



*Research article*

## **Estimating leaf area of *Jatropha nana* through non-destructive allometric models**

Running Title: Allometric estimations of *Jatropha nana*

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**Abstract:** *Jatropha nana* is an endemic, threatened under-shrub possessing significant economic potential. In spite of this economic importance, there has been no allometric equation described till date to estimate its leaf area. We address this lacuna by describing a method of estimating the leaf area directly, without using expensive leaf meters. For the two leaf morphotypes of *J. nana* (monolobed and trilobed), developing and mature leaves were collected from field and an allometric relationship was developed, using length (L), width (W) and LW as independent variables. For the described equations, the coefficient of determination ( $R_a^2$ ) between true leaf area and leaf dimensions ranged from 0.8356 to 0.9963. After applying certain statistical criteria we report that the equations  $\hat{Y} = 0.6942*(LW)$  and  $\hat{Y} = 0.6426*(LW)$  are the best for estimating the monolobed and trilobed leaf area respectively. These equations could be further simplified by considering only one leaf dimension (W). Thus the equation  $\hat{Y} = 1.2890*(W)^{1.8794}$  for monolobed leaves and  $\hat{Y} = 1.1089*(W)^{1.8030}$  for trilobed leaves estimate leaf area of *J. nana* with high precision, accuracy, random dispersion pattern of residuals and without any bias. These equations will be vital for addressing several further questions on this under-researched species.

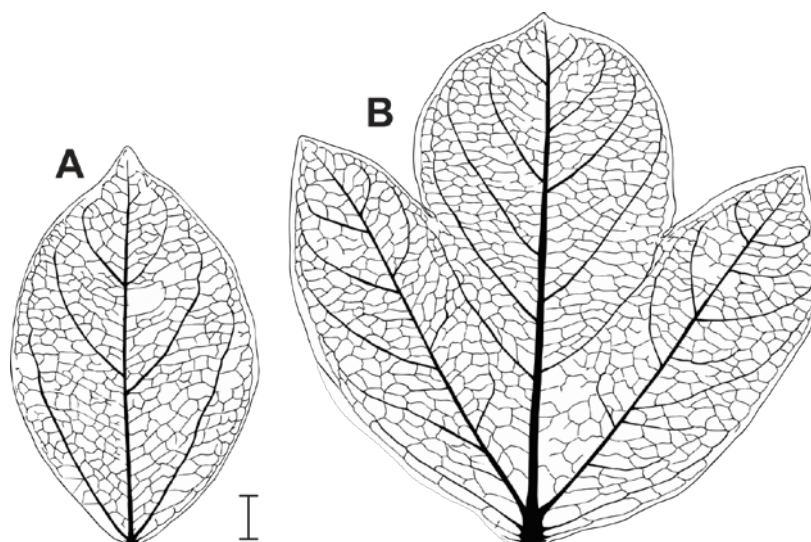
**Keywords:** Euphorbiaceae; *Jatropha*; India; leaf area; measurement

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## 1. Introduction

The genus *Jatropha* (Euphorbiaceae) shows a pan-tropical distribution and several species of this genus are well known for their economic value including biofuel properties [1]. Out of the 12 *Jatropha* species recorded in India, four are endemic to the country: *Jatropha heynei* N.P. Balakr., *Jatropha maheshwarii* Subram. & Nayar, *Jatropha nana* Dalz & Gibs., and *Jatropha villosa* Wight [2,3].

*Jatropha nana* is one such endemic under-shrub possessing significant economic importance [4]. The seeds of this species were found to possess about 46.5% oil content [5], which is much higher than the oil content of the popularly cultivated *Jatropha curcas* L. Owing to this high oil content and several other desirable characters, *J. nana* has gained tremendous economic potential for inter-specific hybridization with *J. curcas* [3]. Along with the larvicidal potential against selected pests, this species is also known to be ethno medicinally important and is reported to be used as a counter-irritant in ophthalmia [3,6]. The leaves of this species are either entire or trilobed [4], here referred to as monolobed and trilobed, respectively (Figure 1).



**Figure 1.** Drawings of the monolobed (A) and trilobed (B) leaves of *Jatropha nana* collected during the monsoon season from the three hills in Pune city, India. Length (L) and Width (W) were measured for both the leaf morphotypes. Scale = 10 mm.

*J. nana* was considered to be a narrow endemic to the Maharashtra state of India [7] until recently when the species was also discovered in the West-Bengal, Jharkhand and Bihar states of India indicating a wider yet fragmented distribution [4,8]. Its global threat status is currently assessed as ‘vulnerable’ due to its limited area of occupancy, the continuing decline in the area of extent, quality of habitat as well as number of mature individuals [8].

Leaf area (one-sided surface area of an individual lamina) has been widely used as a proxy for plant condition and fitness at several scales, right from individual plants to biomes [9–11]. Leaf area being a measure of leaf size, plays an important role in studies spanning from ecophysiology to agriculture and ecology [12]. Out of all the functional traits, leaf area was found to be one of the six most important traits that drive plant form and function [13]. This trait has been widely used to

describe a range of variables including: growth, productivity, photosynthetic efficiency [14], soil characteristics including salinity and acidity, transfer and exchange of heat, carbon, nutrients and water, which in turn affect plant yield [11,15]. Measuring leaf area of individuals directly is both, laborious, expensive as well as a time consuming task and often constrained by logistical factors. Hence, simple linear measurements like leaf length (L), leaf width (W) are used in allometric equations to model the true leaf area [16]. Further, such non-destructive methods like allometry have been preferred over traditional destructive ones [11]. Given the fact that *J. nana* is a threatened species, such non-destructive methods are best suited for the species. Along with the economic aspect which has been extensively researched, accurate estimation of leaf area is also useful in understanding ecology of the species, for example responses of plants against herbivory [17].

Such non-destructive allometry methods for determination of leaf area have been a subject of intensive research especially for plants with high economic value [11,16,18,19]. Within the genus *Jatropha*, there are some studies available on allometric equations for leaf area of *J. curcas* [15,20,21], but no such report is available for its congeneric *J. nana* in spite of its potential economic value. Hence, the current initiative aims to develop an allometric model to accurately predict leaf area of *J. nana*. For this, we evaluate the currently used models to estimate leaf area of *J. curcas* [15] and propose a reliable and accurate model using non-destructive measurements of L and/or W for estimating the leaf area for the two leaf morphotypes of *J. nana*.

## 2. Materials and methods

The leaves of *J. nana* were collected from three natural population in Pune city, India, namely the Vetil hills (18°31'31"N; 73°49'11"E), the Pashan-Baner hills (18°32'56"N; 73°47'09"E), and the NDA hills (18°30'1.0"N; 73°46'50.3"E) in August 2016, during the monsoon season. Average elevation of all the three locations is around 600 m a.s.l. and the vegetation type of these hills is tropical southern dry mixed deciduous as per classification of Champion and Seth [22], (although, now classified as a mesic savanna) and intermixed with plantations of exotic trees. The *J. nana* individuals that were selected for collection of leaves on field belonged to various age-classes and were assumed to encompass wide genetic variability.

For model construction, a total of 575 healthy leaves (developing and mature) were harvested of both, monolobed ( $n = 244$ ) and trilobed ( $n = 331$ ; Figure 1) morphotypes from the study site. The leaves were randomly sampled from different parts of the plants and measured so as to develop the best fitting model for predicting the leaf area of *J. nana*. The leaves were then scanned using a scanner (HP, PSC1410, Palo Alto, CA with resolution of 1200 x 1200 dpi) and images were analysed using the Image Pro<sup>®</sup> Plus (version 4.5.0.29, Media Cybernetics, Silver Spring, USA). The maximum leaf length (L) (from lamina tip to the point of the petiole intersection to the midrib) and leaf width (W) (the widest linear length perpendicular to the midrib) and leaf area were measured to the nearest of 0.00001 cm using the aforementioned software. The collected leaves covered the broadest range as far as possible (range details in results). However, sample size of monolobed leaves was less than that of trilobed leaves because the monolobed morphotype was relatively uncommon on the field.

Several linear and non-linear regression models using W and/or L were made for each morphotype (*i.e.*, monolobed and trilobed leaves). Here, we report only the best equations, based on biological pattern and coefficients of determination. For selecting the models, the following statistical criteria were used: (i) F-test, (ii) coefficients of determination adjusted to samples, (iii)

stability and standard error of estimates, (iv) dispersion pattern of the residuals. In parallel, we used data exploration protocol by Zuur et al. [23] to verify statistical assumptions (*i.e.*, normality and independence of errors), adding the Durbin-Watson criteria [24] and Akaike Information Criterion (AIC) [25] to select accurate and parsimonious equations. All these criteria allowed us to evaluate the bias, precision, simplicity and accuracy of the models [26].

To evaluate if the same model/s can accurately estimate the leaf area for both the leaf morphotypes, a statistical null hypothesis was set up:  $H_0: \beta_1 = \beta_2 = \beta_n$  (where  $\beta_1, \beta_2, \beta_n$  are regression coefficients). That is, the null hypothesis postulates that there is no difference in coefficients based on the difference between the sum of squares of complete model, and rejection of this  $H_0$  implies the evidence to accept the alternative hypothesis – that there is significant difference between the models for the two leaf morphotypes [27].

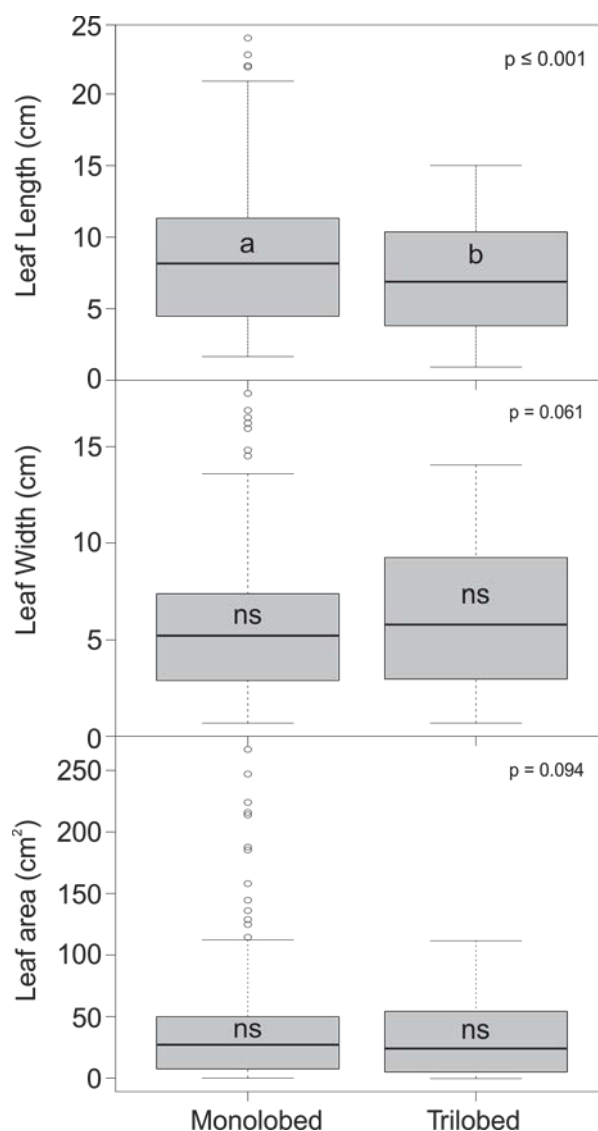
The statistical analyses were performed using the softwares Statistica version 8.0 (StatSoft, Tulsa, OK, USA), DataFit version 8.0.32 (Oakdale Engineering, Oakdale, PA, USA), SigmaPlot for Windows version 11.0 (Systat Software, Inc. Germany) and R package version 3.3.3 (R Development Core Team).

### 3. Results

For this study, a total of 244 monolobed and 331 trilobed leaves were collected. The range of leaf areas sampled varied from 1.12 cm<sup>2</sup> to 274.9 cm<sup>2</sup> for monolobed leaves and 0.78 cm<sup>2</sup> to 115.44 cm<sup>2</sup> for the trilobed leaves (Table 1). In general, the morphological variation of the monolobed leaves was higher than that of the trilobed leaves (Figure 2). Out of the three leaf parameters (leaf area, width and length), only leaf length was significantly different between the two morphotypes ( $\alpha = 0.001$ ; Figure 2). It is important to note that the morphology of trilobed leaves was partially similar to the morphology of *J. curcas* leaves, but that of the monolobed leaves was not (Figure 1).

**Table 1.** Means  $\pm$  standard deviations (SD), minimum (Min) and maximum (Max) values for the leaf length, width and leaf area of *Jatropha nana*.

Leaf morphology	n	Leaf length (cm)			Leaf width (cm)			Leaf area (cm <sup>2</sup> )		
		$\bar{x}$	Min	Max	$\bar{x}$	Min	Max	$\bar{x}$	Min	Max
Monolobed	244	8.11 $\pm$ 4.40	1.63	23.32	5.50 $\pm$ 3.17	0.75	17.14	40.23 $\pm$ 44.90	1.12	274.90
Trilobed	331	6.99 $\pm$ 3.56	0.92	14.64	6.02 $\pm$ 3.40	0.78	13.59	34.58 $\pm$ 31.54	0.78	115.44



**Figure 2.** Box plots for leaf length, leaf width and leaf area for two leaf morphotypes of *Jatropha nana*. Box plots show the interquartile range and median (central line); whiskers indicate the 10th and 90th percentiles. Significant differences between the values of width, length and leaf area are indicated by different letters according to two sample t-test ( $p \leq 0.01$ )

For both leaf morphotypes, twelve new equations were generated along with three previously described ones for *J. curcas* [15], which were both linear and power equations and with or without intercept at zero (total fifteen equations). Table 2 describes the equations generated for monolobed leaves while Table 3 describes the equations for trilobed leaves. We demonstrate that all these equations could be considered very accurate if the selection criteria were based solely on coefficient of determination adjusted for the degrees of freedom ( $R_a^2$ ), since the  $R_a^2$  for all tested models was significant ( $\alpha \leq 0.001$ ), exceeding 83.56%. However, when we analysed the variation and bias of the estimated area with respect to the observed area for individual leaves (Figure 3), we found that equations #13, #14 and #15, proposed for *J. curcas* [15] were inadequate for the estimation of the *J. nana* leaf area. This was because the equations proposed by Pompelli et al. [15] would strongly underestimate the *J. nana* leaf area, independent of the equations used. Equations #14 and #15 were

biased, which lead to a significant underestimation of leaf area. Equation #14 underestimated, on average, the leaf area by 96%, while equation #15 underestimated, on average, the leaf area by 127% independent of the leaf morphotypes. These differences between estimated and observed leaf areas were significantly different from zero (biased) (Figure 3) and for this reason, these equations were eliminated from further considerations. Equation #15 apparently could accurately estimate the monolobed leaf areas of *J. nana*; however, analysis of the dispersion of residuals (Figure 4) showed that 100% of the residuals were less than zero, making this equation completely inadequate for the estimation.

Twelve new equations were generated specifically for *J. nana*, using linear and power models. Figure 3 shows that equations #2, #4, #6, #8 and #12 that present a high  $R_a^2$  are biased, leading to a significantly different estimated leaf area than the true leaf area. For both leaf morphotypes the equations #2, #6, #8 and #12 underestimated the leaf area. On the other hand, equation #4 caused overestimation and underestimation of the monolobed and trilobed leaves, respectively. For this reason, all these equations were eliminated from further considerations.

**Table 2.** Statistical models, regression coefficients (0 and 1), standard errors of estimates (SE), coefficients of determination adjusted for the degrees of freedom ( $R_a^2$ ), mean square error ( $MS_{res}$ ), calculated F ( $F_{calc}$ ), P value, and equations of leaf area as a function of linear dimensions of leaves [length (L) and width (W)] for the monolobed *Jatropha nana* leaves.

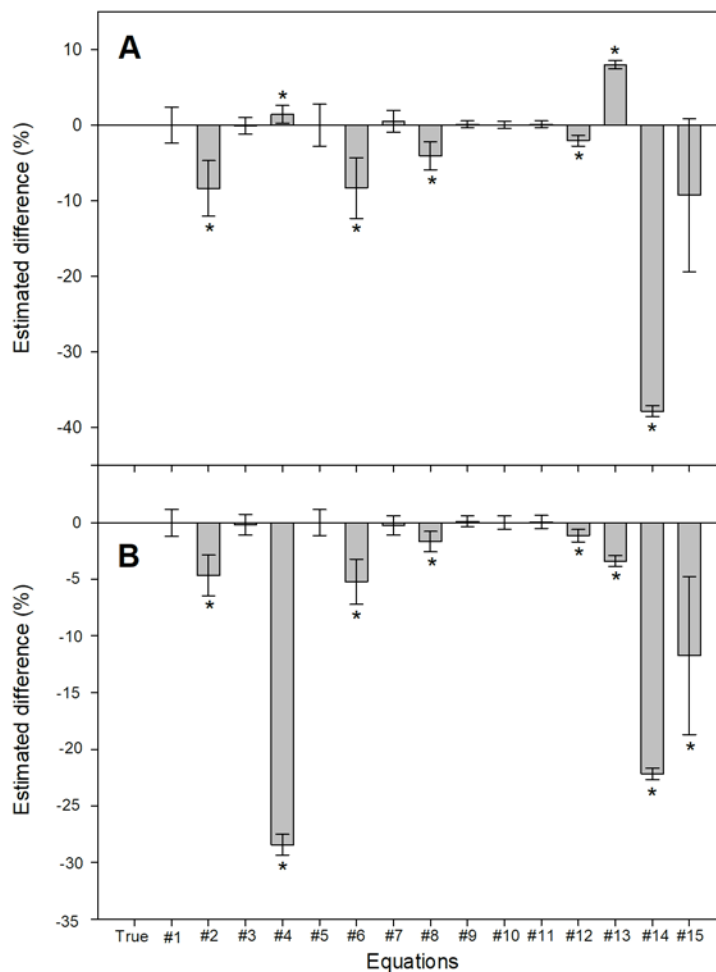
Equation Number	Model	Coefficients		SE	$R_a^2$	$MS_{res}$	$F_{calc}$	P	Estimator of LA ( $\hat{Y}$ )*
		$\beta_0$	$\beta_1$						
#1	$Y = \beta_0 + \beta_1 * W + e_i$	-33.5985	13.4191	14.4191	0.8973	50,314	2,124	<0.0001	$\hat{Y} = -33.5985 + 13.4191 * (W)$
#2	$Y = \beta_1 * W + e_i$	---	8.8343	22.1261	0.8617	118,964	1,568	<0.0001	$\hat{Y} = 8.8343 * (W)$
#3	$Y = \beta_0 * W^{\beta_1} + e_i$	1.2890	1.8794	6.6633	0.9782	10,745	10,835	<0.0001	$\hat{Y} = 1.2890 * (W)^{1.8794}$
#4	$Y = W^{\beta_1} + e_i$	---	1.9818	7.0305	0.9756	12,011	9,707	<0.0001	$\hat{Y} = (W)^{1.9818}$
#5	$Y = \beta_0 + \beta_1 * L + e_i$	-36.6194	9.4722	16.7238	0.8618	67,684	1,516	<0.0001	$\hat{Y} = -36.6194 + 9.4722 * (L)$
#6	$Y = \beta_1 * L + e_i$	---	5.9850	24.1813	0.8356	142,090	1,273	<0.0001	$\hat{Y} = 5.9850 * (L)$
#7	$Y = \beta_0 * L^{\beta_1} + e_i$	0.3794	2.0851	8,4760	0.9647	17,386	6,604	<0.0001	$\hat{Y} = 0.3794 * (L)^{2.0851}$
#8	$Y = L^{\beta_1} + e_i$	---	1.7304	11.0304	0.9399	29,566	3,799	<0.0001	$\hat{Y} = (L)^{1.7304}$
#9	$Y = \beta_0 + \beta_1 * LW + e_i$	-0.1450	0.6953	2.7509	0.9963	1,831	64,752	<0.0001	$\hat{Y} = -0.1450 + 0.6953 * (LW)$
#10	$Y = \beta_1 * LW + e_i$	---	0.6942	2.7473	0.9938	1,824	117,238	<0.0001	$\hat{Y} = 0.6942 * (LW)$
#11	$Y = \beta_0 * LW^{\beta_1} + e_i$	0.6767	1.0050	2.7452	0.9963	1,834	65,022	<0.0001	$\hat{Y} = 0.6767 * (LW)^{1.0050}$
#12	$Y = LW^{\beta_1} + e_i$	---	0.9297	4,2200	0.9912	4,327	27,375	<0.0001	$\hat{Y} = (LW)^{0.9297}$
#13	$Y = W^{\beta_1} + e_i$	---	1.8929	0.0010	0.9817	126,763	64,410	<0.0001	$\hat{Y} = (W)^{1.8929}$
#14	$Y = L^{\beta_1} + e_i$	---	1.9644	0.0014	0.9682	132,670	36,514	<0.0001	$\hat{Y} = (L)^{1.9644}$
#15	$Y = LW^{\beta_1} + e_i$	---	0.9660	0.0004	0.9888	128,812	105,911	<0.0001	$\hat{Y} = (LW)^{0.9660}$

\* Equations #13, #14 and #15 were described by Pompelli et al. (2012) for *Jatropha curcas* L.

**Table 3.** Statistical models, regression coefficients, standard errors of estimates (SE), coefficients of determination adjusted for the degrees of freedom ( $R_a^2$ ), mean square error ( $MS_{res}$ ), calculated F ( $F_{calc}$ ), P value, and equations of leaf area as a function of linear dimensions of leaves [length (L) and width (W)] for the trilobed *Jatropha nana* leaves.

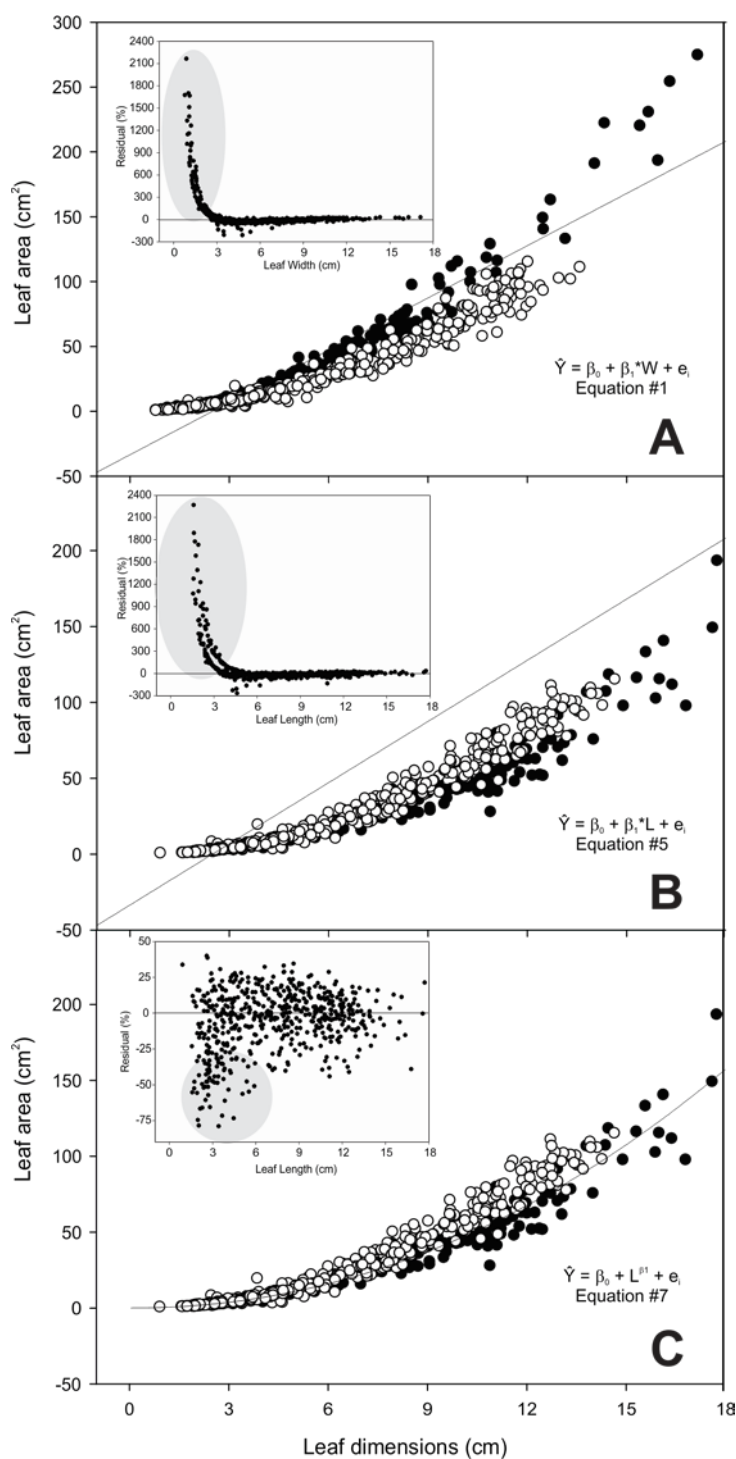
Equation Number	Model	Coefficients		SE	$R_a^2$	$MS_{res}$	$F_{calc}$	P	Estimator of LA ( $\hat{Y}$ )*
		$\beta_0$	$\beta_1$						
#1	$Y = \beta_0 + \beta_1 * W + e_i$	-19.2897	8.9466	8.3366	0.9303	22,865	4409	<0.0001	$\hat{Y} = -19.2897 + 8.9466 * (W)$
#2	$Y = \beta_1 * W + e_i$	---	6.5177	12.6316	0.9243	52,654	4214	<0.0001	$\hat{Y} = 6.5177 * (W)$
#3	$Y = \beta_0 * W^{\beta_1} + e_i$	1.1089	1.8030	6.4211	0.9588	13,565	7657	<0.0001	$\hat{Y} = 1.1089 * (W)^{1.8030}$
#4	$Y = W^{\beta_1} + e_i$	---	1.8474	6.4299	0.9586	13,643	7634	<0.0001	$\hat{Y} = (W)^{1.8474}$
#5	$Y = \beta_0 + \beta_1 * L + e_i$	-25.3963	8.5807	8.0201	0.9355	21,162	4790	<0.0001	$\hat{Y} = -25.3963 + 8.5807 * (L)$
#6	$Y = \beta_1 * L + e_i$	---	5.6941	14.0402	0.9072	65,052	3348	<0.0001	$\hat{Y} = 5.6941 * (L)$
#7	$Y = \beta_0 * L^{\beta_1} + e_i$	0.6174	1.9631	5.8897	0.9653	11,413	9163	<0.0001	$\hat{Y} = 0.6174 * (L)^{1.9631}$
#8	$Y = L^{\beta_1} + e_i$	---	1.7640	6.2897	0.9604	13,055	7993	<0.0001	$\hat{Y} = (L)^{1.7640}$
#9	$Y = \beta_0 + \beta_1 * LW + e_i$	0.0439	0.6421	3.3733	0.9886	3,744	28,606	<0.0001	$\hat{Y} = 0.0439 + 0.6421 * (LW)$
#10	$Y = \beta_1 * LW + e_i$	---	0.6426	3.3684	0.9918	3,740	63,568	<0.0001	$\hat{Y} = 0.6426 * (LW)$
#11	$Y = \beta_0 * LW^{\beta_1} + e_i$	0.6601	0.9943	3.3716	0.9886	3,754	28,636	<0.0001	$\hat{Y} = 0.6601 * (LW)^{0.9943}$
#12	$Y = LW^{\beta_1} + e_i$	---	0.9065	3.8317	0.9853	4,845	22,096	<0.0001	$\hat{Y} = (LW)^{0.9065}$
#13	$Y = W^{\beta_1} + e_i$	---	1.8929	0.0010	0.9817	126,763	64,410	<0.0001	$\hat{Y} = (W)^{1.8929}$
#14	$Y = L^{\beta_1} + e_i$	---	1.9644	0.0014	0.9682	132,670	36,514	<0.0001	$\hat{Y} = (L)^{1.9644}$
#15	$Y = LW^{\beta_1} + e_i$	---	0.9660	0.0004	0.9888	128,812	105,911	<0.0001	$\hat{Y} = (LW)^{0.9660}$

\* Equations #13, #14 and #15 were described by Pompelli et al. (2012) for *Jatropha curcas* L.



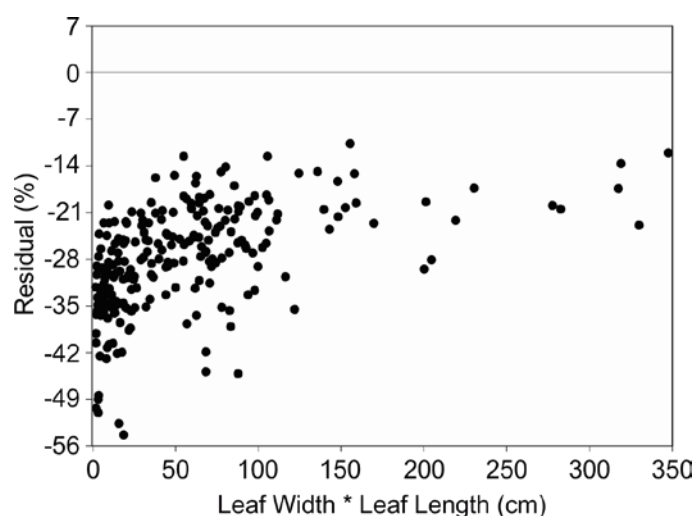
**Figure 3.** Deviation of the estimated area from the observed area for individual leaves. Leaf areas for the monolobed (A) and trilobed (B) *Jatropha nana* leaves were estimated using several models in which 0 and 1 are the coefficients. Vertical bars denote means and spreads denote 99% confidence intervals of the difference (distribution of t-test). For details of the equations and its equation number, see Table 2. Equations #13 #14 and #15 were described by Pompelli et al., 2012 for *Jatropha curcas* L. and are used here for comparison. The asterisks (\*) denotes biased equations.





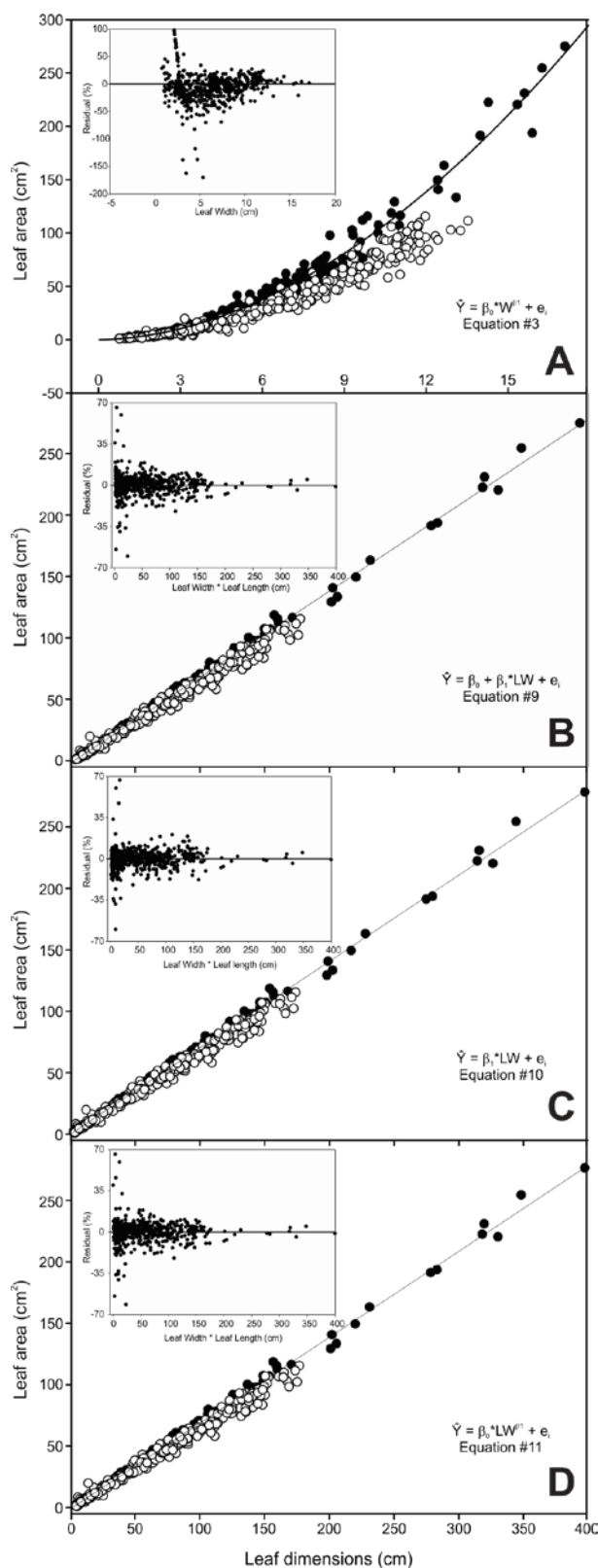
**Figure 4.** Regression curves for estimated leaf area and leaf dimensions: width (A), length (B and C) for both monolobed (black circles) and trilobed (white circles) leaves using linear (A and B) and power (C) models. The dispersion pattern of residuals for the respective models is shown in the insets. The shaded area denotes the region of highly biased data in the equations causing an overestimation (A and B) or underestimation (C) of *Jatropha nana* leaves.

The relationship between estimated leaf area and leaf dimensions (Figure 4) clearly showed that even if the dispersion of the leaf dimensions fits well with the true leaf area, the residuals (Figure 5, insets), that is the difference between the true leaf area and estimated leaf area, followed a non-normal tendency (*i.e.*, heterocedasticity), also not satisfying the conditions established by Durbin-Watson tests at  $\alpha = 0.05$ ). A more detailed analysis showed that equations #1 and #5 presented good estimates for larger leaves, but underestimated the smaller leaves by 42% and 44.3%, respectively. On the other hand, equation #7 presented a normal deviation when estimating larger leaves, but presented a strong overestimation, by approximately 35%, when the estimation occurs in leaves smaller than 6 cm in length. All these three equations showed a non-normal dispersion of errors as validated by the Durbin-Watson test. Further, as we aimed to describe an equation that can estimate the *J. nana* leaf area independent of leaf size, all these equations were summarily eliminated from further considerations.

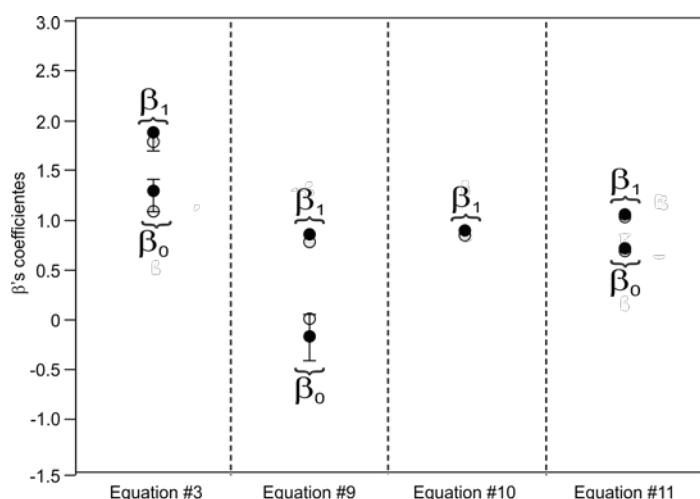


**Figure 5.** Dispersion pattern of residuals for the areas of *Jatropha nana* leaves estimated by equation #15. Note that all residuals were less than zero.

Considering what is presented above, we can see that only four equations remain: equations #3, #9, #10 and #11. Figure 6 shows that the dispersion of the width (Figure 6A) or the dispersion of  $L*W$  (Figure 6B-D) in relation to the true leaf area is perfectly explained by linear (Figure 6B and 6C) or power equations (Figure 6A and 6D). The residuals of all these equations followed a normal dispersion as validated by the Durbin-Watson test ( $p \geq 0.05$ ). Thus, equation #3 could estimate the monolobed and trilobed leaf areas with approximately 98% and 96% accuracy, respectively. Equations #9, #10 and #11, all using  $L*W$  as an independent variable, using linear or power equations, could accurately estimate the monolobed leaf area with about 99.6%, 99.4% and 99.6% accuracy, whereas these three equations could estimate the trilobed leaf area with 98.9%, 99.2% and 98.9% of accuracy. It should be noted that all these equations presented stability of the parameters of the models (Figure 7). In addition, these equations presented high accuracy and lack of bias, as demonstrated above.



**Figure 6.** Regression curves for estimated leaf area and leaf dimensions: width (A) and width \* length (B-D) for both monolobed (black circles) and trilobed (white circles) leaves of *Jatropha nana* using a linear (B and C) and power (A and D) models. Dispersion pattern of residuals for the respective models is shown in the insets. Note that the residuals follow a normal distribution.



**Figure 7.** Statistical comparison of the  $\beta_0$  and  $\beta_1$  coefficients estimated using different models for distinct leaf morphotypes: monolobed (black circles;  $n = 244$ ) or trilobed (white circles;  $n = 331$ ). Vertical bars denote 99% confidence interval for  $\beta$  coefficients means.

It is thus clear, that the best equations were always generated with  $L*W$ , because they presented the best statistical summaries. Among all  $L*W$  equations, #9, #10 and #11 presented the best performance (*i.e.*, in terms of accuracy, bias and precision) for the adhesion tests of the regression models among all the fifteen equations tested here. Because of the absence of bias, homoscedastic residual scatter, high stability of estimated coefficients and the absence of other penalties, the equation #10 ( $\hat{Y} = \beta_1 * LW$ ) would be most suitable for the estimation of the *J. nana* leaf area because it presented the lowest AIC [25] as compared to equations #9 and #11. This equation presented an AIC 68.22% [lower than the highest value presented in this study (*i.e.*, equation #6)] for the estimation of the monolobed leaf areas and a value of 54% of the AIC for estimations of the trilobed leaf area. Moreover, equation #10 is simple, precise and also not greatly penalized by the AIC criteria. Further, the model described by equation #10 could be simplified by using only one leaf dimension (equation #3;  $\hat{Y} = \beta_0 * W^{\beta_1}$ ). This equation could be used with accuracy, with the advantage of reducing field work by up to 50%, since only one linear dimension ( $W$ ) needs to be measured instead of two.

The linear regression of all models that estimate the leaf area with  $L*W$  were significantly different ( $\beta_0 \neq \beta_1$ ;  $\alpha = 0.001$ ) between the leaf morphotypes and hence we fail to accept the null hypothesis ( $F_{\alpha=0.001}; 2.574$ ). From this data, it is thus evident that the equations proposed for monolobed leaves did not estimate the accurate and unbiased leaf area for trilobed leaves and vice versa (Table 4).

**Table 4.** Variance analysis for linear models ( $Y = \beta_0 + \beta_1 X$ ), where  $X$  is  $L \times W$ , using the set of leaves adjustment of the two morphotypes ( $n = 575$ ). The dependent and independent variables were log-transformed for the analysis, following recommendations for statistical standardization of the data for variance reduction Zuur et al. (2010).

Variation font	Degrees of Freedom	Sum of squares	Mean square	$F_{calc}$
Parameters	4	6060.9572	-	
Reduction ( $\beta_s$ )	2	6059.9361	-	
Reduction ( $H_0$ )	2	1.0211	0.5105	29.5303**
Residual	570	9.8546	0.0173	
Total	574	6070.8118		

$$F_{0.01\%}(2.572) = 4.6422$$

#### 4. Discussion

Through this work, we demonstrate that it is possible to accurately estimate the leaf area of *J. nana* using specific allometric equations. To our knowledge, this is the first study that describes allometric equations for this species. However, when we consider the genus *Jatropha*, there have been several articles that describe allometric equations for the estimation of leaf area [15,20,21,28]. In spite of several studies on this genus, most of them lack the detailed examination of underlying assumptions of the models used to estimate leaf area; and any violation of these assumptions can render the inferences drawn as invalid [29]. Pompelli et al. [15] described that the previously reported models for *J. curcas* leaf area had flaws, and the sample size was lower than the minimum required to accurately estimate the leaf area.

As mentioned earlier, the current study species is threatened and hence the sample size for the leaves had to be restricted. Nonetheless, we demonstrated that even with limited sample size, the selected equations estimated leaf area with high accuracy. Many studies have described that the best equation must be validated with another and independently sample of leaves [15,19,21,30–33]. Knowing that the validation of the proposed models is extremely important, it was not possible for us to collect new samples to validate the models due to the fact that *J. nana* is threatened with the risk of extinction [8]. Thus our sample size of leaves was restricted to 575 leaves considering both the morphotypes and their developing and mature phases. The fact that monolobed leaves were relatively uncommon on field was also partly responsible for the lower sample size for this morphotype as compared with trilobed leaves. Nonetheless, we followed the minimum sample size (about 500 leaves) that is recommended for accurately estimating leaf area of *J. curcas* [15].

Extensive literature is available on the use of the same equation to estimate leaf area for different varieties of the same species [34–36] or different species of the same genus [19] or a generalised equation at familial level for different grass species [37,38]. However, there have been no studies to prove the efficacy of such generalizations. Even though the trilobed leaves of *J. nana* are morphologically similar to the leaves of *J. curcas*, we have shown here that the equations proposed for *J. curcas* [15] do not estimate *J. nana* leaf area accurately and without bias. Because of its high seed oil content and other characters, *J. nana* is one of the important target species for inter-species hybridization for crop improvement of *J. curcas* [3] and the present equations will be of great use in the near future to quantify the growth and fitness of the hybrids against several experimental treatments.

Of the twelve developed models, the best ones were determined according to selection criteria described in materials and methods and in accordance with Pompelli et al. [15], Antunes et al. [19] and Peters [39]. With respect to these criteria, equations #2, #4, #6, #8 and #12 had an estimated difference significantly different from zero (biased) (Figure 3). Considered together, these results indicate that these equations do not satisfactorily estimate leaf area of *J. nana*.

At a cursory glance, equations #1, #5 and #7 could accurately estimate the leaf area of *J. nana*, independent of the leaf morphotypes. However, the analysis of the residuals showed that the first two equations underestimate the leaf area of small leaves by approximately 43%, but indicate that they were able to accurately estimate the larger leaves. Equation #7 differed slightly from the other two equations because it caused a strong overestimation of the leaf area of small leaves. Based on this data, even these equations could be used but only for leaves with width greater than 6 cm, *i.e.* only for leaves approximately in the advanced process of expansion. These equations, must therefore be used with caution and these points were also previously recommended for *J. curcas* [15] and *Coffea* spp. [19].

In this study, we showed that the power models were able to accurately estimate the *J. nana*, leaf area, independent of whether W or L\*W were used. In this context, we recommend the equation  $\hat{Y} = 1.2890*(W)^{1.8794}$  for estimating the monolobed leaf area and  $\hat{Y} = 1.1089*(W)^{1.8030}$  for estimating the trilobed leaf area using only one dimension, *i.e.*, width. The equation #11 could also be used with high accuracy, but in this case two leaf dimensions (*i.e.*, length and width) should be measured in the field. The use of only one dimension for the field sampling of leaves was described for many agricultural crops [18,34,40–44] including trees [15,19–21,28,45]. However, the two foliar dimensions gave the best results for several species [16,45–50]. In this sense, equation #10 is important because it shows a good statistical summary, coefficient of determination adjusted to degrees of freedom ( $R_a^2$ ), one of the smallest standard error of estimates (SE), the residual sum of squares ( $QM_{res}$ ) and the lowest AIC value. Given a set of candidate models for the data, the preferred model is the one with the minimum AIC value [25]. AIC rewards goodness of fit (as assessed by the likelihood function), but it also includes a penalty that is an increasing function of the number of estimated parameters [51]. The penalty discourages overfitting, because increasing the number of parameters in the model almost always improves the goodness of the fit, making the use of the model more complex. Equation #10 is a linear equation of easy execution, even in field conditions, since it is enough to multiply the product of the length by width by the correction factor. Thus, it is suggested that equations  $\hat{Y} = 0.6942*(LW)$  (for monolobed leaves) and  $\hat{Y} = 0.6426*(LW)$  (for trilobed leaves) are the best for accurate and unbiased estimation of *J. nana* leaf area.

Figure 7 shows that separate regression models that estimate leaf area from W and the L\*W product did not differ significantly ( $\alpha \leq 0.01$ ) among leaf morphotypes. Thus, data for these leaf morphotypes could be potentially pooled and a single regression model could be developed for the combined data. However, according to Vasconcellos et al. [52], a single equation for different conditions can only be valid if the null hypothesis  $H_0$  is true for  $\beta_1 = \beta_2 = \dots = \beta_h$  (where models h are identical) vs. the alternate  $H_a$ :  $\beta_h \neq \beta_{h'}$  for at least one  $h \neq h'$ , where at least one model differs from the others. Since we fail to accept the null hypothesis in this case, we can conclude that the statistical difference between the model coefficients ( $\beta_0$  and  $\beta_1$ ) between the monolobed and trilobed leaves does not allow the calibration of a single equation for the two morphotypes and the regression models should be adjusted for each morphotype.

## 5. Conclusion

Although the leaf morphology of the congeneric *J. curcas* and *J. nana* are partly similar, equations published for the former species could not accurately estimate the leaf area of the later. This study describes allometric equations for the accurate leaf area estimation for the two leaf morphotypes (monolobed and trilobed) of *J. nana*. The equations were validated by different statistical criteria and show high coefficient of determination, stability of coefficients, low sum of squares and were also simple. The allometric equations described here will help in measuring leaf area in a cost-effective manner and facilitate further research on the physiology and ecology of this understudied potentially economic species.

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## Conflict of interest

The authors declare no conflict of interest.

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