Colegio de Postgraduados

Assessment of *Hemileia vastatrix* Severity in *Coffea arabica* L., using Image Analysis (Pliman)

Enríquez-López, Sandra Lizveth[1]; Alvarado-Castillo, Gerardo[1*]; Cerdán-Cabrera, Carlos Roberto[1]; Argumedo-Delira, Rosalba[2]; Escamilla-Prado, Esteban[3]

[1] Universidad Veracruzana, Facultad de Ciencias Agrícolas, Doctorado en Ciencias Agropecuarias. Xalapa, Xalapa-Enríquez, Veracruz, México. C. P. 91090.

[2] Universidad Veracruzana, Instituto de Química Aplicada, Xalapa, Xalapa-Enríquez, Veracruz, México. C. P. 91190.

[3] Universidad Autónoma Chapingo, Centro Regional Universitario Oriente, Huatusco, Huatusco, Veracruz, México. C. P. 94100.

* Correspondence: gealvarado@uv.mx

ABSTRACT

Objective: To determine the severity index of *Hemileia vastatrix* on coffee leaves using the Pliman package in R Studio, aiming to optimize quantitative evaluations and improving disease monitoring.

Methodology: Sampling was conducted at Los Barreales farm located in Teocelo, Veracruz, where leaves from the Catuaí amarillo coffee variety, both infected by *Hemileia vastatrix* and healthy, were selected. The leaves were photographed to capture the leaf surface, and the images were subsequently processed in Photoshop to calculate the healthy and rust-affected areas. Subsequently, the RGB color index of the images was analyzed using the Pliman package in R Studio. Various indices were evaluated, and the NGRDI was selected for the automatic quantification of affected and healthy areas. Finally, an analysis of variance (ANOVA), followed by Tukey's test, was performed to compare significant differences among the samples.

Results: Indices based on specific combinations of RGB colors effectively highlighted subtle differences in leaf reflectance, facilitating the detection of disease symptoms, particularly with the NGRDI index.

Study Limitations: While reflectance spectroscopy is highly accurate, it can be expensive and requires specialized equipment.

Conclusions: Computational tools offer precise and rapid disease detection, providing critical support for integrated pest and disease management strategies in agriculture.

Keywords: Leaf disease, rust, image processing.

Citation: Enríquez-López, S. L., Alvarado-Castillo, G., Cerdán-Cabrera, C. R., Argumedo-Delira, R., & Escamilla-Prado, E. (2025). Assessment of *Hemileia vastatrix* Severity in Coffea arabica L., using Image Analysis (Pliman). *Agro Productividad*. https://doi.org/10.32854/agrop.v18i2.3252

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: November 10, 2024.
Accepted: January 05, 2025.
Published on-line: March XX, 2025.

Agro Productividad, 18(2). February. 2025. pp: 149-158.

INTRODUCTION

Coffee (*Coffea arabica* L.) is one of the most important beverages worldwide and a crop of significant commercial, economic, and social value (Gichuru *et al.*, 2012). However, coffee production has been severely affected by coffee leaf rust (*Hemileia vastatrix* Berkeley and Broome), which is considered the primary disease causing substantial economic losses (Cabral *et al.*, 2009; Gichuru *et al.*, 2012; Alvarado-Castillo *et al.*, 2017). The main symptoms include small yellow spots on the underside of the leaves, whose appearance is influenced by climatic conditions, facilitating the sporulation and dispersal of uredospores, eventually leading to plant death (Huaman, 2021; Muttappagol *et al.*, 2022).

For early disease detection, standard area diagrams based on visual estimations have been developed, allowing for the assessment of disease severity and serving as crucial tools for decision-making to identify the most effective treatments (Barbedo, 2014). However, these visual estimations can be subjective and depend on the observer's experience, potentially leading to inaccuracies (Avelino *et al.*, 2015).

In an effort to standardize visual estimations, the National Coffee Research Center (CENICAFÉ) developed diagrams of rust-affected areas for technical assistants and coffee growers in Colombia (López-Vásquez *et al.*, 2018). In Mexico, the National Service for Agrifood Health, Safety, and Quality (SENASICA) established a seven-level evaluation scale ranging from 0 to 6, with damage percentages varying between 0% and 70% (Calderón, 2016). Recently, plant disease assessment has advanced toward the use of digital image analysis, enabling faster processes and more accurate quantitative evaluations (Mutka & Bart, 2015; Bock *et al.*, 2020; Gallego-Sánchez *et al.*, 2020).

Reliable and accurate estimations are essential for predicting yield loss, forecasting epidemics, evaluating crop resistance to diseases, and understanding the biological processes of pathogens. Some of the software tools used for these evaluations include ImageJ with the rust script (Schneider *et al.*, 2012), Quant (Bock *et al.*, 2022), and R Studio with the Pliman package, which estimates rust severity in crops such as wheat and soybean (Olivoto *et al.*, 2022). These programs are based on the analysis of RGB (red, green, and blue) values from digital photographs to distinguish plant diseases by quantitatively differentiating symptomatic areas from healthy ones (Bock *et al.*, 2020). However, there is no literature reporting the severity analysis of coffee rust.

The objective of this study was to determine the severity index of *H. vastatrix* on coffee leaves using the Pliman package in R Studio, with the aim of improving quantitative evaluations and enhancing disease monitoring in susceptible varieties.

MATERIALS AND METHODS

Study Area

The sampling was conducted at Los Barreales farm, located in the municipality of Teocelo, Veracruz (19° 23' 37.3" N, 96° 59' 01.0" W; 19° 23' 39.7" N, 96° 59' 12.4" W and 19° 23' 34.0" N, 96° 59' 14.9" W; 19° 23' 29.5" N, 96° 58' 59.0" W).

Leaf Sampling and Photography

A total of 144 coffee leaves from the Catuaí amarillo variety were collected, consisting of 72 leaves infected by *H. vastatrix* and 72 healthy leaves. Photographs of the leaf surface were taken using a white sheet as a neutral background. A Sony A6400 camera was positioned 15 cm away vertically with the help of a tripod. The integrated flash was used, and a Sigma 30 mm lens was employed with the following settings: aperture f/9, exposure time 1/60 s, ISO 500, and focal length 30 mm.

Image Processing

An initial manual classification was performed using Adobe Photoshop to delineate the areas of healthy tissue (S), infected tissue (I), and background (F). Four validation stages were conducted with increasingly precise samples, aiming to determine the best estimation of tissue infected by *H. vastatrix* on coffee leaves.

First Validation (p1): A tissue sample of S, I, and F areas was selected from the image (Figure 1A). Second Validation (p2): 90 samples of healthy tissue, 54 samples of infected tissue, and 64 samples of the background were selected (Figure 1B). Third Validation (p3): 122 samples of healthy tissue, 74 samples of infected tissue, and 187 samples of the background were obtained (Figure 1C). Each sample set was combined into an independent image using Photoshop to calculate the healthy area and the asymptomatic area affected by rust on the coffee leaves (Figure 1).

The fourth stage (p4) involved the analysis of the RGB indices performed by the Pliman script, which makes estimations using the red, green, and blue colors with the following equation $(G-R)/(G+R)$ $(green-red)/(green+red)$ to highlight the infected tissue from the healthy tissue on coffee leaves (Olivoto *et al.*, 2022). It was observed that the HI indices reveal healthy tissue, NGRDI detects pustules, and NDGBI neither shows pustules nor healthy tissue (Figure 1D). Twenty-five indices are shown (Figure 2A) with different estimations for detecting *H. vastatrix* pustules, using different codes, from which NGRDI was selected due to being the most precise (Figure 2B). The infected leaf by *H. vastatrix* is shown (Figure 2C). Then, the selected code was analyzed with Pliman, which detected all pustules, as seen in Figure 2D in black. A threshold graph was created to define the accuracy of the NGRDI index in which the pustules of rust were best detected, and it was established at 0.045 (Figure 3).

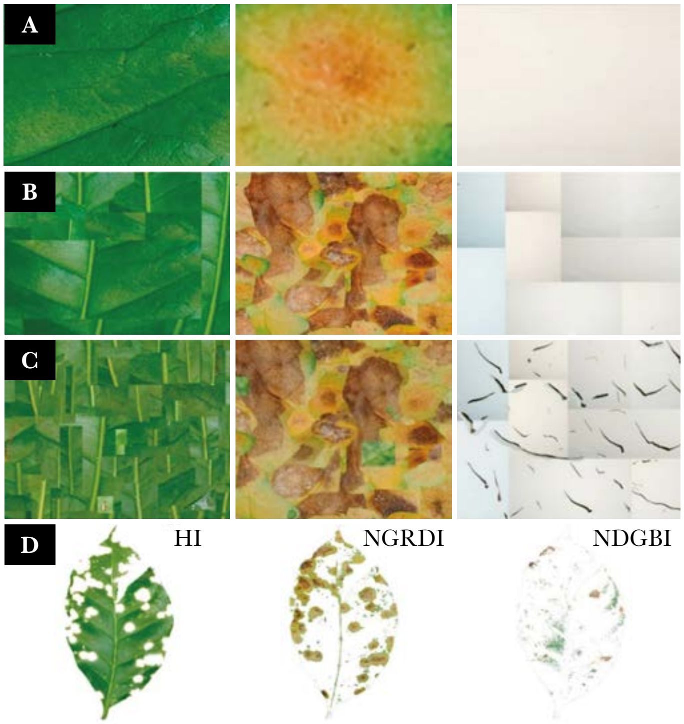

Figure 1. Sample images of healthy tissue, infected tissue, and background for analysis in Pliman.

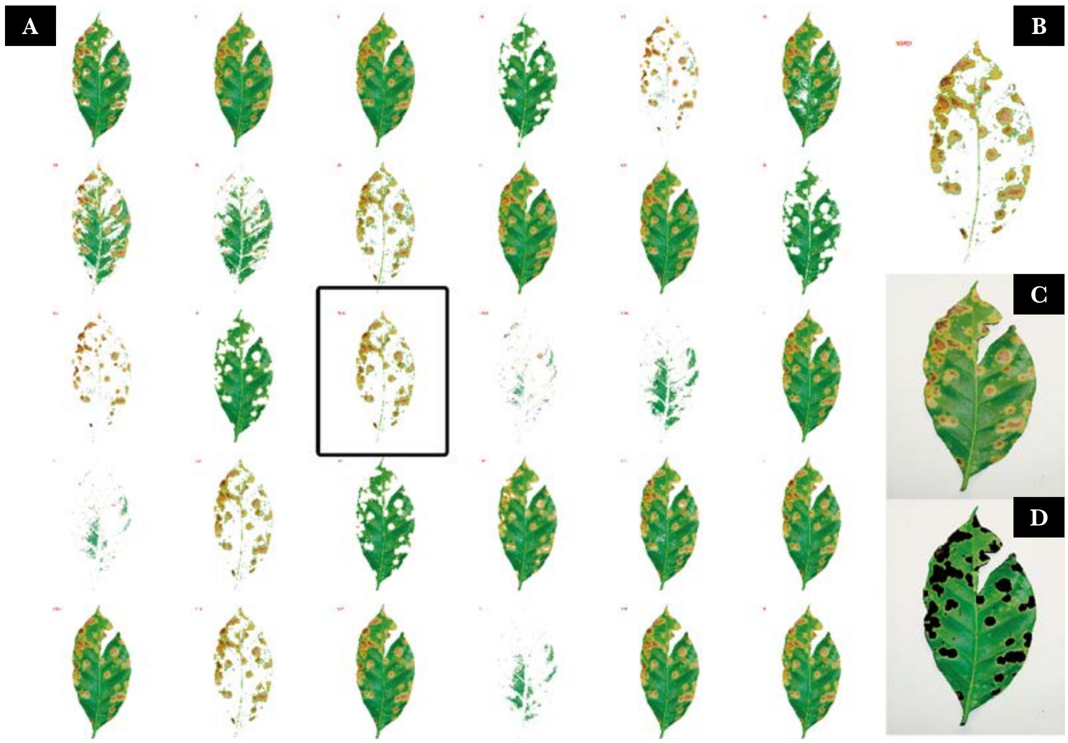

Figure 2. A) Comparison of different RGB indices, with the selected one for detecting *H. vastatrix* pustules on coffee leaves shown in the black box. B) The index that best detected the pustules was NGRDI. C) Original photograph of coffee pustules. D) Detection of the pustules with the selected code.

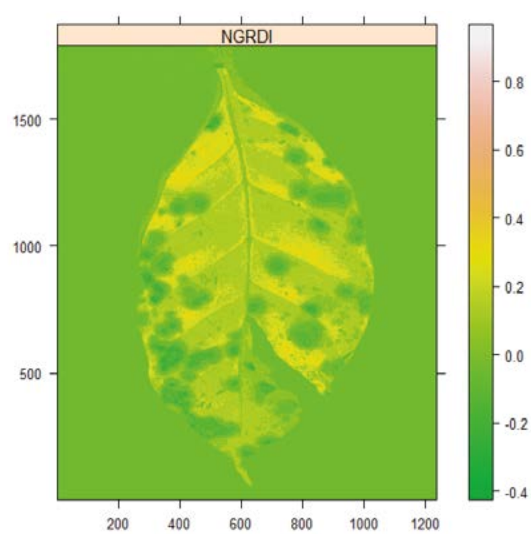

Figure 3. Color threshold based on RGB colors (Red, Green, and Blue) that allows the identification of *H. vastatrix* pustules with the highest accuracy.

The Pliman R package

This package is designed to perform various analyses on plant leaf photographs, including measuring area, counting objects in an image, calculating the symptomatic area of the disease, extracting RGB values for each object in an image using different color indices, and calculating object measurements (Olivoto *et al.*, 2022).

Pliman script (R Studio software) for *H. vastatrix*

Once the samples for healthy tissue (S), infected tissue (I), and background (F) were established, the selection of leaf areas was carried out automatically. The current stable version of the package (0.3.0) requires R 4.1.0 and can be installed directly through the R console using: install.packages, following the methodology of Olivoto *et al.* (2022).

Statistical analysis

An analysis of variance (ANOVA) and a Tukey's test were performed to compare significant differences between tests with a p-value <0.05 in R Studio to compare the different validation stages and precision of the results.

RESULTS AND DISCUSSION

Effectiveness of color indices in plant disease detection

The use of color indices for detecting the severity of *H. vastatrix* is relatively new. In this study, 25 indices based on RGB (Red, Green, and Blue) colors were obtained, and the NGRDI code was selected, as it detected the coffee rust pustules most effectively. According to Olivoto *et al.* (2022), indices based on specific combinations of RGB colors can highlight subtle differences in leaf reflectance, facilitating the detection of disease symptoms. In particular, the NGRDI index has proven to be effective in this study for highlighting infected tissue, which is consistent with other studies that have used vegetation indices to detect stress and diseases in plants (Rivadeneyra and Huamán, 2021). In other studies, NGRDI is used as part of a method to improve the detection of grassy weeds in rice fields (Barrero and Perdomo, 2018).

It is important to note that the severity analysis of *H. vastatrix* was carried out using the ImageJ program with the Rust script through the free Fiji image software, which is one of the most widely used programs for measuring rust pustules in wheat. It is also recognized in the scientific community because it allows the evaluation of diseases by transforming color using pixel-based threshold measurements to calculate leaf area in certain cereal crops (Gallego-Sánchez *et al.*, 2020). However, for this study, it was not effective, as it failed to detect small coffee rust pustules and also encountered difficulties in distinguishing leaves from their background when using a single threshold (Easlon and Bloom, 2014; Alheeti *et al.*, 2021). It is worth mentioning that each leaf (healthy and infected) was analyzed for infected tissue (Figure 4A; Figure 5A) and healthy tissue (Figure 4B; Figure 5B) to determine if the tests correctly detected the tissue. Both healthy leaves (Figure 4) and infected ones (Figure 5) showed that the p4 test was significantly different from the p1 test ($p<0.05$).

This could be due to the image crop captures of healthy tissue (S), infected tissue (I), and background (F). In the first stage (p1), only one sample was selected, compared to the third stage (p3), where 122, 74, and 187 image captures were obtained, respectively. It is worth noting that, although no significant differences were observed between p3 and p4, the latter does not require image samples of the different tissues, since the indices

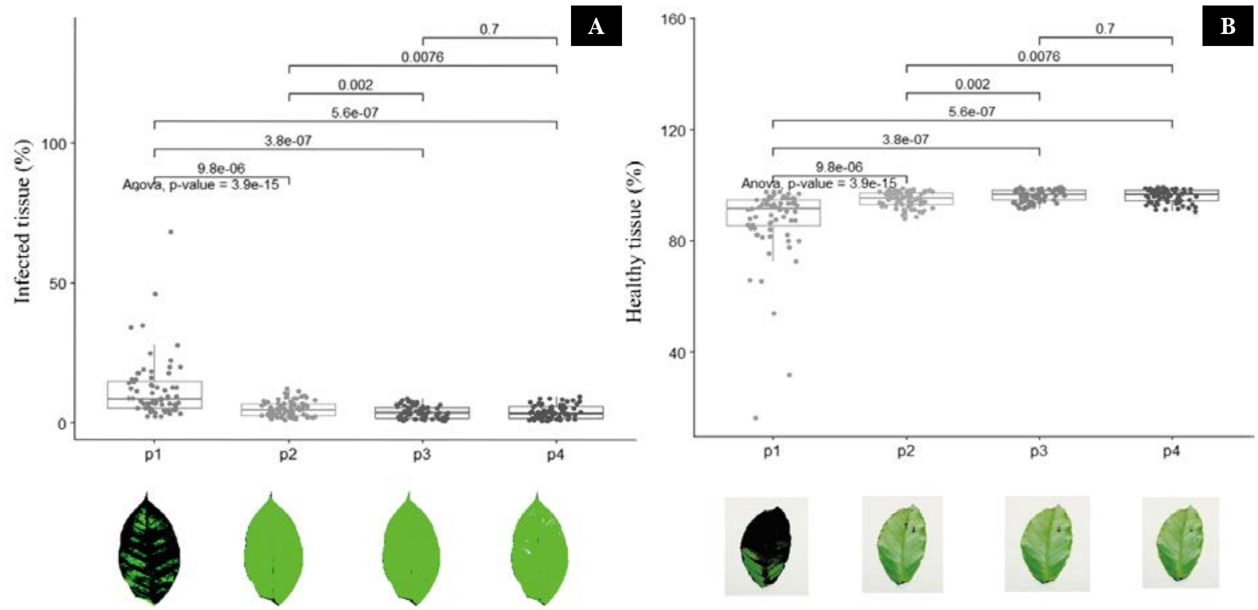

Figure 4. In healthy leaves, infected and healthy tissue are observed from the different training tests of Pliman (p1-p2-p3-p4). Significant differences between the tests are indicated at the top ($p<0.05$).

used by Pliman only consider the RGB values of a photograph to distinguish healthy and infected tissue. This allows for reduced working time, as the image processing can be done more quickly.

In the second test, p1 and p2 detected the veins as symptomatic, because *H. vastatrix* has a yellow coloration very similar to that of this tissue (Figure 5A), so this type of error is likely the reason for quantifying false positives, according to Barbedo (2014), who removed the petiole and veins of some coffee leaves using a two-pixel radius to prevent the algorithm from incorrectly counting the veins as symptomatic tissue. Therefore, it was necessary to take precise digital captures and include them in the sample image of healthy tissue, so that Pliman could perform a better analysis and the program could detect the symptoms of the coffee leaves more accurately. Similar phenomena have also been reported in the accuracy of manual disease severity assessments performed by individual evaluators, such as Leaf Doctor (Pethybridge and Nelson, 2015; Bock *et al.*, 2020). Thus, as the samples increase and parameters are optimized, it is consistent with the literature that suggests that a greater number of samples and parameter refinement improve the accuracy in disease detection.

The use of the Pliman package in R to automate image analysis is becoming increasingly common in plant pathology. Pliman allows for complex image analysis, such as measuring areas, counting objects, and extracting RGB values, in an efficient and precise manner. The methodology applied in this study demonstrates how computational tools can facilitate the accurate and rapid detection of diseases, which is essential for integrated pest and disease management in agriculture.

In p1, the affected area percentage ranges from 26.61% to 78.38%. This suggests that these leaves are severely affected by *H. vastatrix*. However, this result is inaccurate as

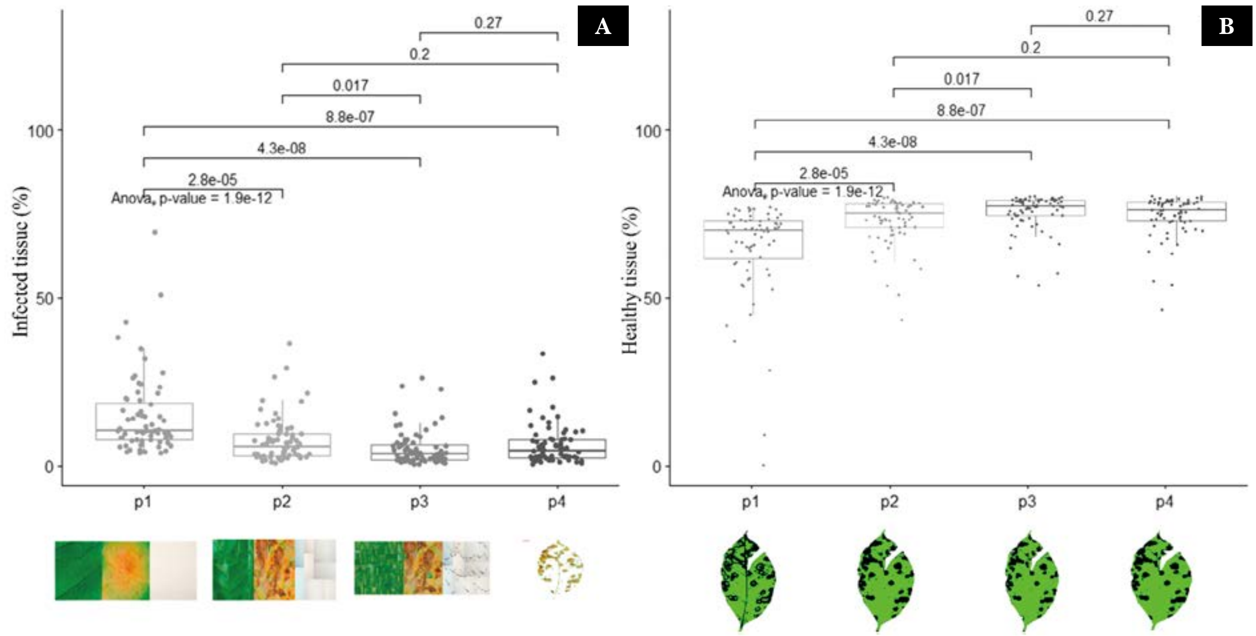

Figure 5. In coffee leaves infected by *H. vastatrix*, infected tissue and healthy tissue are observed from the different training tests of Pliman (p1-p2-p3-p4). Significant differences between the tests ($p<0.05$) are indicated at the top.

healthy tissue is detected as infected. In p2, the leaves show a lower percentage of infected tissue compared to the first test, with percentages ranging from 0.89% to 36.36%. In p3, the affected areas are smaller, with percentages ranging from 0.53% to 25.84%. In p4, the range is from 0.48% to 33.44% (Figure 6).

In the research, these classifications are generally derived from visual assessments and the use of infected area diagrams, which are used as evaluation tools; however, they lack accuracy, precision, and reliability. In coffee, visual estimation has been used (Melo *et al.*, 2020), and in Mexico, SAGARPA proposes seven classes in its diagrammatic scale for severity in leaves with a percentage less than 0% and greater than 70% (Calderón, 2016). In Colombia, CENICAFE has a series of 15 images for visual estimation, which ranges between 0.05% and 80.0%, assigning a class mark of midpoint points at 1.0%, 2.0%, 4.0%, 8.0%, 16.0%, 32.0%, and 64.0% severity (López-Vásquez *et al.*, 2018). In this study, six evaluations of the affected area were carried out in the four tests to detect the symptoms of *H. vastatrix*, and the most accurate evaluation was p4, with the highest percentage of 33.44% and the lowest at 0.48%.

Therefore, with this test, a reliable, quantitative, and precise evaluation is being conducted to detect the presence of *H. vastatrix* in coffee leaves, which can be reproducible for continued monitoring of rust in the field (Maloof *et al.*, 2013), determining the loss of leaves, fruit, and branch death, as well as plant death, improving the understanding of the disease's epidemiology, and evaluating the effect of treatments (Bock *et al.*, 2022). The method proposed by Pliman is ideal for situations where speed is important or when a large number of images must be processed. This package can improve the accuracy of *H. vastatrix* detection quantitatively and strengthen the disease monitoring in susceptible varieties.

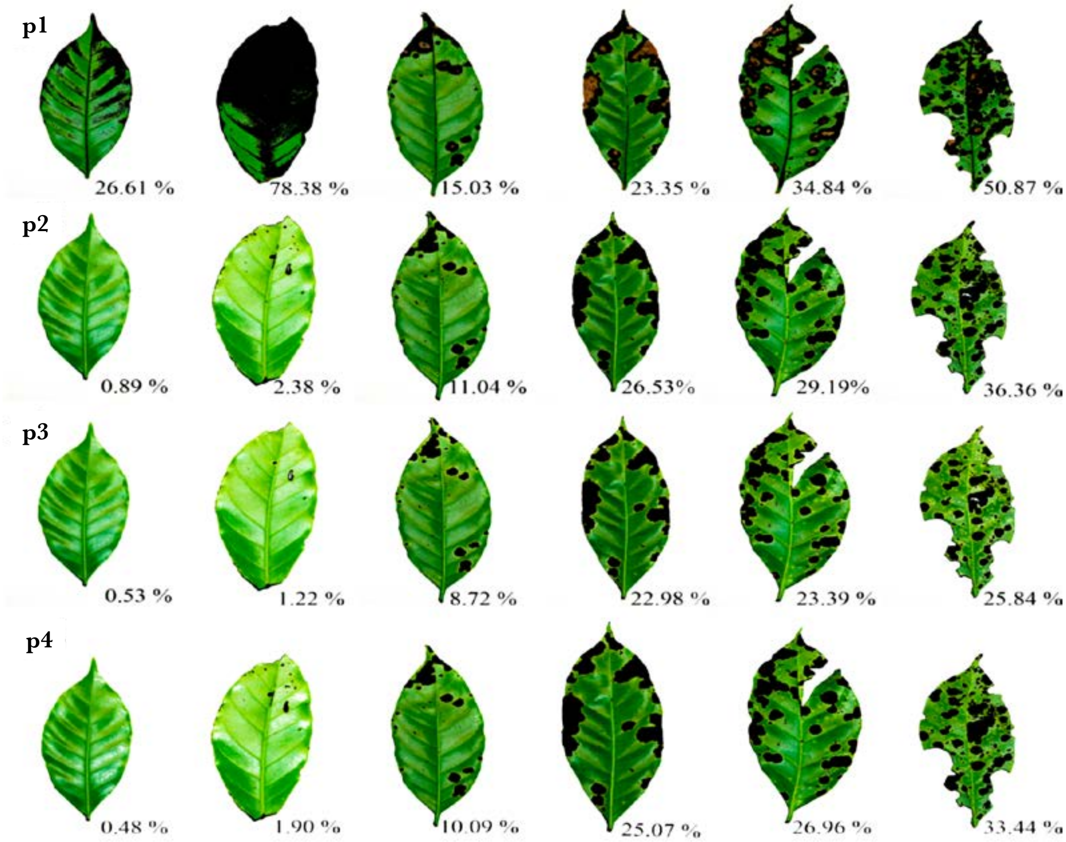

Figure 6. Diagram of the severity percentage of *H. vastatrix* in the different validation tests (p1-p4), comparing the accuracy of pustule detection.

Comparing this approach with traditional disease detection methods, such as visual inspection and reflectance spectroscopy, the methodology based on NGRDI and image analysis presents clear advantages in terms of speed, accuracy, and the ability to process large volumes of data. Visual inspection can be subjective and dependent on the observer's experience, while reflectance spectroscopy, although precise, can be expensive and requires specialized equipment.

Despite the positive results, it is important to consider some limitations. The methodology may be influenced by variations in lighting and environmental conditions during the image capture process. Additionally, while NGRDI was accurate in this study, its effectiveness may vary with other coffee varieties or plant species. Future research could focus on validating this index under different conditions and with other plant species to generalize its applicability.

CONCLUSIONS

The results obtained demonstrate that the NGRDI index is an effective tool for the detection of coffee rust, providing superior and objective precision in identifying infected areas. With the Pliman package, the severity of *H. vastatrix* in coffee leaves was assessed, and it was determined that the best validations were p3 and p4.

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