG® device (WELLBURN, 1994; ZHANG *et al*., 2022). Similar results were reported by Wenneck *et al*. (2021) in Green pea (*Pisum sativum* L.), in which SPAD-type sensors also demonstrated stronger correlations with chlorophyll *b* than with chlorophyll *a* and carotenoids. Therefore, as suggested by Zhang *et al*. (2022), these findings may reflect the intrinsic optical sensitivity of such devices to chlorophyll *b*, as well as its specific distribution within the mesophyll tissue present in leaves.

However, the statistical approach adopted for model fitting also appears to affect the interpretation of pigment estimation. In the present study, linear models performed satisfactorily, in agreement with previous studies that have emphasized the widespread use and effectiveness of linear regression for this purpose (NASCIMENTO *et al*., 2021; SHIBAEVA *et al*., 2020). By contrast, Wenneck *et al*. (2021) reported a superior fit for polynomial regression models. Such divergence may be associated with differences in sample size (*n*) and biological variability, as polynomial models may be more suitable for capturing nonlinear trends in smaller datasets, particularly those comprising fewer than 50 observations (FÁVERO *et al*., 2021), whereas linear models may better reflect the intrinsic logarithmic response behavior of SPAD and ClorofiLOG® based measurements in larger-scale studies.

For example, unlike the pattern observed in the present study, Villa e Vila *et al*. (2022) reported an inverse relationship between SPAD values and chlorophyll *a* concentration in okra leaves (*Abelmoschus esculentus* L.). This response may be linked to species-specific morphological characteristics or to potential saturation effects in thicker or senescent leaves, as previously discussed Manetas *et al*. (1998). Such evidence underscores the need to standardize sampling procedures in order to ensure greater consistency and reliability in measurements obtained with non-destructive optical sensors r (TAIZ *et al*., 2017).

Therefore, from a practical perspective, non-destructive tools like the ClorofiLOG® sensor may aid in monitoring chlorophyll content and assessing the physiological status of medicinal plants. Increasing evidence suggests that chlorophyll dynamics, as part of primary metabolism, are closely linked to secondary metabolite synthesis (WANG *et al*., 2025; VISHWAKARMA *et al*., 2022). In *M. laevigata*, Souza *et al*. (2025a) and Souza *et al*. (2025b) found significant associations between chlorophyll levels and the accumulation of flavonoids and coumarins, the plant's main bioactive compounds (GASPARETTO *et al*., 2010). Consequently, leaf chlorophyll content can be employed as a non-invasive proxy for inferring phytochemical potential. Nevertheless, because this study was conducted under greenhouse conditions during a single vegetative stage, the extrapolation of these models to open-field environments and different phenological phases requires further validation.

Conclusions

The ClorofiLOG® index had strong potential as a non-destructive tool for estimating foliar pigment content in *Mikania laevigata*, particularly for total chlorophyll and chlorophyll *b*. The high correlation coefficients and predictive metrics obtained could validate the application of this optical sensor as an efficient indicator of plant physiological status during the vegetative stage. Nonetheless, because the calibration was developed in a controlled greenhouse environment during a single developmental phase, direct application under broader field conditions requires complementary assessments. Future research should investigate the integration of spectral and biochemical parameters across distinct environmental scenarios to enhance predictive accuracy and assist crop management in this medicinal species.

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References

ALVARES, C.A.; STAPE, J.L.; SENTELHAS, P.C.; GONÇALVES, J.L.M.; SPAROVEK, G. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, n. 6, p. 711–728, 2014. Available at: https://doi.org/10.1127/0941-2948/2013/0507. Accessed on: Nov. 15, 2025.

ALVES, L.; DESCHAMPS, C. Effect of nitrogen doses on morphological parameters and content of cumarina in accesses of *Mikania glomerata* Sprengel (guaco). **Revista de Ciências Agroveterinárias**, v. 24, n. 2, p. 276–288, 2025. Available at: https://doi.org/10.5965/223811712422025276. Accessed on: Nov. 15, 2025.

AZEVEDO, S. G. D.; OLIVEIRA, L. P. H.; MANZALI, S. I.; CAR, S. A. **Fitoterapia contemporânea:** tradição e ciência na prática clínica. 2nd ed. Rio de Janeiro: Guanabara Koogan, 2018. p. 286–289.

FÁVERO, L.; SOUZA, R.; BELFIORE, P.; CORRÊA, H.; HADDAD, M. F. Count Data Regression Analysis: Concepts, Overdispersion Detection, Zero-inflation Identification, and Applications with R. **Practical Assessment, Research, and Evaluation**, v. 26, n. 1, p. 13, 2021. Available at: https://doi.org/10.7275/44nn-cj68. Accessed on: Apr. 11, 2026.

FLOR, I. C.; RODRIGUES, A. R.; SILVA, S. A.; PROENÇA, B.; MAIA, V. C. Insect galls on Asteraceae in Brazil: richness, geographic distribution, associated fauna, endemism and economic importance. **Biota Neotropica**, v. 22, e20211234, 2022. Available at: https://doi.org/10.1590/1676-0611-bn-2021-1234. Accessed on: Nov. 16, 2025.

GASPARETTO, J. C.; CAMPOS, F. R.; BUDEL, J. M.; PONTAROLO, R. *Mikania glomerata* Spreng. e *M. laevigata* Sch. Bip. ex Baker, Asteraceae: estudos agronômicos, genéticos, morfoanatômicos, químicos, farmacológicos, toxicológicos e uso nos programas de fitoterapia do Brasil. **Revista Brasileira de Farmacognosia**, v. 20, p. 627–640, 2010. Available at: https://doi.org/10.1590/s0102-695x2010000400025. Accessed on: Nov. 16, 2025.

HAWKINS, T. S.; GARDINER, E. S.; COMER, G. S. Modeling the relationship between extractable chlorophyll and SPAD-502 readings for endangered plant species research. **Journal of Nature Conservation**, v. 17, p. 123–127, 2009. Available at: https://doi.org/10.1016/j.jnc.2008.08.001. Accessed on: Nov. 16, 2025.

HISCOX, J. D.; ISRAELSTAM, G. F. A method for the extraction of chlorophyll from leaf tissue without maceration. **Canadian Journal of Botany**, v. 57, n. 12, p. 1332–1334, 1979. Available at: https://doi.org/10.1139/b79-163. Accessed on: Nov. 15, 2025.

MANETAS, Y.; GRAMMATIKOPOULOS, G.; KYPARISSIS, A. The use of the portable, non-destructive, SPAD-502 (Minolta) chlorophyll meter with leaves of varying trichome density and anthocyanin content**. Journal of Plant Physiology**, v. 153, p. 513–516, 1998. Available at: https://doi.org/10.1016/S0176-1617(98)80265-9. Accessed on: Nov. 20, 2025.

MUKAKA, M. M. Statistics corner: a guide to appropriate use of correlation coefficient in medical research. **Malawi Medical Journal**, v. 24, n. 3, p. 69–71, 2012. Available at: https://doi.org/10.4236/jwarp.2015.77047. Accessed on: Nov. 15, 2025.

NASCIMENTO, G. H.; MORAES, L. C.; MOREIRA, S. G.; MARTINS, F. A. D.; PIMENTEL, G. V. Fertilization factor associated with nitrogen sufficiency index for nitrogen topdressing fertilization in common bean. **Pesquisa Agropecuária Tropical**, v. 51, e69160, 2021. Available at: https://doi.org/10.1590/1983-40632021v5169160. Accessed on: Nov. 20, 2025.

PADILLA, F. M.; GALLARDO, M.; PEÑA-FLEITAS, M. T.; DE SOUZA, R.; THOMPSON, R. B. Proximal optical sensors for nitrogen management of vegetable crops: a review. **Sensors,** v. 18, n. 2083, 2018. Available at: https://doi.org/10.3390/s18072083. Accessed on: Nov. 20, 2025.

PARRY, C.; BLONQUIST JR, J. M.; BUGBEE, B. In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. **Plant, Cell & Environment**, v. 37, n. 11, p. 2508–2520, 2014. Available at: https://doi.org/10.1111/pce.12324. Accessed on: Apr. 11, 2026.

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. D. S.; GOLIAS, H. C.; AZEVEDO, J. L.; PAMPHILE, J.A. 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**, v. 64, 103530, 2025. Available at: https://doi.org/10.1016/j.bcab.2025.103530. Accessed on: Nov. 16, 2025.

SHIBAEVA, T. G.; MAMAEV, A. V.; SHERUDILO, E. G. Evaluation of a SPAD-502 Plus chlorophyll meter to estimate chlorophyll content in leaves with interveinal chlorosis. **Russian Journal of Plant Physiology**, v. 67, p. 690–696, 2020. Available at: https://doi.org/10.1134/S1021443720040203. Accessed on: Nov. 16, 2025.

SINGH, P. A.; BAJWA, N.; CHINNAM, S.; CHANDAN, A.; BALDI, A. An overview of some important deliberations to promote medicinal plants cultivation. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 31, 100400, 2022. Available at: https://doi.org/10.1016/j.jarmap.2022.100400. Accessed on: Nov. 16, 2025.

SOUZA, J. O.; OLIVEIRA, E. F.; SANTOS, M. E. S.; KIRSTEN, C. N. *Mikania glomerata* Spreng (Asteraceae): its therapeutic use and potential in the COVID-19 pandemic. **Revista Fitos**, v. 16, p. 270–276, 2022. Available at: https://doi.org/10.32712/2446-4775.2022.1292. Accessed on: Nov. 16, 2025.

SOUZA, M. E. A.; DIAS, V. O.; RODRIGUES, P. H. V.; ALTIZANI-JÚNIOR, J. C.; ZAMARRENHO, L. G.; DE LIMA, J. A.; BERRETA, A. A. Colored shade nets and foliar ProLyks® application modulate plant growth and secondary metabolism in *Mikania laevigata* Sch. Bip. ex-Baker. **Research, Society and Development**, v. 14, n. 9, e4514949425, 2025a. Available at: https://doi.org/10.33448/rsd-v14i9.49525. Accessed on: Nov. 16, 2025.

SOUZA, M. E. A.; DIAS, V. O.; RODRIGUES, P. H. V.; ALTIZANI-JÚNIOR, J. C.; AMBROSANO, G. B.; ZAMARRENHO, L. G.; DE LIMA, J. A.; BERRETTA, A. A. Spectral light quality and D-limonene modulate plant growth and pharmacological compounds in Mikania laevigata Sch. Bip. ex Baker. **Preprints**, v. 2025, 2025061461, 2025b. Available at: https://doi.org/10.20944/preprints202506.1461.v1. Accessed on: Nov. 20, 2025.

TAIZ, L.; ZEIGER, E.; MØLLER, I. M.; MURPHY, A. **Plant physiology and development.** 6th ed. Sunderland, MA, USA: Sinauer Associates, Inc., 2017. 761p.

VILLA e VILA, V.; WENNECK, G. S.; TERASSI, D. S.; REZENDE, R.; SAATH, R. SPAD index as an indirect estimate of chlorophyll content in okra plants. **Revista de Agricultura Neotropical**, v. 9, n. 2, e6787, 2022. DOI: https://doi.org/10.32404/rean.v9i2.6787. Accessed on: Nov. 15, 2025.

VISHAWKARMA, M.; KULHARE, P. S.; TAGORE, G. S. Estimation of chlorophyll using SPAD meter. **International Journal of Environment and Climate Change**, v. 13, n. 11, p. 1901–1912, 2023. Available at: https://doi.org/10.9734/ijecc/2023/v13i113348. Accessed on: Nov. 20, 2025.

WANG, Y.; GUAN, T.; YUE, X.; YANG, J.; ZHAO, X.; CHANG, A.; YANG, C.; FAN, Z.; LIU, K.; LI, Y. The biosynthetic pathway of coumarin and its genetic regulation in response to biotic and abiotic stresses. **Frontiers in Plant Science**, v. 16, 1599591, 2025. Available at: https://doi.org/10.3389/fpls.2025.1599591. Accessed on: Nov. 16, 2025.

WELLBURN, A. R. 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**, v. 144, n. 3, p. 307–313, 1994. Available at: https://doi.org/10.1016/S0176-1617(11)81192-2. Accessed on: Nov. 15, 2025.

WENNECK, G. S.; VILLA E VILA, V.; SAATH, R.; REZENDE, R.; LOPES PEREIRA, G.; CONTI MONDANEZ, B. M. Leaf pigments in pea crop: relationship between spad index and chlorophyll Content. **Revista Brasileira de Engenharia de Biossistemas**, v. 15, n. 3, p. 391–400, 2021. Available at: https://doi.org/10.18011/bioeng2021v15n3p391-400. Accessed on: Nov. 15, 2025.

WILLIAMS, P. C.; SOBERING, D. C. Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds. **Journal of Near Infrared Spectroscopy**, v. 1, n. 1, p. 25–32, 1993. Available at: https://doi.org/10.1255/jnirs.3. Accessed on: Nov. 15, 2025.

ZHANG, R.; YANG, P.; LIU, S.; WANG, C.; LIU, J. Evaluation of the methods for estimating leaf chlorophyll content with SPAD chlorophyll meters. **Remote Sensing**, v. 14, n. 20, 5144, 2022. Available at: https://doi.org/10.3390/rs14205144. Accessed on: Nov. 16, 2025.