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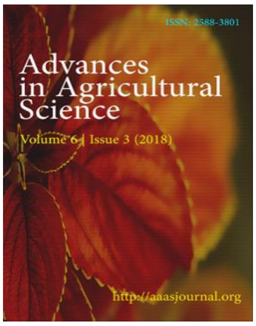

Research Article Open Access

Rapid leaf area measurement methods for Peppermint (*Mentha piperita* L.) grown under tropical condition

Olumide Samuel Daramola [1,3*], Faucett Olagundoye Olasantan [2], Adewale Waheed Salau [2], Patience Mojibade Olorunmaiye [1,3], Joseph Aremu Adigun [1,3], Tunrayo Tinuoye Joseph-Adekunle [2], Omobolanle Adewale Osipitan [1,3]

[1] *Department of Plant physiology and Crop Production, Federal University of Agriculture, Abeokuta, Nigeria.*

[2] *Department of Horticulture, Federal University of Agriculture, Abeokuta, Nigeria.*

[3] *Northeast Research and Extension Centre, University of Nebraska-Lincoln USA.*

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[*] Corresponding Author;
E. Mail:
olumidedara01@gmail.com

ABSTRACT

Leaf area (LA) is a valuable key for plant physiological studies, therefore accurate and simple models for LA determination are important for many experimental comparisons. Field experiment was conducted at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta (07°15'N, 03° 25'E) in the forest-savannah transition zone of South West Nigeria in 2013 to estimate leaf area (LA) of peppermint *(Mentha piperita* L.) using functions between plant LA and fresh weight (FW), dry weights (DW) and leaf dimensions (width-W and length-L) to identify appropriate functions for use in models estimating leaf area of peppermint. Leaf samples were randomly selected from the lower, middle and upper parts of the plant at 30, 60, 90 and 120 days after transplanting (DAT). Leaf length, width, L^2, W^2, product of these dimensions and leaf fresh and dry weights of 150 leaf samples were assessed and compared with actual leaf area measured by graph tracing method, to test their accuracy and reliability using Y = a + bX model. There was a highly significant correlation (r = 0.6 to 0.9) between actual leaf area and the corresponding leaf length, width, L^2, W^2, product of these dimensions and leaf fresh and dry weights. Regression analyses of LA versus FW, DW, L, W, L^2, W^2 and the products of these dimension revealed several models that could be used for estimating the area of individual peppermint leaf. Among the models, one based on length dimension (LA = a + bL) r = 0.9, R^2 = 0.96, RMSE = 0.03 was the most accurate. To validate this model, actual leaf area of 60 leaf samples obtained by graph tracing method was compared with leaf area estimated by the model at 30, 60, 90 and 120 DAS in another trial in 2014 wet season. The leaf area estimated by the models strongly agreed with the measured value of leaf area as evident from high value of R^2 (0.99) and low RMSE (0.03). The validation of the models indicates that model (LA = a + bL) was accurate and reliable to determine the leaf area of peppermint and therefore would be very useful for field workers dealing with large samples.

Introduction

Peppermint (*Mentha piperita* L.) is a spicy herb of the Lamiaceae plant family, which has about 3500 species distributed among 210 genera (Blank *et al*., 2004). It is cultivated as edible herb in many countries and valued mainly for its fresh or dried leaves, and essential oil extracted from its leaves. The leaves are usually harvested and used for fresh consumption, flavorants, herbal preparations or condiments (Wink, 2003; Beemnet *et al*., 2010). Although peppermint is more common in temperate regions, it has been found adaptable to tropical conditions. Previous research on peppermint as a new crop in Nigeria revealed a great potential for its adaptability and cultivation (Joseph-Adekunle and Daramola, 2014). The leaves of peppermint are the commercially important plant parts, and by estimating leaf area (LA), the production could be predicted. Also, LA is a key factor for physiological studies involving plant growth, transpiration, light interception, photosynthetic efficiency, evaporation and also responses to fertilizers and irrigation (Blanco and Folegatti, 2005) as well as essential oil extracted from the leaves of peppermint. Thus, LA strongly influences growth and productivity and its estimation can be a fundamental component of crop growth models (Rouphael *et al*., 2010).

Various methods have been reported for measuring the leaf area of crops. These include using a planimeter (Fallovo *et al*., 2008), fixing a camera with image analysis software (Granier *et al*., 2002), tracing individual leaves on graph paper (graph method) and measuring of weight of leaves (Montero *et al*., 2000). However, most of these methods require sophisticated tools, which are costly and not easily available in most developing countries. Besides, other methods devised to facilitate the measurement of LA such as graph tracing and photographing is time-consuming, labour intensive and requires the excision of leaves from the plants which results in canopy damage and reduction in photosynthetic surface area of intact plant leaves and might also cause problems to other measurements (Cristofori *et al*., 2007). An appropriate method of leaf area measurement in peppermint must not reduce or damage the leaves, which is the major product for which the crop is grown.

The use of regression models for estimating leaf area can provide simple, quick, accurate, reliable, inexpensive, rapid, and non-destructive alternative method to within 0.05 accuracy (Raju *et al*., 1991; Uzun and Celik, 1999). Such models eliminate the need for leaf area meters and also save time as compared to cumbersome geometric reconstructions (NeSmith, 1992). Furthermore, this method can allow the replication of measurements during the growth periods, reduces variability in experiment as compared to destructive sampling (NeSmith, 1992) and are useful in studying plant activities, which requires a non-destructive method of leaf area measurement and also when the number of available plants is limited (Pinto *et al*., 2004).

The usual procedure of this method involves measuring lengths, widths and areas of samples of leaf and then calculating several regression equations to estimate areas of subsequent leaf samples (Pouono *et al*., 1990; Pinto *et al*., 2004). Although the importance of developing models as a rapid measurement of leaf area in agronomic and physiological studies is well known and established for other crops in the literature (Bhatt and Chanda, 2003; Lu *et al*., 2004; Gamper, 2005) such models have not yet been established for estimating the leaf area of peppermint in Nigeria and elsewhere. This study was therefore undertaken with the objective of developing the best matching regression equation for estimating areas of intact peppermint leaves using functions between plant LA and plant vegetative characteristics.

Materials and Methods

The study was conducted at the Teaching and Research Farm of the Federal University of

Table 1. Descriptive statistic of the leaf parameters measured during the experiment in 2013.

	Leaf Area (cm^2)	Length (cm)	Width (cm)	L^2 (cm^2)	W^2 (cm^2)	L + W (cm)	L×W (cm)	$L^2×W^2$ (cm^2)	$L^2 + W^2$ (cm^2)	FW (g)	DW (g)
Min	3	1.2	1.1	1.4	1.2	1.3	2.3	2.7	1.7	0.04	0.01
Max	20.4	10	6.7	100	44.8	6.7	16.7	144.8	4489	0.31	0.07
Mean	8.9	4.4	2.4	21.9	6.6	11.9	6.8	28.5	236.6	0.1	0.03
SD	3.7	1.4	0.6	14.7	4.1	2.0	7.4	390.6	18.4	0.06	0.01
Var	14.0	2.0	0.4	216.4	16.8	4.2	55.3	1525.5	341.0	0.04	0.0002

Table 2. Fitted coefficient (b), constant (a), root mean square error (RMSE) correlation coefficient (r) and coefficients of determination (r^2) values of the models used to estimate peppermint leaf area (LA) of single leaves from length (L) and width (W) measurements.

		Fitted coefficient and constant				
	Model tested	**a**	**b**	**R^2**	**r**	**RMSE**
1.	LA = a + bL	0.9877	0.3767	0.96	0.9	0.03
2.	LA = a + bW	1.4123	0.0128	0.72	0.8	0.09
3.	LA = a + bL^2	-0.7279	0.2854	0.74	0.8	0.7
4.	LA = a + bW^2	1.0595	0.0661	0.54	0.7	0.2
5.	LA = a + bLW	0.7404	0.1373	0.67	0.8	0.3
6.	LA = a + b (L + W)	3.4113	0.0434	0.87	0.9	0.05
7.	LA = a + b ($L^2 + W^2$)	0.1317	0.3515	0.72	0.8	0.6
8.	LA = a + bL^2W^2	-143	4.226	0.23	0.6	0.7
9.	LA = a + bFW	0.0134	0.0014	0.71	0.8	0.05
10.	LA = a + bDW	0.0026	0.0003	0.89	0.9	0.04

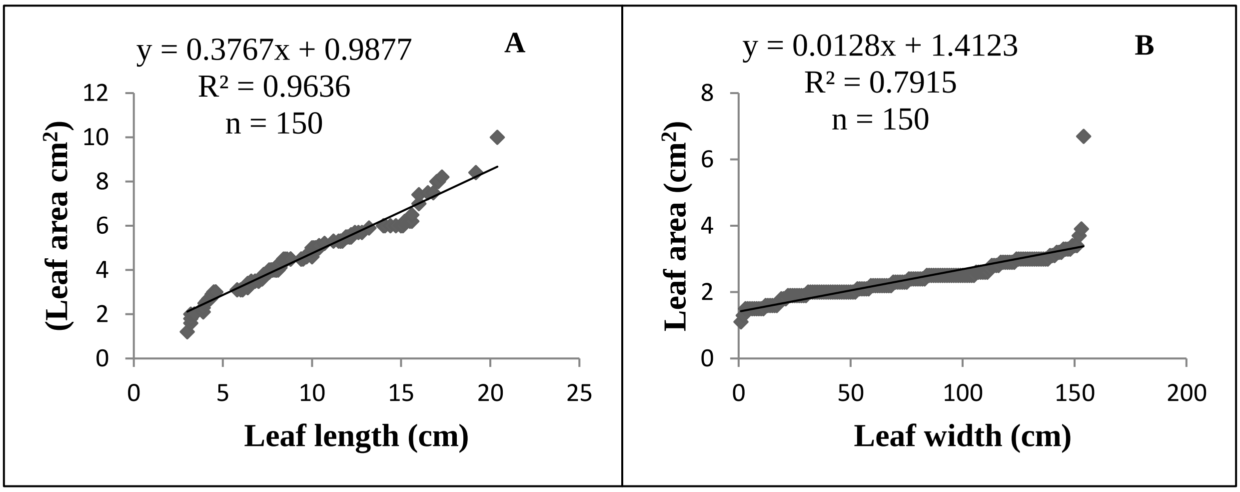

Figure 1. A - Relationship between leaf area (LA) and leaf length (L) and B- between (LA) and leaf width (w)

Agriculture, Abeokuta (07 15’N 03 25’E 159 m above sea level) Ogun State in the Forest-Savanna transition agro ecological zone of South West Nigeria during 2013 and 2014 rainy seasons. Peppermint vines cuttings of 10 cm length with 5 nodes each were planted in seed trays each containing 21 kg of sieved and sterilized top soil. The site of the experiment was disc-ploughed and harrowed at two weeks interval, and leveled manually. Vigorous six weeks old seedlings were later transplanted to the field with planting density of 50 x 30 cm to give 48 plants per plot. The experimental design was arranged in a randomized complete block design with three replications and individual plot sizes of 2.1 × 2.5 m^2. The soil at the experimental sites was sandy loam with high proportion of sand (89.8), 5.4% silt, 4.8 % clay, 0.35% nitrogen and pH of 6.2. The growing conditions were optimum (max. and min. temperature 33 ºC and 29 ºC, respectively and RH: 69%). Leaf sampling was done at 30, 60, 90 and 120 days after transplanting (DAT) and 150 fully expanded, healthy leaves were collected from three replications (50 leaves per replications) from the lower, middle and upper parts of 12 tagged plants in the middle of each plot. Immediately after cutting, leaves were placed in plastic bags and were transferred to the University’s horticultural laboratory. The length (L, in cm), width (W, in cm) and area (A, in cm^2) of single leaves were determined. Leaf length (L) was measured from lamina tip to the point of intersection of the lamina and stem and width (W) were measured from tip to tip between the widest lamina with a simple ruler. The LA of the 150 leaves were estimated by graph paper tracing. The corresponding leaf dry weights were obtained after oven drying at 70 ºC for 48 h and weighed. The actual leaf area (dependent variable) was then regressed on their linear measurements (independent variables), including, L, W, L^2, W^2, the products of these dimension (L+W, L×W, L/W, $L^2 \times W^2$, $L^2 + W^2$) and also with dry weight of leaves to identify appropriate functions for use in models estimating leaf area of peppermint. Root mean square error (RMSE) and the values of the coefficients (b) and constants (a) were also reported. The estimated LA was determined by fitting the equation and the final model was selected based on the combination of the highest coefficient of determination (R^2) and lowest RMSE. In addition, for two-dimensional models, involving L and W, the variance inflation factor (VIF) and the tolerance value (T) as stated by Rouphael *et al*. (2010) and Souza *et al*. (2015) were used to test the collinearity. If the VIF value was higher than 10 or if T value was smaller than 0.10, then collinearity may have more than a trivial impact on the estimates of the parameters, and consequently one of them should be excluded from the model. VIF lie between 1.6-5.0 (< 10) and T between 0.2-0.6 (T > 0.10), indicating that L and W can be used without collinearity and can both be included in the model. All statistical analysis and testing of model was done using GENSTAT discovery package

Results

The averages, maximum, minimum and standard deviation for leaf area, length (L), width (W), L^2, W^2, the products of these dimension (L+W, L×W, L/W, $L^2 \times W^2$, $L^2 + W^2$) and the fresh and dry weights of single leaves of peppermint at different sampling stages are shown in (Table 1). High amplitude (difference between minimum and maximum values) was observed for each measured variable (1.2 cm ≤ length ≤ 10.0 cm, 1.1 cm ≤ width ≤ 6.7 cm, 1.4 cm^2 ≤ $length^2$ ≤ 100, 1.2 cm^2 ≤ $width^2$ ≤ 44.8, 2.3 cm ≤ length + width ≤ 16.7, 1.3 cm^2 ≤ length × width ≤ 67.0 cm^2, 2.6 cm^2 ≤ $length^2$ + $width^2$ ≤ 144.8, 1.7 cm^2 ≤ $length^2$ × $width^2$ ≤ 4487, 0.04 g ≤ fresh weight ≤ 0.3 g and 0.01 g ≤ dry weight ≤ 0.07 g) in leaves used for the mathematical generation of models of leaf area estimation (Table 1).

There was a highly significant positive correlation between actual leaf area (LA) and Leaf length, leaf width and functions of these dimensions using Y = a + bX, (r = 0.6 - 0.9) (Tables 2). Similarly, significant correlation was observed between LA and leaf fresh and dry weights described by Y = a +

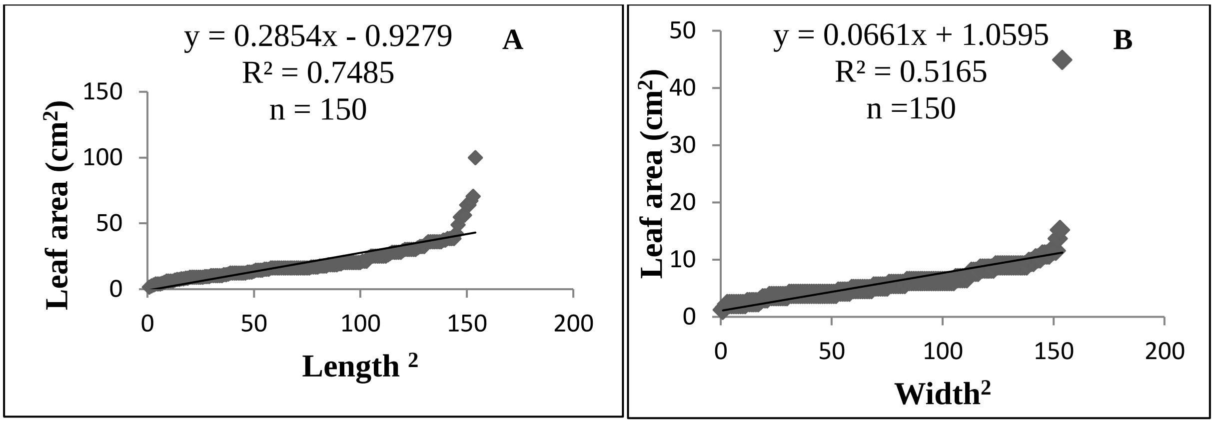

Figure 2. A - Relationship between leaf area (LA) and leaf length (L^2) and B- between (LA) and leaf width (W^2) of single leaves of peppermint

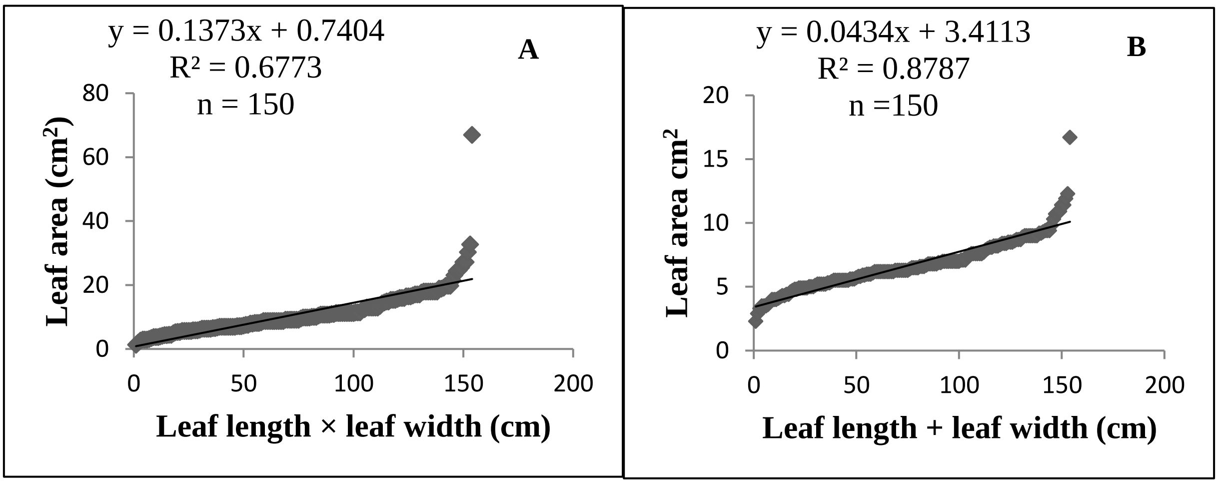

Figure 3. A -Relationship between leaf area (LA) and leaf length × leaf width and B- between (LA) and leaf length + leaf width of single leaves of peppermint

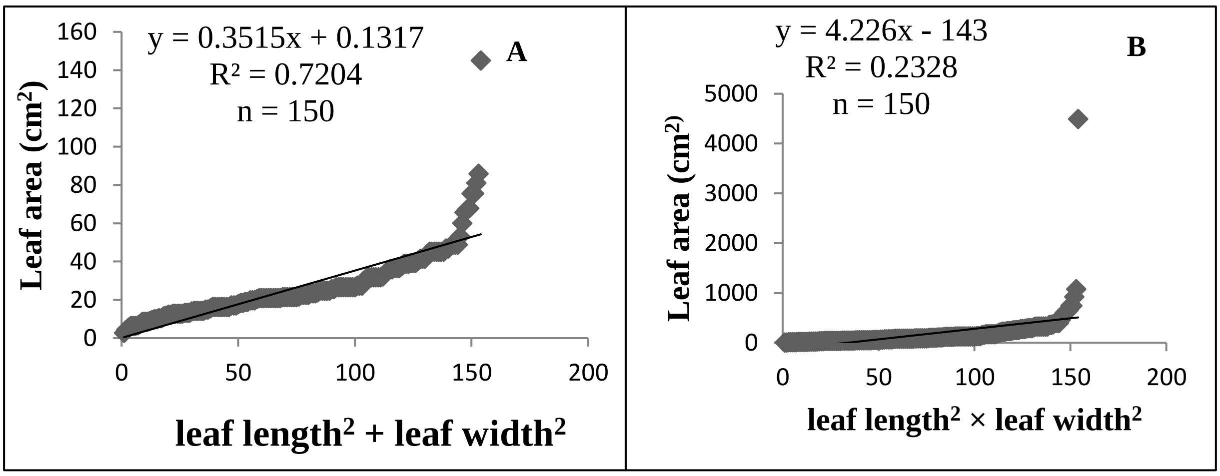

Figure 4. A -Relationship between leaf area (LA) and leaf length^2 + leaf width^2 and B- between (LA) and leaf length^2 × leaf width^2 of single leaves of peppermint

bX (r = 0.8 and 0.9, respectively) (Tables 2). Root mean square error, and coefficients of determination of the models developed are also shown in Table 2. Based on selection criteria previously described (highest R^2, and lowest RMSE) we selected the best model for estimating leaf area of peppermint. Except for models 3, 4, 5 and 8, all other models produced a coefficient of determination (R^2) equal to or greater than 0.72 (Tables 1). From the result of this study, models 2, 3, 4, 5, 8 and 9 are less acceptable for estimating leaf area of peppermint due to their lower coefficient of determination (R^2; 0.72, 0.74, 0.54, 0.67, 0.23, 0.71 respectively) and higher RMSE values (0.09, 0.7, 0.2, 0.3, 0.7 and 0.05, respectively) while models 1, 6 and 10 are more acceptable for estimating leaf area of peppermint due to their higher coefficient of determination (R2; 0.96, 0.87 and 0.89, respectively) and lower RMSE values (0.03, 0.05 and 0.04, respectively) (Table 2).

Model validation

To validate the best model, 60 leaves of peppermint were taken from different experiment during 2014 rainy season to compare leaf area estimated by the linear model Y = a + bX with actual leaf area as determined by graph tracing method. Actual leaf areas and leaf length were determined by the previously described procedure. Leaf area of individual leaves was predicted using the best model from the calibration experiment and was compared with the actual leaf area. Regression analyses was conducted and comparisons was made between measured versus calculated leaf area of leaves collected from different experiment during 2014 by using the best model (LA = a + bL) where LA is individual leaf area (cm^2), L is the leaf length (cm)

The leaf area estimated by the model strongly agreed with the measured value of leaf area of the leaves as evident from high value of R^2 (0.9) and low RMSE (0.03) (Figures 5). The validation of the model indicates that peppermint leaf area could be measured rapidly and accurately by using the developed model.

Discussion

Leaf area is one of the important growth factor for plants especially in vegetables and lack of accurate model is a limitation for calculating LA (Kandiannan *et al*., 2009). Linear regression model (Y = a + bX) has been found to be accurate in leaf area estimation of pawpaw (*Carica papaya*) by Aiyelaagbe and Fawusi (1988), and pumpkin (*Cucurbita maxima*) by Salau and Olasantan (2004). Result of the current study showed that area of peppermint leaves is well correlated to its length (LA = a + bL), the addition of its length and width (LA = a + b (L + W), and its dry weight (LA = a + bDW) with high R^2 values 0.96, 0.87 and 0.89, respectively. Souza *et al*. (2015) have earlier reported that values of R^2 and RMSE are important to validate a regression model. The close relationship between measured leaf area and calculated leaf area based on combination of leaf length and width found in this study is in agreement with those reported Kandiannan (2009) in ginger and Potdar (1991) in banana. In this study, leaf length solely provided the best variable to calculation leaf area of peppermint with highest R^2 value. This result is in line with that of Cho *et al*. (2007) who stated that a single variable of either leaf length or leaf width has a good correlation with leaf area. Similar result was also reported by Mousavi *et al*., (2011) for basil (*Ocimum bacilicum* L.). In agreement with the result of this study, Ma *et al*., (1992) also showed estimate of leaf area from leaf dry weight.

Out of the best three models, model 6- (LA = a + b (L + W) with relationship between length and width would require more measurements (length and width), which would probably imply the use of higher working hours and a higher margin of error. Model 10- (LA = a + bDW) on the other hand, involving the relationship of leaf area with leaf dry weight is a destructive method because it will involve excision of leaves from the plant. Such method may not be the most appropriate because it will reduce the number of peppermint leaves which is the major product for which the crop is grown.

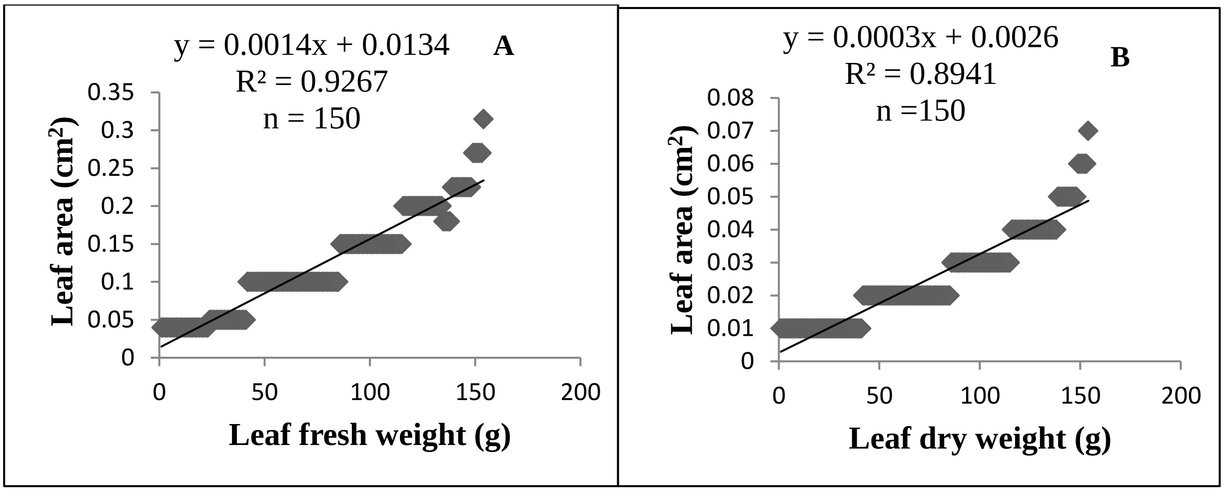

Figure 5. A -Relationship between leaf area (LA) and leaf fresh weight and B- between (LA) and leaf dry weight of single leaves of peppermint

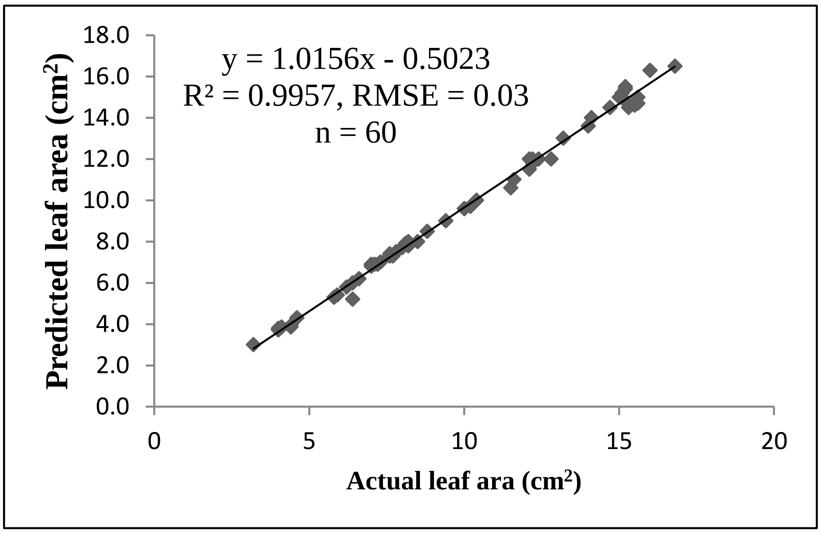

Figure 5. Comparison of actual and predicted leaf area in peppermint (n=60)

Therefore, from the result of this study, the best method for estimating the leaf area of peppermint is model 1- (LA = a + bL) with the highest coefficient of determination (R^2 =0.96) and lowest RMSE (0.03). The validation of the model showed a strong agreement between predicted and measured data and could be estimated quickly, accurately, and non-destructively by using the developed model. The reliability of these models for estimating leaf area of peppermint in this study proved highly satisfactory.

Conclusion

The results from this study indicate that leaf area determination of peppermint could be estimated from the relationship with leaf length using linear equation Y = 0.9877 + 0.3767L (R^2 = 0.96). This model would enable researchers to make non-destructive measurements and repeated measurements on the same leaves. This method showed high correlation in estimation of leaf area and can accurately estimate the leaf area of single leaves without the use of any expensive instrument. The leaf area of peppermint could also be estimated from the relationship with both leaf length and width using linear equation Y = 3.4113 + 0.0434 (L + W) (R^2 = 0.87) and from the relationship with dry weight using linear equation Y = 0.0026 + 0.0003DW. However, estimation of leaf area of peppermint from model involving relationship with both length and width requires more measurements, use of higher working hours and a higher margin of error while using model with the relationship with dry weight is a destructive method with consequence excision of peppermint leaves.

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