Agricultural Sciences, 2019, 10, 796-806
http://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Modeling of the Leaf Area of *Maytenus obtusifolia* Mart. from Scanned Images

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How to cite this paper: Oliveira, V. de S., dos Santos, K.T.H., Pinheiro, A.P.B., Santos, G.P., Santos, J.S.H., Chisté, H., Schmildt, O., Arantes, S.D., Czepak, M.P., Fernandes, A.A. and Schmildt, E.R. (2019) Modeling of the Leaf Area of *Maytenus obtusifolia* Mart. from Scanned Images. *Agricultural Sciences*, **10**, 796-806.
https://doi.org/10.4236/as.2019.106061

Received: May 22, 2019
Accepted: June 25, 2019
Published: June 28, 2019

Abstract

The leaf has a vital role in the functions of the plant, being responsible for photosynthesis and gas exchange. Thus, the objective of this study was to fit a mathematical equation model to estimate the leaf area of *Maytenus obtusifolia* Mart. through the linear dimensions of the leaves. For that, six hundred and fifteen healthy leaves were collected from plants belonging to the Federal University of Espírito Santo, São Mateus Campus, in the municipality of São Mateus, located in the north of the State of Espírito Santo, Brazil. All leaves were digitized and the images processed using the ImageJ® software, obtaining the measurements of the maximum length of the main midrib (*L*), the maximum width of the leaf blade (*W*) and the real leaf area (*RLA*) of each sheet. Subsequently, the product of length and width multiplication (*LW*) was also obtained. 500 sheets were randomly separated for the generation of models of mathematical equations and their respective coefficient of determination (R^2), where *RLA* was used as dependent variable as function of *L*, *W* or *LW* as independent variable. Based on the models generated, a 115 leaf sample was used for validation, where the *L*, *W* and *LW* values of this sample were replaced in the adjusted equations, thus obtaining the estimated leaf area (*ELA*). A comparison of the means of *RLA* and *ELA* was performed by Student's *t* test at 5% probability. We also calculated the mean absolute error (*MAE*), the root mean square error (*RMSE*) and the Willmott index (*d*). The best equation was defined by the following criteria: non-significant values of *RLA* and *ELA* averages, R^2 and index d closest to unit, and *MAE* and *RMSE* values with greater proximity to zero. The quadratic model equation represented by $ELA = 0.18122798 + 0.72847767(LW) + 0.00002789(LW)^2$

generated by multiplying the length with the width (*LW*) is the most suitable for the estimation of the leaf area of *Maytenus obtusifolia* Mart., in a fast, safe and non-destructive way.

Keywords

Maytenus obtusifolia Mart, Non-Destructive Method, Mathematical Equations

1. Introduction

The leaf is the organ that performs vital functions, being responsible for carrying out the photosynthesis and the gas exchanges of the plant. For this reason, the determination of leaf area and fundamental in ecological, agronomic and eco-physiological studies may be essential in the understanding of the behavior of plants in relation to light interception, photosynthetic efficiency, evapotranspiration and responses to the use of irrigation and fertilizers, directly influencing the growth and development [1] [2].

Among the forms reported for the determination of leaf area are direct methods and indirect methods. The direct methods, in spite of their good accuracy, are mostly destructive, besides requiring time and sophisticated equipment, which implies a higher cost for the analysis [2]. On the other hand, the indirect methods allow a precise and fast estimate of the leaf area, without the need of leaf destruction, and can be used throughout the crop cycle, being possible the successive measurements in the same plant [3].

From the indirect methods used to determine the leaf area, the use of models of statistical equations generated through measures of foliar dimensions such as length, width, or combination of both measures has been widely used, promoting reliable estimates of leaf area of many species. Notorious are the studies that seek the adjustment of mathematical equations that estimates the leaf area of various crops through the linear dimensions of its leaves as reported by several authors *Cucumis sativus* L. [4], *Vicia faba* L. [5], *Helianthus annuus* L. [6], *Crambe abyssinica* [7], *Jatropha curcas* [8], *Coffea canephora* [9], *Vitis vinífera* L. [10], *Rosa hybrida* L. [11], *Crotalaria juncea* [12], *Litchi chinensis* Sonn. [13], *Ormosia paraensis* Ducke [2], guava [14], *Artocarpus heterophyllus* [15] and *Plectranthus barbatus* Andrews [16].

However, no work was found in the literature involving the species *Maytenus obtusifolia* Mart. due to the importance of the species, which is a shrub with a height of less than 5 m, belongs to the Celastraceae family, abundant in the Restinga region of southeastern Brazil and has its leaves used in folk medicine as analgesic, anti-inflammatory and antiulcerogenic [17] [18]. The aim of this study was to propose, test and adjust mathematical model equations that accurately estimate the leaf area of this species in a fast and non-destructive way through the linear dimensions of the leaves.

2. Material and Methods

A total of 615 healthy leaves with no signs of mechanical damage and all cardinal points were collected from plants of *Maytenus obtusifolia* Mart. belonging to the Federal University of Espírito Santo, São Mateus Campus, in the municipality of São Mateus, located in the north of the State of Espírito Santo, Brazil, with the following geographical coordinates: 18˚40'36" south latitude and 39˚51'35" of East longitude. The climate of the region is characterized by rainfall during summer and dry winter, being classified according to köppen with tropical AW (tropical humid) [19].

All the leaves were packed in a plastic bag and transported to the laboratory where, after petiole removal, they were scanned with an HP Deskjet F4280® flatbed scanner and saved in Tag Image File Format (TIFF), with 75 dpi quality in order to guarantee greater accuracy in reading the data. From the generated images, it was possible to obtain the maximum length (L in cm), measured along the main midrib, considering the distance between the insertion of the petiole to the other end of the leaf, the maximum width (W in cm) (**Figure 1**) and the real leaf area (RLA in cm^2) of each leaf, with the aid of ImageJ® software [20]. Subsequently, the product of the multiplication of the width with the length (LW in cm^2) was obtained.

Descriptive statistics analysis was used to ascertain the behavior of the values of the variables *L*, *W*, *LW* and *RLA*. The values of maximum, minimum, mean, amplitude, standard deviation (SD) and coefficient of variation (CV) of the evaluated dimensions were then obtained.

Nine models of regression equations (**Table 1**) were fitted and tested based on a sample of 500 sheets, which were: three linear first degree; three quadratic and three power, where the linear dimensions L and W, as well as their combination (*LW*), were the independent variables (x) and RLA was dependent variables (y). Then, the coefficient of determination (R^2) for each adjusted equation was calculated between the values of x and y.

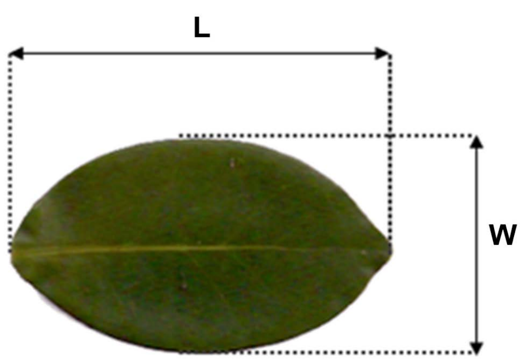

Figure 1. Representation of the length (L) along the midrib and the maximum width (W) of leaves of *Maytenus obtusifolia* Mart.

Table 1. Denomination and representation of equation models adjusted for area estimation of leaflets of *Maytenus obtusifolia* Mart.

Denomination	Representation
Linear	$ELA = \hat{\beta}_0 + \hat{\beta}_1 x$
Quadratic	$ELA = \hat{\beta}_0 + \hat{\beta}_{11} x + \hat{\beta}_2 x^2$
Power	$ELA = \hat{\beta}_0 x^{\hat{\beta}_1}$

For the validation of the nine models, the values of *L*, *W* and *LW* of a sample of 115 separate leaves for this purpose were substituted in the adjusted equations, obtaining the estimated leaf area (*ELA*) for each model in cm^2. A comparative analysis was performed between the means of *RLA* and *ELA* to test the similarity between both by Student's t test at 5% probability. We also calculated the mean absolute error (*MAE*), the root mean square error (*RMSE*) and the Willmott index (*d*) [21] for all proposed models, using expressions 1, 2, 3.

$$MAE = \frac{\sum_{i=1}^{n} \left| ELA - RLA \right|}{n} \quad (1)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} \left(ELA - RLA \right)^2}{n}} \quad (2)$$

$$d = 1 - \left[\frac{\sum_{i=1}^{n} \left(ELA_i - RLA_i \right)^2}{\sum_{i=1}^{n} \left(\left| ELA_i - \overline{RLA} \right| + \left| RLA_i - \overline{RLA} \right| \right)^2} \right] \quad (3)$$

where, *ELA* are the estimated values of leaf area by the proposed equations; *RLA* are the actual leaf area values; $\overline{RLA}$ is the average of the values of the real leaf area; *n* is the number of leaves used in the validation, *I* = 115 in the present study.

The best equation estimating the leaf area of *Maytenus obtusifolia* Mart., as a function of the linear measurements of the leaf surface was defined by the following criteria: non-significant values of the comparison of the means of *ELA* and *RLA*, R^2 and index d closest to the unit and values of *MAE* and *RMSE* with closer proximity to zero. All statistical procedures were performed using software *R* [22], with scripts developed for the ExpDes.pt version 1.2 package [23].

3. Results and Discussion

The characteristics analyzed presented high values of amplitude, indicating the use of leaves with different sizes (**Table 2**). This high amplitude can be easily represented by the high values of the coefficient of variation, exceeding 36% in the sample used for the modeling and 19% in the sample used for the validation, being this one indicative of leaves in different phenological stages, making the use of equations feasible for the entire plant cycle.

According to [24] for the modeling of equation that aim to estimate the leaf area, should use leaves of various stages of development of the plant because this

Table 2. Descriptive statistics with value minimum, maximum, mean, amplitude, standard deviation (*SD*) and coefficient of variation (*CV*) of the variables: length (*L*); width (*W*); product of the length and width (*LW*) and real leaf area (*RLA*) of leaves of *Maytenus obtusifolia* Mart.

Variable	Unit	Minimum	Max	Average	Amplitude	*SD*	CV
500 leaves were used for modeling							
L	cm	1.32	11.25	5.14	9.93	1.87	36.40
W	cm	0.73	5.82	2.90	5.09	1.05	36.19
LW	cm^2	0.96	61.65	16.82	60.69	12.25	72.86
RLA	cm2	0.73	45.02	12.45	44.29	8.95	71.94
115 leaves for validation							
L	cm	1.90	7.19	4.51	5.29	0.87	19.40
W	cm	1.13	3.98	2.44	2.85	0.52	21.55
LW	cm^2	2.15	28.67	11.41	26.52	4.51	39.56
RLA	cm^2	1.71	19.89	8.56	18.18	3.22	37.61

practice allows to achieve greater precision in the models tested. In addition, the high variability of leaf size, belonging to different stages of plant growth and development, contribute to a wide spectrum of use of the models generated for a crop [12].

Therefore, the conditions in which the data were obtained for the adjustment of the equations must be taken into account, since as long as the data are not extrapolated, these equations can be used in the estimation of leaf area of seedlings of the same species, as well as plants of similar ages and climatic conditions, otherwise, the estimated values of the leaf area from a given variable can be biased and imprecise [2].

Note, in **Figure 2** that the dispersion diagram of the measurements of the leaf surface measurements of the length, width and the combination of both, presented a trend of linear and non-linear behavior when related to the actual leaf area observed from the leaves. In this way, linear models of first degree, quadratic and power were tested to fit the best equation for estimation of the leaf area of *Maytenus obtusifolia* Mart.

Table 3 shows the linear equations of the first degree, quadratic and power models for the estimation of leaf area of *Maytenus obtusifolia* Mart. and their respective coefficient of determination (R^2). Note that, in general, all models had a high degree of correlation between *RLA* values and *L, W* and *LW* measurements, with R^2 higher than 0.95 for all equations, being the three *LW* based models presented R^2 values identical and superior to the models generated based on only one linear dimension (*L* or *W*).

According to [25] values of R^2 lower than 0.90 indicate less precision of the models in the estimation of leaf area, however, according to these authors, the

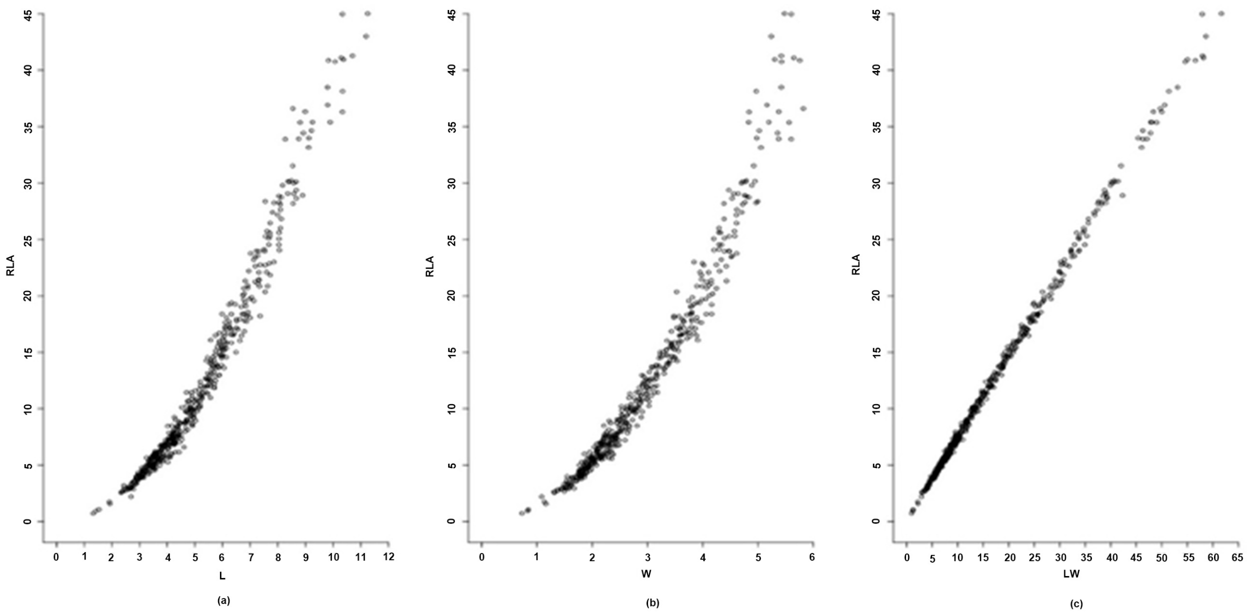

Figure 2. Scatter plot of the actual leaf area in relation to length (L) (A), width (W) (B) and product of length with width (LW) (C).

Table 3. Equation with linear adjustment of first degree, quadratic and power and its respective coefficient of determination (R^2) using the real leaf area (RLA) as dependent variable, in function of length (L), width (W), product of length with width (LW) of leaves of *Maytenus obtusifolia* Mart.

Model	Equation	R^2
Linear	$ELA = -11.61031 + 4.68093(L)$	0.9562
Linear	$ELA = -11.60517 + 8.28574(W)$	0.9453
Linear	$ELA = 0.169901 + 0.729869(LW)$	0.9980
Quadratic	$ELA = -2.19508 + 1.03495(L) + 0.31174(L)^2$	0.9794
Quadratic	$ELA = 0.41870 - 0.08303(W) + 1.28765(W)^2$	0.9760
Quadratic	$ELA = 0.18122798 + 0.72847767(LW) + 0.00002789(LW)^2$	0.9980
Power	$ELA = 0.5120(L)^{1.8883}$	0.9787
Power	$ELA = 1.3688(W)^{1.9612}$	0.9760
Power	$ELA = 0.7656(LW)^{0.9886}$	0.9980

choice of the best equation model should not be based solely on high R^2 values. Therefore, other parameters must be used as a criterion for the selection of the most satisfactory equation, for this reason, the validation of the models becomes essential for the determination of the best fit.

After validation of the models using 115 leaves (Table 4), it was observed that the mean values of the real leaf area (RLA) and estimated leaf area (ELA) by the equations did not differ statistically by Student's t test at 5% probability for all proposed models. For this reason, the equations allow a good estimation of the leaf area of *Maytenus obtusifolia* Mart. This finding is important because it

Table 4. Real leaf area (*RLA*) and estimated leaf area (*ELA*) of linear equations of first degree, quadratic and power for the independent variables length (*L*), width (*W*) and product of length and width (*LW*), besides the value of p, mean absolute error (*MAE*), root mean square error (*RMSE*) and Willmott d index of leaves of *Maytenus obtusifolia* Mart. used for validation.

Modelo	Variável	*RLA*	*ELA*	Valor p*	*MAE*	RMSE	*d*
Linear	L		9.4894	0.0589	1.4017	1.6936	0.9466
Linear	W		8.6082	0.9349	1.1288	1.3998	0.9655
Linear	LW		8.4975	0.8717	0.2640	0.3464	0.9971
Quadratic	L		9.0405	0.2765	0.7676	1.0024	0.9766
Quadratic	W	8.5669	8.2321	0.4458	0.6320	0.8178	0.9847
Quadratic	LW		8.4971	0.8710	0.2635	0.3460	0.9971
Power	L		9.0684	0.2388	0.7423	0.9787	0.97675
Power	W		8.2104	0.4209	0.6517	0.8407	0.9841
Power	LW		8.4889	0.8566	0.2711	0.3523	0.9971

Note. *p values higher than 0.05 indicate that the observed leaf area (OLA) and the estimated leaf area (ELA) do not differ by Student t-test.

allows to infer similarity of the real leaf area of the leaves with the leaf area found by the equations when using linear length, width or combination dimensions.

However, the unidimensional models in which they used *L* or *W* as independent variable presented higher values of *MAE* and *RMSE*, in addition to index d more distant of one. These models generated with only a linear dimension are simpler, making the work easier in practice by requiring only a measurement of the sheet [15]. On the other hand, according to [26], are less predictive in the determination of leaf area, showing they less accurate for most species, being are only used in specific cases. The results also show that although the *LW* based models presented equal values of R^2, the quadratic model equation has MAE and *RMSE* values closer to zero and values of index d with greater proximity to the unit, which suggests greater precision of this model in the estimation of the leaf area of *Maytenus obtusifolia* Mart.

In relation to the use of digitized images as done in the present study for the evaluation of the leaf area, according to [27] this is a method that presents some difficulties, since it requires the removal of the leaves of the plant, besides software for the processing of the images and the calculation of the desired dimensions. However, after the determination of the equation models there is no need for further destruction of the leaves for further analysis, and for future research these equations can be used with precision in the estimation of the leaf area.

In this way, the quadratic model regression based on the product of length multiplication with the width (*LW*) of the sheets represented by equation $ELA = 0.18122798 + 0.72847767(LW) + 0.00002789(LW)^2$ and R^2 of 0.9897 (**Figure 3**) showed to be more accurate for the estimation of the leaf area of *Maytenus obtusifolia* Mart. when compared to the other models tested. When

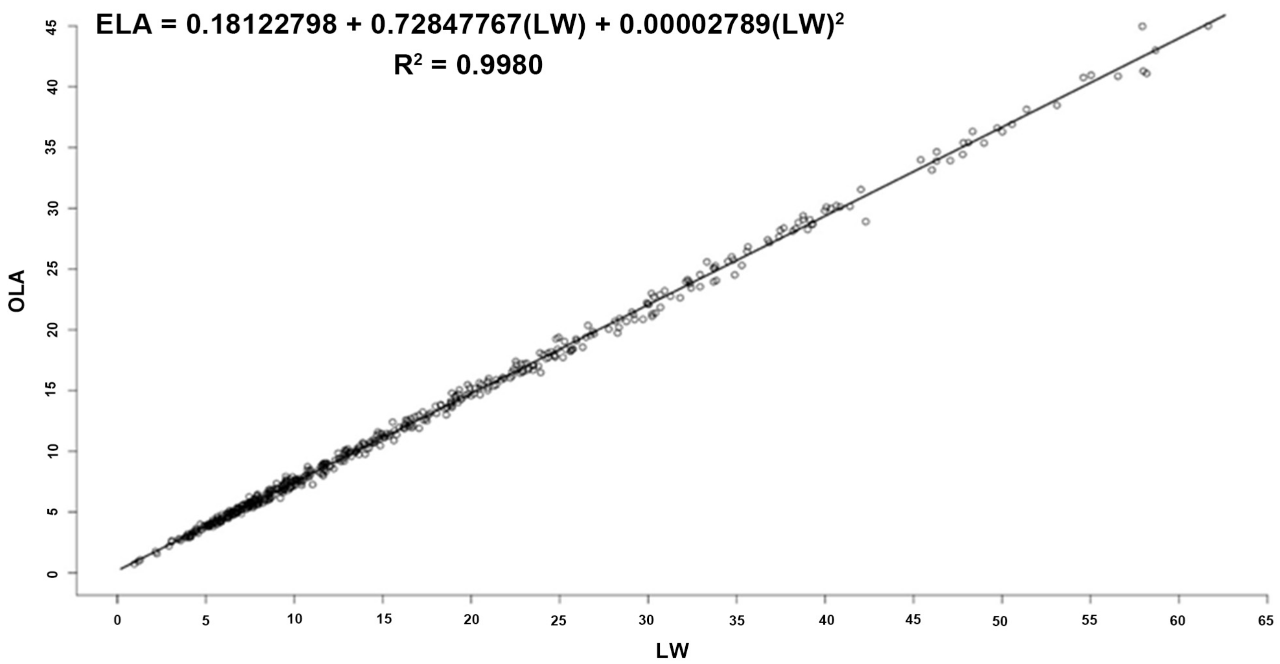

Figure 3. Adjusted equation of quadratic model and its coefficient of determination (R^2), using the real leaf area (*RLA*) as dependent variable, as a function of the product of length and width (*LW*) of leaves of *Maytenus obtusifolia* Mart.

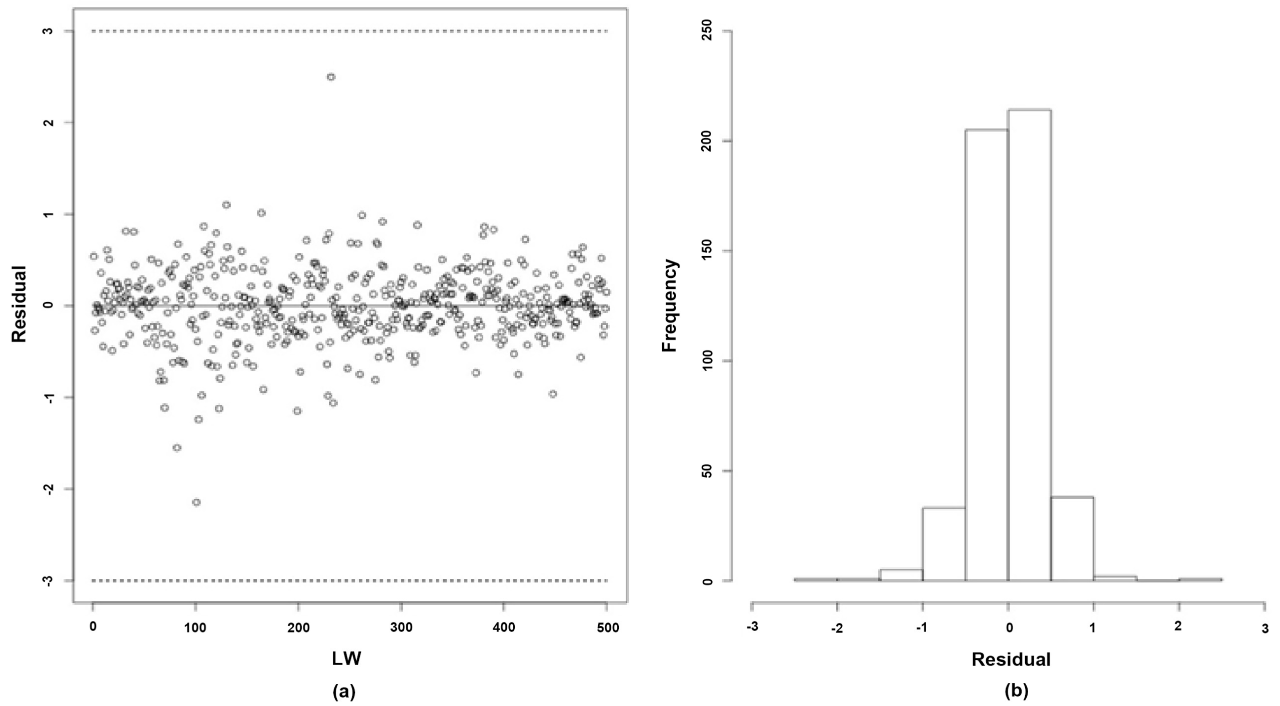

Figure 4. Distribution (a) and frequency histogram (b) of residue of the quadratic model equation, using the real leaf area (*RLA*) as dependent variable, as a function of the product of length and width (*LW*) of *Maytenus obtusifolia* Mart leaf.

analyzing the results of the residual distribution, note that the points were homogeneously distributed (Figure 4(a)) and that the frequency followed the trend of normality (Figure 4(b)). According to [2] is indicative of satisfactory statistical analyzes for this model, following a trend of biological parameters.

Thus, the use of non-destructive methods such as modeling equations is extremely important in studies that want to make plant growth assessments without destroying them [28]. On the other hand, this practice is not possible when it is necessary to highlight the leaves of the plant, which may be an obstacle in experiment with a limited number of plants [5]. In this way, the researcher needs alternative methods like the mathematical equations for the excussion of the studies. For this reason, the use of models of mathematical equations is an important tool in studies that require the determination of leaf area, since this feature is directly linked to the photosynthetic performance of the plants, influencing its development. Thus, it is essential to investigate this methodology for the different plant species [2].

4. Conclusion

The models based on individual measures (L or W) were less adequate in estimating the leaf area of *Maytenus obtusifolia* Mart., being the quadratic model which was represented by equation $ELA = 0.18122798 + 0.72847767(LW) + 0.00002789(LW)^2$ and R^2 of 0.9897 generated with the multiplication of the length and width (LW), most appropriate, accurately obtaining the area of the leaves of this species, in a fast, safe and non-destructive manner.

Acknowledgements

CNPq, CAPES and FAPES for financial support.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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