EVALUATION OF THREE METHODS FOR ESTIMATING LEAF AREA INDEX OF COWPEA (*Vigna unguiculata*)

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ABSTRACT

Leaf Area Index (LAI) is a concept that cuts across agricultural sciences and agricultural engineering with an encapsulating feature in environmental engineering. It is one of the most difficult to quantify properly owing to large spatial and temporal variability. This paper discusses briefly LAI and the use of three methods which are non-destructive in determination of its value for cowpea, namely: the empirical formulae, the graphical and the image processing methods. Cowpea seeds were planted and samples were marked for determination of LAI by the three methods. The results showed that image processing as a more accurate and promising method compared to the other two.

Keywords: *Leaf Area Index, Empirical Formula, Graphical, Image Processing, Statistical Analysis*

Symbols

LAI,	Leaf area index
LA,	Leaf area of each leaf in cm²
L,	Maximum width of the central leaflet of each plant in cm.
A,	Area of the soil occupied per plant in cm².
A_t,	Area of traced leaf portion in cm²
A_p,	Area of picture frame in cm²

1.0 INTRODUCTION

Leaf Area Index (LAI) was first defined as the total one sided area of photosynthetic tissue per unit ground surface area (He et al., 2007). For deciduous trees with flat leaves, this definition is useable because both sides of a leaf have the same surface area. However, if foliage elements are not flat, but wrinkled, bent or rolled, the one sided area is not clearly defined. The problem exists for coniferous trees, as needles may be cylindrical or hemi-cylindrical (Gower and Norman, 1991). Some researchers therefore proposed a projected leaf area in order to take into account the irregular form of needles and leaves (Jonckheere et al., 2004; Bolstad and Gower, 1990). Myeni et al. (1997), consequently defined LAI as the maximal projected leaf area per unit ground surface. On the basis of canopy-atmosphere interface where most of the energy fluxes exchange. Breda (2003) defined LAI as the total one-sided area of leaf tissue per unit ground surface area; this definition was applied in Hosoi and Omasa (2007).

Leaf Area Index (LAI) according to Tewolde et al. (2005), is a key input in the analysis of crop growth and productivity, water use, and in the management of weeds and other pests. LAI is critical to understanding many aspects of crop development, growth, and management (Wilhelm et al., 2000). The interaction between the vegetative surface and green vegetation as a whole with the atmosphere in terms of radiation uptake, precipitation, interception, energy conversion, momentum and gas exchange, is substantially determined by the vegetative surface (Monteith and Unsworth, 1990). De-Jesus et al. (2001), therefore reported that a determinant factor in mechanisms such as radiation interception, water and energy exchange of a crop is the leaf area. This interaction allows the agricultural and environmental Engineer to blend in their hydrological study. Green plants are essential to man in that they bring an ecological balance in the environment there is therefore the need to understand the interaction between crop growth and environment hence accurate measurements of LAI are essential.

Ecophysiologists, botanists, agronomists, farmers, foresters, ecologists, modelers, require information about canopy LAI which is difficult to quantify due to its spatial (horizontal and vertical) and temporal variability (Wilhelm et al., 2000; Breda, 2003). Dependence of LAI on species composition, development stage, seasonality, prevailing site conditions, management practices, planting density, environmental factors, combined with the difference in assessment methods, may therefore lead to under or over estimation of LAI values (Tewolde et al., 2005).

There are two distinct methods for LAI estimation, the direct and indirect methods (Breda, 2003). The direct methods are the most precise but have the disadvantage of being extremely time consuming and as a consequence making a large-scale implementation only marginally feasible (De-Jesus et al., 2001). De-Jesus et al. (2001) had an empirical approach in measuring the maximum width of the leaf with respect to the area it covered on the soil. All direct methods were destructive in nature except the empirical formulae method. In the indirect methods the leaf area is inferred from observations of another variable. Hence, they are generally faster to compute, amendable to automation and thereby allow for a larger spatial sample to be obtained. It is divided into two (2) categories: indirect contact LAI and indirect non-contact LAI.

The indirect methods infer LAI are non destructive (Pelsen, 2007) and measures LAI from the transmission of radiation through canopy, and are based on statistical and probabilistic approach to foliar element distribution and arrangement in the canopy (Breda, 2003). A number of research work have been reported on the indirect method for the determination of vegetation indices in automated crop imaging especially using image processing for spectra analysis in remote sensing, these include, Barr et al., (2004); Wang et al., (2005); Yang et al., (2003); Hague et al., (2006); Zheng and Moskal, (2009); and Meyer and Neto, (2008).

This research was set up to evaluate three (3) methods of measuring LAI using Cowpea (*Vigna unguiculata*) which is a tropical lowland grain legume grown in Africa. It grows erect up to 80 cm in height having pods up to 12 cm long and seeds up to 6 mm (Langer, 1982). The evaluation was done with Microsoft SPSS 10 to analyze data obtained from the three methods employed in LAI determination viz; empirical formulae, graphical and the image processing methods.

2.0 MATERIALS AND METHODS

The experiments were carried out at the Department of Agricultural and Environmental Engineering, Faculty of Technology, University of Ibadan, Ibadan, Nigeria.

2.1 Collection and Analysis of Soil Samples

Four (4) soil samples were collected from four (4) locations within the University of Ibadan: the Faculty of Agriculture, the Faculty of Technology, Independence Hall and Parry road. The samples were labeled and referred to as Agriculture, Technology, Indy and Parry samples. The physical characteristics, percentage sand, silt and clay were determined using the Bouyoucos Hydrometer method while the USDA soil textural classes were Sandy, Sandy-clay loam, Sandy, and Loamy sand soil respectively.

2.2 Seedbed Preparation and Experimental Design

Ife-Brown Cowpea seeds with a maturity period of 60 days obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria were used for this study.

A randomized block design was used with one treatment and three replications on a plot 7 m by 7 m demarcated into two sections by 1m spacing into 3 m by 7 m each. A hundred and twenty holes were dug 20 cm deep at regular intervals of 30 cm on each row (that is 10 holes per row for 6 rows on each 3m by 7m plot, giving a total of 12 rows for the entire 7m by 7m plot). Two hundred and forty seeds were sown, two seeds per hole. Each 3m by 7m consisted of 6 rows, 3 m long and spaced 1 m apart. The plot was maintained with conventional agronomic practices, no fertilizer was used, and manual weed removal was carried out daily throughout experimental period. Spraying of insecticide was done to prevent damage to planted crop.

Data used to determine water application amount was obtained from IITA (International Institute of Tropical Agriculture) weather station, Ibadan. The following data were imputed into CROPWAT 4 Windows Programme to calculate reference crop evapotranspiration. Minimum and maximum Temperature (^{0}C), Humidity (%), Wind speed (km/hr), Sunshine hours, Solar Radiation (MJ/m^2/day), Altitude (228m above Mean Sea Level) and Latitude (7.26^{0}N). Water applied was based on actual crop evapotranspiration from equation 1.

$$ET = Kc \times ETo \quad \ldots\ldots(1)$$

Where; ET = Actual evapotranspiration for specific crop (mm/day);

ET_O = potential evapotranspiration or reference crop evapotranspiration (mm/day);

K_C = crop coefficient (1.152 for green and dry beans mid stage growth in sub-humid climate with minimum Relative Humidity approximately 45%).

On the pilot scale experimental field, actual daily crop water requirement for Cowpea computed from CROPWAT was applied with graduated watering can.

One plant was selected from each replicate of each treatment, making a total of 12 plants. These were marked and the leaf area of all the leaves on each marked plant was estimated daily for the initial and mid stage crop growth period which lasted 14 days using three methods; empirical, graphical and image processing. A total of 168 readings for each method were obtained.

The leaves were not growing significantly on a daily basis. However, the 8th, 14th and 21st days shows significant differences in leave growth. The initial and mid stage growth period of Cowpea does not witness remarkable leaf drops. Throughout the experimental period, less than 10 leaves dropped on both the marked and un-marked plants.

2.3 The Empirical Formula Method

This is also known as the central leaflet method. The maximum width of the central leaflet of each marked leaf was measured in situ using a precision steel rule and the leaf area was calculated using the equation developed for beans by De-Jesus et al., (2001).

$$LA = 2.1371\left(L^{1.9642}\right) - 2.7013 \quad \ldots\ldots(2)$$

where; LA = Leaf area of each leaf in cm²

L = maximum width of the central leaflet of each plant in cm.

Hence for every marked plant the leaf area index was obtained by dividing the sum of the leaf areas of all leaves of each marked plant by the area of the soil occupied per plant. Thus

$$LAI = \frac{\sum LA}{A} \quad \ldots\ldots(3)$$

Where; A = area of the soil occupied per plant in cm².

This area was not field measured. The area is given as the area of picture frame and is the same for the three methods compared. The Tekxon 410 digital camera (8 mega pixel with 4x optical and digital zoom) used to take the pictures was set to the same magnification (zoom distance) throughout the experimentation period for uniformity.

3.0 THE GRAPHICAL METHOD

In this approach, plan view pictures of every marked plant were taken with a Tekxon 410 digital camera. In this case, because of large amount of data to be processed only one plant was selected from each replicate of each treatment. These pictures were printed on 1cm² square grid papers. From the graph sheet, the area of the combined leaves of the plant was calculated. The LAI of each marked plant was thus obtained by dividing the area of the traced leaf portion by the measured area of the picture frame.

$$LAI = \frac{A_t}{A_p} \qquad (3)$$

Where; A_t is the area of traced leaf portion in cm^2 and A_p is the area of picture frame in cm^2

3.1 Image Processing Method

The digital images of the samples obtained in the graphical method were processed using Paint Shop Pro 7.02 to convert the images to portable pixel map (ppm) format. This is a digitized image file. A programme was written in FORTRAN based on the principles of thresholding and eight-connectivity for edge detection in image processing. The ratio of the area covered by the picture element (pixels) on the portions of the image representing the leaf to that of the whole image was obtained as the number of pixels representing the leaf to the total number of pixels. Extracts of the predominant green features of the cowpea leaves from the entire picture gave the area covered by the leaves thus differentiating them from the background. Salford FORTRAN online graphic display was used during the execution of the programme. LAI was calculated as the ratio of total number of pixels of the predominant green portion of picture to the total number of pixels of the entire picture.

3.2 Evaluation and Analysis

The values from the three methods for estimating LAI were analyzed using SPSS for windows. The analyses employed a two-way test, one-way test and correlation test. ANOVA was used and the two-way test checked the significance between the block and treatment while the one-way test checked only for the treatment. The correlation employed the Pearson correlation and the significant 2-tailed method for the analysis of the three methods.

4.0 RESULTS AND DISCUSSION

The physical characteristics, percentage sand, silt and clay and the soil textural classes obtained are presented in Tables 1 and 2. Figure 1 shows the original image of one of the samples and the image processing output obtained from the online graphics display.

The variation of the LAI with days was plotted for the cowpea planted on Agriculture soil (Figure 2). The functional relationships obtained from the regression analysis with the t-statistics of each order showing level of significance are presented in the following equations;

Empirical

$LAI = 0.0778 - 0.00198d + 0.0023d^2 - 0.000228d^3 + 7.05^{-6}d^4$ ($R^2 = 0.987$)

t-stat (11.70) (-0.349) (1.553) (-1.571) (1.471)

Graphical

$LAI = 0.172 - 0.0792d + 0.0275d^2 - 0.0027d^3 + 8.47^{-5}d^4$ ($R^2 = 0.939$)

t-stat (3.62) (1.95) (2.62) (2.61) (2.48)

Image Processing

$LAI = 0.142 - 0.052d + 0.0219d^2 - 0.00243d^3 + 8.41^{-5}d^4$ ($R^2 = 0.946$)

t-stat (4.24) (0.102) (0.016) (0.009) (0.007)

where; d is the number of days

The best lines of fits were obtained using a fourth order polynomial fit. However from the equations, the fourth derivatives are negligible although the third order polynomial fit did not show good fit. The LAI can then be estimated using the equations shown. The empirical method showed almost a straight line trend with a positive slope throughout (Figure 2). This was because it used all the leaves neglecting overlap of leaves for its computation. The other two methods showed polynomial trends of LAI values with days probably because they take overlaps and leaf shapes into consideration.

Table 1: Colour, Appearance and Texture of the Soil Samples

Soil Sample Location	Colour	Appearance/texture
Agriculture	Black	Finely divided
Technology	Brick red	Stony
Parry road	Black	Finely divided
Independence hall	Light black	Stony and cement like

Table 2: Clay, Silt and Sand Content in each Soil Sample

Soil sample location	% Clay	% Silt	% Sand	Soil class
Independence hall	6.8	6.0	87.2	Sandy soil
Technology	24.8	6.0	69.2	Sandy- clay loam
Parry road	4.2	7.0	88.8	Sandy soil
Agriculture	11.2	2.0	68.8	Loamy- sand

Table 3: Block and Treatments

		Value label		N
Block	1	I		84
	2	II		84
			Total	168
Treatments	1	Agriculture		42
	2	Independence		42
	3	Parry road		42
	4	Technology		42
			Total	168

Where; N is sample size

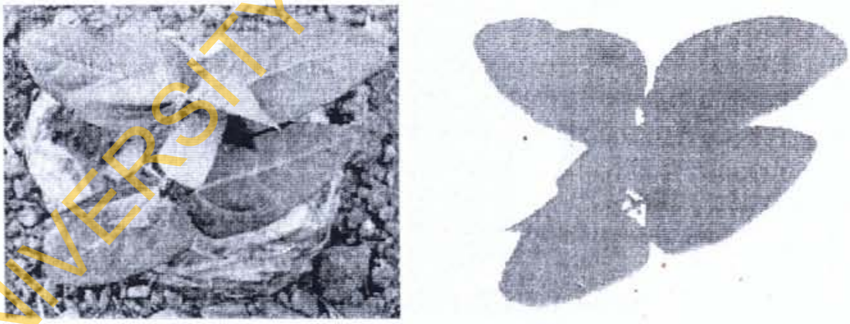

Figure 1: Sample image and image process analysed image on day 14

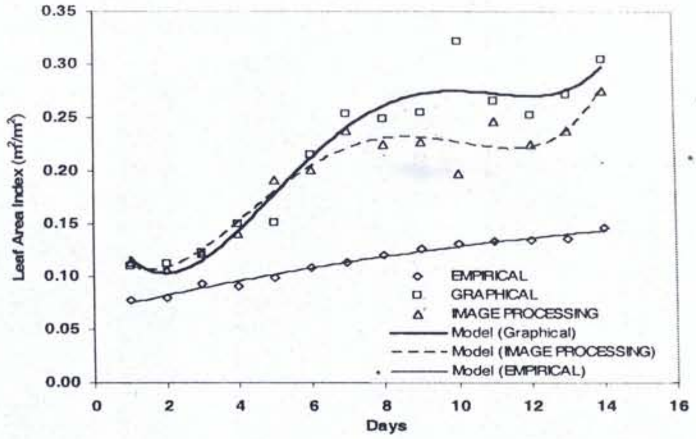

Figure 2: LAI for plant 1 showing functional relationship curves

In this regard the graphical and image processing methods can be taken as better estimates since they reflect real time situations as opposed to apparent values. Further analyses through statistical analysis were used to obtain the variations within blocks and treatments (Table 3).

The results of the descriptive statistics from the SPSS analysis of the LAI obtained for the blocks and the combined blocks are as presented in Figures 3-5.

4.1 Empirical Formula Method

The results of the LAI obtained across blocks and treatments subjected to two-way test are presented in Table 4. There was a significant difference in the LAI values (that is, F-calculated is greater than F-tabulated) between the subject effects.

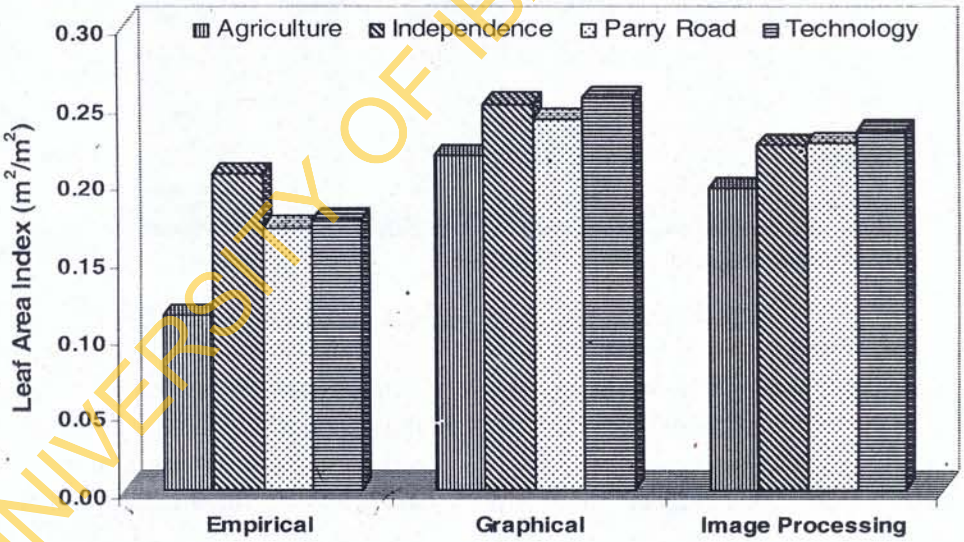

Figure 3: Average LAI for Block I

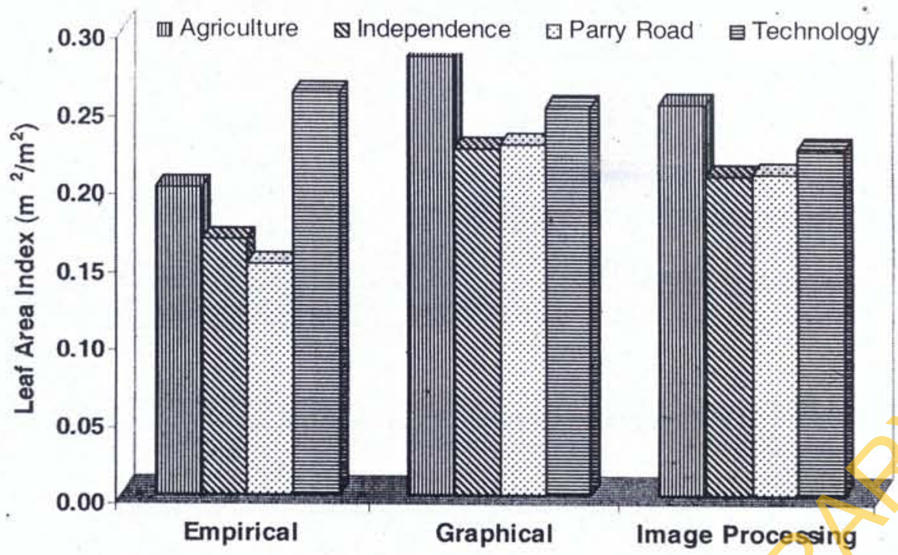

Figure 4: Average LAI for Block II

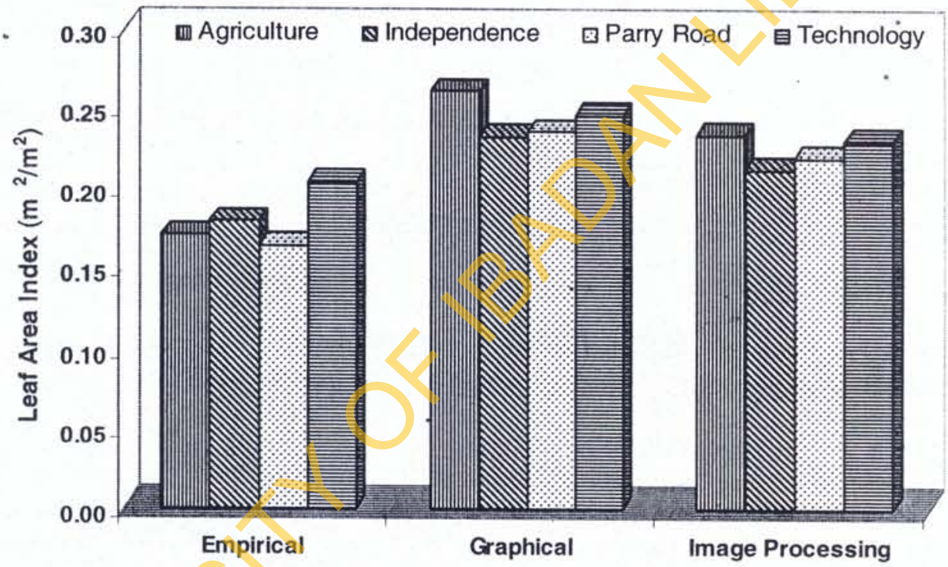

Figure 5: Average LAI for the Combined Blocks

A follow up test using the Duncan Multiple Test which is a post hoc test carried out for the treatments revealed that Technology had a significant difference from the other three (Table 5). This follow up test is always done when there is a significant difference in the ANOVA. This means that the LAI values obtained when using this method showed a significant change during the daily readings.

A similar trend indicating significant difference in the LAI values was obtained when the results were subjected to one-way test (Table 6). This was also subjected to the Duncan test similar to the two-way test. Technology again had a significant difference as presented in Table 7.

Table 4: Two-Way Test of LAI[b] Values between Subject Effects

	Type III Sum of Squares	Df	Mean Square	F cal	Sig.
MODEL	5.461[a]	5	1.092	380.664	0.000
BLOCK	0.028	1	0.028	9.890	0.002
TREATMENT	0.047	3	0.016	5.469	0.001
ERROR	0.468	163	0.003		
TOTAL	5.929	168			

a. $R^2 = 0.921$ (Adjusted $R^2 = 0.919$), b. Method = Empirical Formulae

Table 5: Post Hoc Test of LAI[b] Values of Treatments in Homogenous Subsets

Duncan[a]

	N	SUBSET, α =0.05	SUBSET, α =0.05
		1	2
PARRY	42	0.1636	
AGRICULTURE	42	0.1707	
INDEPENDENCE	42	0.1788	
TECHNOLOGY	42		0.20356
SIG.		0.222	1.000

Means for groups in homogeneous subsets are displayed based on Type III Sum of Squares. The error term is Mean Square (Error) = 2.869E-03, a. Uses Harmonic Mean Sample Size = 42.000, b. Method = Empirical Formulae

Table 6: One-Way Test of LAI[a] Values between and within Groups

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.038	3	0.013	4.211	0.007
Within Groups	0.496	164	0.0033		
Total	0.534	167			

a. Method = Empirical Formulae

Table 7: Post Hoc Test of LAI[b] Values in Homogenous Subsets

Duncan[a]

	N	SUBSET, α =0.05	SUBSET, α =0.05
		1	2
PARRY	42	0.1637	
AGRICULTURE	42	0.1707	
INDEPENDENCE	42	0.1788	
TECHNOLOGY	42		0.2036
SIG.		0.222	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 42.000, b. Method = Empirical Formula

Table 8: Two-Way Test of LAI[b] Values between Subjects Effects

	Type III Sum of Squares	Df	Mean Square	F cal	Sig.
MODEL	10.164[a]	5	2.033	457.776	0.000
BLOCK	0.0109	1	0.001	0.248	0.620
TREATMENT	0.0234	3	0.008	1.759	0.157
ERROR	0.724	163	0.004		
TOTAL	10.888	168			

a. $R^2 = 0.934$ (Adjusted $R^2 = 0.931$), b. Method = Graphical

Table 9: Two-Way Test of LAI[b] Values between Subjects Effects

	Type III Sum of Squares	Df	Mean Square	F cal	Sig.
MODEL	8.428[a]	5	1.686	488.902	0.000
BLOCK	0.0004	1	0.0004	0.122	0.727
TREATMENT	0.0124	3	0.0041	1.201	0.311
ERROR	0.5620	163	0.0034		
TOTAL	8.990	168			

a. $R^2 = 0.937$ (Adjusted $R^2 = 0.936$), b. Method = Image Processing

4.2 *Graphical and Image Processing Methods.*

The two way tests showed no significant difference in the LAI values for Graphical (Table 8) and Image Processing methods (Table 9). The one way test also showed no significant difference in the LAI values for Graphical method (Table 10) and Image processing method (Table 11).

4.3 Correlation Tests

To create a relationship among the three methods for estimating LAI, the Pearson Correlation and the significance 2-tailed tests were carried out on the results. Correlation results in Table 12 show only Pearson Correlation giving meaningful values. Low positive correlations were achieved between Empirical Formulae and the other two (Graphical and Image Processing methods) with R^2 equals 0.356 and 0.357 respectively. While a high positive correlation was achieved between the Graphical and Image Processing methods ($R^2 = 0.802$).

From the results and analyses presented, Empirical formula method showed a significant difference from the other two methods because of its shortcomings of having negative values of leaf areas for leaf widths of 0.1cm to 1.2cm consequently having reduction in LAI values. This made the formula inaccurate, because no matter the size of the leaf an index must be obtained with respect to the area it covers even if it is negligible. In this light, the Graphical and Image processing methods gave higher LAI's. Inaccuracy of this empirical formula method may be attributed to the fact that the formula has not been calibrated for different varieties (species) of Cowpea available.

All the results showed that the Graphical and Image Processing methods have higher LAI's as compared to the Empirical formula. The treatments in the Graphical and Image processing methods follow a pattern where the values of LAI have increments in descending order for Agriculture, Technology, Parry and Independence. This pattern was not achieved in the Empirical Formula method.

Consequently, results from both the Graphical and Image processing methods were well correlated unlike with the graphical method which was more subjected to human error. The Image processing method where every process is computerized and hence errors are minimized would stand out as the best for estimating LAI.

Table 10: One-Way Test of LAI[a] Values between and within Groups

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.024	3	0.0078	1.783	0.152
Within Groups	0.725	164	0.0044		
Total	0.749	167			

a. Method = Graphical

Table 11: One-Way Test of LAI[a] Values between and within Groups

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.012	3	0.0042	1.191	0.315
Within Groups	0.562	164	0.0034		
Total	0.575	167			

a. Method = Image Processing

Table 12: Correlations of the Three Methods

		Empirical Formula	Graphical	Image Processing
Empirical Formula	Pearson correlation	1.000	0.597*	0.598*
	Sig. (2-tailed)		0.000	0.000
Graphical	Pearson correlation	0.597*	1.000	0.896*
	Sig. (2-tailed)	0,000		0.000
Image Processing	Pearson correlation	0.598*	0.896*	1.000
	Sig. (2-tailed)	0.000	.0.000	

- N = 168, Correlation is significant at the 0.01 level (2-tailed)

5.0 CONCLUSION

From the results obtained in this research, it can be deduced that the image processing method is the best of the three methods for measuring or estimating LAI for cowpea plant in tropical environment. It appeared to be more accurate than the other methods since it is computer enhanced rather than being manual thereby subjective to human error. It saves time, less laborious, it is non destructive and can be extended in measuring LAI in forests with minimal errors. Further research works can be conducted on calibrating the empirical formulae method for the different species of Cowpea.

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