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Morphometric Study of the Genus *Indigofera* Linn. (Leguminosae-Papilionoideae) in South-Western Nigeria

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Abstract: The relationship among seven species of *Indigofera* (Leguminosae-Papilionoideae) is described using eight quantitative parameters based on both herbarium and fresh specimens. The results of the multivariate analyses (Principal Component Analysis (PCA) and Cluster Analysis) revealed that three out of the eight quantitative parameters utilized accounted for differences among the taxa. *Indigofera macrophylla* Schum and Thonn. and *Indigofera nummulariifolia* (Linn.) Livera ex Alston were found to be closer with a stronger coefficient of agglomeration (13.390) than *I. hirsuta* Linn. and *I. suffruticosa* Mill. with 2839.673 coefficient of agglomeration. In this study, number of leaflet, leaf length and leaf width have been found to significantly contribute to the delimitation of the seven species of *Indigofera.* These characters can therefore be effectively employed in the taxonomic treatment of other *Indigofera* species that were not included in the present study.

Key words: Morphometrics, morphology, *Indigofera*, Leguminosae, Papilionoideae, numerical taxonomy

INTRODUCTION

The genus *Indigofera* Linn. is a large genus of about 700 species of flowering plants belonging to the sub-family Papilionoideae in the family Fabaceae/ Leguminosae. The species are native mainly to tropical and sub-tropical regions of the world. The genus is one of the nine genera which are members of the tribe Galegeae (Nwachukwu and Mbagwu, 2007). Leguminales was considered by Bentham and Hooker in 1883 as a whole to constitute one family Leguminosae among the dicotyledons with nine tribes. Galegeae however, is one of these tribes.

Hutchinson and Dalziel (1968) recognized 78 species of *Indigofera* in West Africa. Burkill (1995) recognised 60 species while Soladoye and Lewis (2003) recorded 60 species in Nigeria with over 60% abundance in the Northern region of the country and about 27 species distributed across the South Western area of the country. Most of these species are in the savanna ecological zone with a few present in the rainforest area. The reason for the varying number of taxa in this group of plant could be due to the perceived similarities in structural and reproductive biology of the legumes in general.

Indigofera in Greek means indigo dye which is famous for the natural blue colours obtained from the leaflets and branches of this herb. Indigo plants have a single semi-wood stem, dark green leaves that are oval-shaped in most species and clusters of red flowers that look like butterflies and turn into peapods. The flowers are usually in axillary racemes, less often in open or condensed panicles, dense axillary clusters or solitary in the leaf axils, nearly always quite small; corolla usually red or pink. The upper filament is free and the rest are united into a tube, persistent anthers uniform, almost always apiculate. *Indigofera* fruits are oval shaped and elongated, 4-angled or flattened and often curved with many seeds (Bernard, 1979). The species are mostly shrubs, though some are herbaceous and a few can become small trees up to 5-6 ft in height. The dye which is among the most widely used natural dye in the world is obtained mainly from the leaves through a process of fermentation (Dauril, 1965). However, most are dry-season or winter deciduous. *Indigofera* species posses wide range of uses ranging from several economical and ecological purposes, feed for livestock, ornamental, medicinal plant recipes as well as dye for commercial purposes (Burkill, 1995). The most important of the species are *Indigofera arrecta* and *Indigofera tinctoria*. *Indigofera* species are used as food plants by the *larvae* of some *Lepidoptera* species including Turnip Moth. Several of them and especially *Indigofera tinctoria* and *Indigofera suffruticosa* are used to produce the dye indigo.

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The leafy twigs are the main sources of indigo dye used since very ancient times for dyeing textile blue. The leaves and twigs do not contain indigo but colourless precursors that must be extracted and processed to produce the indigo dye. Indigo has been called the king of dyes (Takawira-Nyenya and Cardon, 2005). An indigo dye obtained from the plant is used by the Yoruba dyers of South Western Nigeria to produce indigo cloth called Adire eleko using both tie and dye method, but the plant is not specifically grown for this purpose as opined by Dalziel (1937). *Indigofera hirsuta* has several medicinal uses in Africa. In Kenya, it is used as chest medicine and in Tanganyika; the whole plant is prepared as an external application for back ache (Burkill, 1995). The people of Ethiopia use the root of *Indigofera spicata* as a toothbrush. The stem of *Indigofera tinctoria* is chewed to cure cough and decoction of leaves is used to cure chest pains, epilepsy, nervous disorders, asthma, bronchitis, fever and complaints of stomach, liver, kidney and spleen- especially in Cameroon (Takawira-Nyenya and Cardon, 2005). The twine paste cures dislocation. Also the Warm leaves dismiss bruises (Ibe and Martin, 2005). *Indigofera trita* has some green manure potential and has been used as a cover crop in Zimbabwe (Burkill, 1995).

Morphometrics also known as numerical taxonomy is the application of various mathematical procedures to numerically encode character state data from organisms under study. Even today the fact can not be denied that morphological characters have their own importance in taxonomy and all systems of classifications suggested are based on principles of morphology, therefore, while plants are considered taxonomically the appearance becomes a major criterion. The practice of numerical taxonomy embraces numbers of fundamental assumption and philosophical attitudes towards taxonomic work. However, it has the power to integrate data from a variety of sources, such as anatomy, cytology, ecology, genetics, geography, physiology, chemistry, etc. The product of this operation is often taken to be unbiased indicators of the similarity or difference between the taxa, which were in turn used to arrange taxa in hierarchy (Quike, 1993). In other words, numerical taxonomy is based on the numerical comparison of large number of equally-weighted characters, scored consistently for all the groups under consideration and in which individuals are grouped on the basis of observable similarities (Subrahmanyam, 2006). This method has been used in classifying many plants as well as interpreting results of the taxonomic studies (El-Gazzar, 2008; Abu Zaida *et al*., 2008).

Inspite of the great importance of *Indigofera* species, only little study has been done on their identification as well as classification of the species scattered in Southwestern Nigeria. Available reports on the genus include: morphological and agronomic characterization of *Indigofera* species using multivariate analysis (Hassen *et al*., 2006); leaf anatomy of eight species of *Indigofera* species (Nwachukwu and Mbagwu, 2007); Tannins, starch grains and crystals in some species of *Indigofera* (Nwachukwu and Edeoga, 2006); Novel reports of glands in Neotropical species of *Indigofera* L. (Leguminosae, Papilionoideae) (Marquiafável *et. al.,* 2008). This study thus examines the differences and similarities in macromorphological characters used in delimiting the seven *Indigofera* species (*Indigofera hirsuta* Linn., *I. spicata* Forssk., *I. suffruticosa* Mill., *I. tinctoria* Linn., *I. trita* Linn., *I. nummulariifolia* (Linn.) Livera ex. Alston and *I. macrophylla* Schum. and Thonn.) which are commonly available in the Southwestern part of Nigeria using both herbarium and freshly collected specimens. It is hoped, that the method of numerical taxonomy (Soladoye, 1982; Sonibare *et al*., 2004; Boratynski *et al*., 2008) which are employed in this study will produce a hierarchical classification of the species with a visual interpretation of the taxonomic relationship existing between them. The present study therefore aims at evaluating morphological variations among seven species of *Indigofera* using numerical methods.

MATERIALS AND METHODS

Plant collection: Freshly collected specimens from the field as well as herbarium specimens of *Indigofera hirsuta* Linn., *Indigofera spicata* Forssk., *Indigofera suffruticosa* Mill. *Indigofera tinctoria* Linn., *Indigofera trita* Linn., *Indigofera nummulariifolia* and *Indigofera macrophylla* from the Forest Herbarium, Ibadan, Nigeria were used for the study. The fresh specimens were collected from Ibadan, Oyo State; Ago-Iwoye, Shagamu, Olokemeji in Ogun State and Ilesha in Osun State while the herbarium specimens were accessions previously collected from different parts of South-Western Nigeria (Table 1). The plant parts, leaves, flowers, fruits and stem were collected using secateurs and cutlass. The fresh specimens were pressed using a plant press, which consists of a wooden frame (for rigidity), blotting paper (to absorb moisture) and folded newspaper (to contain the plant material). The plant press was tightened using straps or twines. The objective of pressing plants is to extract moisture in the shortest period of time, while preserving the

Table 1: Voucher information and distribution of fresh and herbarium specimens of *Indigofera* species studied

Plant species	Location	Voucher No.
I. hirsuta Linn.	Ogun State: Shagamu,Egbado North and Abeokuta Oyo State: Lowe-Ibadan	FHI 82911, FHI 35879, FHI 95989, FHI 7524, FHI 100209
I. spicata Forssk.	Ogun State: Shagamu, Ajebandele-yebu and Ilashe-Egbado Oyo State: FRIN, Ibadan	FHI 95910, FHI 98468, FHI 38330, FHI 78484, FHI 27908
I. suffruticosa Mill.	Oyo State: *IITA, Ibadan and Igbetti Osun State: Ilesha Ogun State: Ago-Iwoye	FHI 78983, FHI 46397, FHI 59896, FHI 17244, FHI 86254
I. tinctoria Linn.	Ogun State: Olokemeji and Olumo rock, Abeokuta Ondo State: Idoani	FHI 63465, FHI 23906, FHI 49830, FHI 49832, FHI 88023
I. trita Linn.	Lagos State: Bar Beach Oyo State: Gambari, Ibadan	FHI 60927, FHI 20195, FHI 27042, FHI 2641, KEW 1950
I. macrophylla Schum. and Thonn.	Oyo State: Ibadan	FHI 94160, FHI 72827, FHI 86740, FHI 101937, FHI 10390
I. nummulariifolia (Linn.) Livera ex. Alston	Oyo State: *FRIN, Ibadan	FHI 63380, FHI 39956, FHI 34119, FHI 71544, FHI 106206

*FRIN: Forestry Research Institute of Nigeria, *IITA: International Institute of Tropical Agriculture

morphological integrity of the plant and to yield material that can be readily mounted on herbarium paper (an acid-free cardstock) for long-term storage. The specimens were pressed immediately upon collection in order to obtain the best specimens. The dried specimens were poisoned to prevent them from fungal and insect attack using a mixture containing 5 L of Methylated spirit, 4-6 drops of phenol and 200 mL of mercury (II) chloride. Some of the specimens were poisoned by dipping the whole plants into a basin containing the poison and others were poisoned using a brush dipped in the mixture to brush both the adaxial and abaxial parts of the plant according to the methodology of Bridson and Forman (1992). The poisoned specimens were then mounted on standard mounting sheet/cardboard (16.5×10.5 cm) using glue and kept in between newspapers for drying. The cardboard provides physical support that allows the specimens to be handled and stored with a minimum of damage. Identification and authentication of the species were done at the Forest Herbarium Ibadan (FHI). Field note indicating location of collection, collector's name, habit, determinavit, name of species and plant description were attached to the mounting sheets along with the voucher specimens which were deposited at the Elikaf herbarium of Olabisi Onabanjo University, Ago-Iwoye, Ogun State (Note that this herbarium is yet to be recognized internationally as it is not listed in Holmgren and Keuken, 1974).

Morphometric studies: Morphometric studies were carried out on five mature living and five herbarium specimens of each *Indigofera* species. Length and width of the leaves were measured using a 30 cm meter rule. The length of the leaf was obtained by spreading the middle leaflet on a flat surface on the laboratory bench, while for the width; the same median leaflet was chosen and measured to ensure uniformity (Olowokudejo, 1990). Flowers from herbarium specimens were revived by boiling in water with two to three drops of teepol (soap solution) before measurements were taken. In all, eight different characters were employed for each of the species and data collected were statistically analyzed and recorded. Measurements were taken for eight selected quantitative characters which are: number of leaflet, leaflet length, leaflet width, racemose length, flower length, fruit length, fruit width, number of seeds using a line ruler and twine. The morphological measurements were recorded on recording sheets using many numbers of specimens per species as were available for each taxonomic operational unit (OTU). The mean and standard deviations were calculated for all the eight characters. The values were then entered into a Microsoft excel spreadsheet and raw data were coded to allow analysis using SPSS 10.0 analysis sheet (SPSS Inc., 1999). For analysis, the ratios of these values were calculated. Principal component analysis was also carried out on the eight selected quantitative measurements. The objective was to determine the characters that contributed strongly to the delimitation of the taxa. These parameters were also subjected to Cluster analysis.

RESULTS

Foliar parameters of seven *Indigofera* species in Nigeria were examined with numerical methods. Voucher information and distribution of fresh and herbarium specimens of the species studied are shown in Table 1. The morphological features employed for delimitation of the 7 species with their means and standard deviations are shown in Table 2. From the Principal Component Analysis carried out on the data set, three out of the eight characters examined accounted for about 95% importance in the delimitation of the taxa (Table 3). Similarity matrix based on correlation of *Indigofera* species presented as Table 4 shows that close resemblance of species could be observed when certain characters are employed. For

Table 2: The mean and standard deviation of morphometric characters used in centimeters

Plant species	No. lflt	L. leng	L. width	Rac. leng	Fl. leng	Fr. leng	Fr. width	No. seed
	(cm)							
I. hirsuta	7.0±1.12	4.00±0.45	2.50±0.21	2.50±0.21	4.5±1.24	16.00±3.14	0.0	0.0
I. macrophylla	0.0	2.60±0.42	0.15±0.012	0.00	3.0±0.12	1.70±0.01	0.0	1.0
I. spicata	8.0±2.35	1.65±0.12	0.00	15.00±0.61	5.0±0.45	18.00±4.23	2.0±0.15	7.0±2.15
I. suffruticosa	1.3±0.12	2.50±0.14	0.90±0.1	2.75±1.02	50.0±4.54	32.30±5.12	2.0±0.05	4.0±1.23
I. tinctoria	14.0±2.45	2.00±0.12	1.20±0.04	0.00	6.0±1.25	32.50±5.25	2.0±0.42	11.0±1.25
I. trita	9.0±2.51	2.15±0.24	1.10±0.05	27.00±4.75	3.5±0.12	2.25±0.75	1.3±0.12	10.0±1.4
I. nummulariifolia	3.0±0.45	3.20±0.78	0.85±0.1	1.30±0.12	3.5±2.00	1.50±0.01	1.5±0.42	0.0

No. lflt: No. of leaflets, L. leng: Leaf length, L. width: Leaf width, Rac. leng: Racemose length, Fl. leng: Flower length, Fr. leng: Fruit length, Fr. width: Fruit width, No. seed: No. of seeds

Table 3: Components extracted from principal component analysis

Component	Initial eigen values			Extraction sums of squared loadings		
	Percentge of variance	Cumulative (%)	Total	Percentage of variance	Cumulative (%)	Total
1	3.356	41.951	41.951	3.356	41.951	41.951
2	1.927	24.089	66.040	1.927	24.089	66.040
3	1.647	20.587	86.627	1.647	20.587	86.627
4	0.705	8.812	95.439			
5	0.286	3.580	99.019			
6	7.848E-02	0.981	100.000			
7	-3.182E-16	-3.978E-15	100.000			
8	-3.475E-16	-4.343E-15	100.000			

Extraction method: Principal component analysis

Table 4: Similarity matrix based on Correlation coefficient of *Indigofera* species

Characters	No. lflt	L. leng	L. width	Rac. leng	Fl. leng	Fr. leng	Fr. width	No. seed
No. lflt	1.000	-0.340	0.307	0.321	-0.379	0.364	0.362	0.766
L. leng	-0.340	1.000	0.715	-0.454	-0.066	-0.244	-0.665	-0.792
L. width	0.307	0.715	1.000	-0.133	-0.018	0.181	-0.342	-0.158
Rac. leng	0.321	-0.454	-0.133	1.000	-0.177	-0.284	0.199	0.559
Fl. leng	-0.379	-0.066	-0.018	-0.177	1.000	0.605	0.394	-0.035
Fr. leng	0.364	-0.244	0.181	-0.284	0.605	1.000	0.533	0.376
Fr. width	0.362	-0.665	-0.342	0.199	0.394	0.533	1.000	0.613
No. seed	0.766	-0.792	-0.158	0.559	-0.035	0.376	0.613	1.000

No. lflt: No. of leaflets, L. leng: Leaf length, L. width: Leaf width, Rac. leng: Racemose length, Fl. leng: Flower length, Fr. leng: Fruit length, Fr. width: Fruit width, No. seed: No. of seeds

Table 5: Factor loading of the eight quantitative characters

Component matrix	Components		
	1	2	3
No. of leaflets	0.630	-0.265	0.702
Leaf length	-0.894	0.104	0.384
Leaf width	-0.363	0.110	0.881
Racemose length	0.503	-0.588	-5.231E-02
Flower length	0.131	0.866	-0.207
Fruit length	0.469	0.766	0.357
Fruit width	0.815	0.349	-0.113
No. of seeds	0.934	-0.173	0.213

Extraction method: Principal component analysis, A 3 components extracted

example, when number of leaflet was correlated with leaf width, the degree of affinity was -0.340 and 0.362 when correlated with fruit width but when the number of leaflet was correlated against itself, it was 1.000. Similarly, when racemose length was correlated with leaf width, the degree of resemblance was -0.133. It was 0.199 when compared with fruit width and 1.000 when correlated against itself. Thus, it is shown that there is significant correlation between number of leaflet and leaf width, number of leaflet and racemose length, leaf width and number of leaflets, leaf width and fruit length, racemose length and fruit

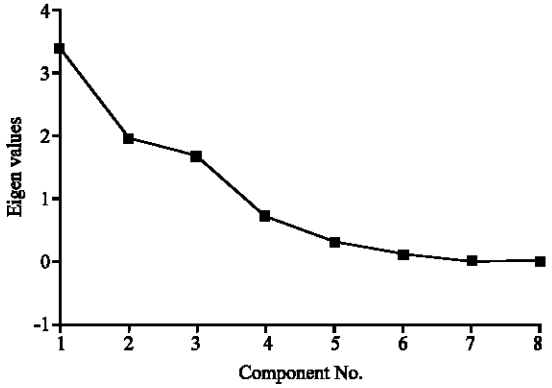

Fig. 1: Scree plot for the eight morphological characters used in the morphometric study of *Indigofera* species

width. The factor loading shown in Table 5 also revealed that some characters carry more weight in the variation than others. Figure 1 shows the scree plot for the extraction of the components that weighs higher than the rest. It gives the figurative representation of the weight of

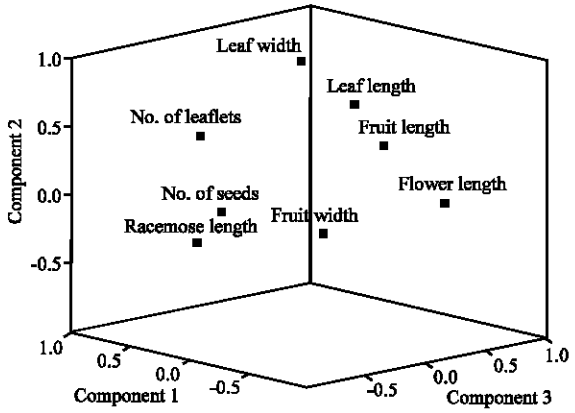

Fig. 2: Component plot in rotated space for the eight morphological characters

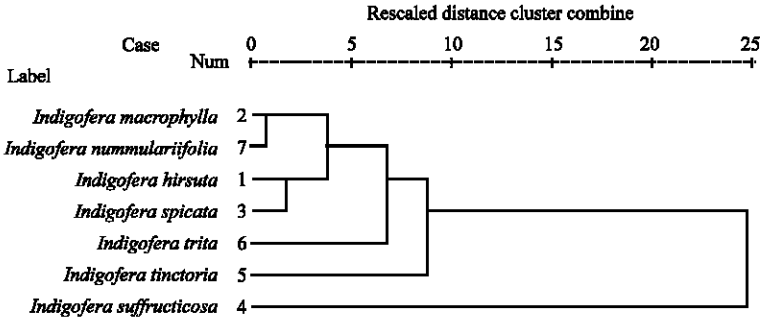

Fig. 3: Dendogram showing the relationship of seven *Indigofera* species based on eight morphological characters (Dendrogram using Average Linkage)

the characters employed. Those above +1 eigen values are very much stronger than those below. From the Fig. 1, it could be observed that characters 1, 2 and 3 weigh higher than the rest five characters employed in the analysis, with eigen value greater than 1. This is also shown in Fig. 2 which shows the component plot in rotated space. It is indicated that leaf width, leaf length and number of leaflet have higher values (above 0.5) than the remaining five characters. It is therefore affirmed that these characters contribute heavily to the delimitation of the taxa.

From the qualitative morphological view shown in Fig. 3, greater affinity exists between *Indigofera macrophylla* and *Indigofera nummulariifolia* than between *Indigofera hirsuta* and *Indigofera spicata* which are distantly related. Further explanation of the differences based on morphometry of *Indigofera* species is observed in Table 6 that shows average linkage between the groups on agglomeration schedule. In Table 6, the cluster that exists between species 2 (*Indigofera macrophylla*) and species 7 (*Indigofera nummulariifolia*) is having the coefficient 13.390 whereas that which exists between species 1 (*Indigofera hirsuta*) and species 4 (*Indigofera suffruticosa*) is 2839.673 showing great degree of variation in their morphometry though their gross morphologies are similar.

Table 6: Average linkage between groups

	Cluster combined			Stage cluster first appears		
Stage	Cluster 1	Cluster 2	Coefficients	Cluster 1	Cluster 2	Next stage
1	2	7	13.390	0	0	3
2	1	3	226.273	0	0	3
3	1	2	421.519	2	1	4
4	1	6	767.191	3	0	5
5	1	5	1019.451	4	0	6
6	1	4	2839.673	5	0	0

DISCUSSION

The methods of numerical taxonomy have been used in classifying many plants as well as interpreting results of taxonomic studies (Sneath and Sokal, 1973; Soladoye, 1982; Chiapella, 2000; Gomez-Campo *et al*., 2001; Sonibare *et al*., 2004; Soladoye *et al*., 2008). Morphometric analysis are commonly performed on

organisms and are particularly useful in analyzing the fossil record. Morphometrics add a quantitative element to descriptions, allowing more rigorous comparisons. In the numerical analysis of seven *Indigofera* species utilizing eight quantitative characters, present results confirm that variations in the vegetative and floral characters among the seven species of *Indigofera* are important, diagnostic and could be used taxonomically in the delimitation of these taxa. Significant correlation existing between number of leaflet and leaf width, number of leaflet and racemose length, leaf width and number of leaflets, leaf width and fruit length, racemose length and fruit width shows that these characters carry more weight in the overall analyses. Of the eight parameters used in the analyses, leaf width, leaf length and number of leaflet have higher values (above 0.5) than the remaining five characters; affirming their usefulness for delimitation purpose. These characters can be effectively employed in the taxonomic treatment of other *Indigofera* species that were not included in the present study. Comparable deductions have been made in earlier studies reported by Nwachukwu (1997) and Stern (2000) on the importance of morphological features in taxonomic classification of plants.

The average linkage between the groups on agglomeration schedule and Cluster analysis show that *Indigofera macrophylla* and *I. nummulariifolia* are more closely related than *I. hirsuta* and *I. suffruticosa*. The variations noted within species may be as result of age. This is because older leaves tend to be leathery and glabrous than younger leaves. The size of the leaflets decreases progressively downward. The size of the fruit and bud length depends on the age of the plant (Irvine, 1961; Burkill, 1995; Delzon and Loustau, 2005). Leaf shape and size may vary within the same plant. It is suggested that light intensity acting on leaf may affect the carbohydrate balance which in turn could affect the length of the cells in the direction of the long axis thereby leading to the difference in the length, shapes and width of the leaves (Aborg, 1943).

Indigofera species examined exhibited variations based on location and localities. This was observed while taking the measurements of some of the characters employed. For example, the range of leaf lengths of *I. suffruticosa* measured from herbarium specimens at the Forest Herbarium Ibadan was 1.9-2.4 cm while those measured in Ago-Iwoye ranged between 2.3-2.9 cm. Also, the number of leaves varied from 7-11 in Ibadan and 5-14 in Ago-Iwoye. Such variations may be due to environmental as well as genetic factors and interaction among them (Gbile, 1976; Nwachukwu and Mbagwu, 2006). The influence of environmental factors, geographical variation in growth includes racial variation which is due to several mechanisms such as mutation, natural selection, hybridization or combination of these. However, knowledge of the existing variations between various morphological characters is vital for any plant taxonomic practice. Marquiafável *et al.* (2008) in their study of eight *Neotropical* species of *Indigofera* noted that distribution and gland types differed between species and that these gland distribution patterns can be used as diagnostic characters. Each of the eight *Indigofera* species analyzed had at least two different trichome types out of the seven types occurring in reproductive and vegetative organs of the taxa. Also, Nwachukwu and Edeoga (2006) in their studies on tannins, starch grains and crystal types in the vegetative organs of eight species of *Indigofera* using a microscope observed that the type and distributions of the phytochemical substances are varied in the species. As observed in this study, information that is obtainable through multivariate techniques such as PCA and Cluster analysis may assist plant taxonomists in the characterization of plant species to identify valuable characteristics, which accounts for morphological variations. It can therefore be concluded that the number of leaflet, leaf length and leaf width have a significant contribution in distinguishing individual members of the genus *Indigofera.* In contrast to the observation in our study however, Hassen *et al.* (2006) in their study of 41 *Indigofera* accessions representing eight species reported that clustering on the basis of morphological traits alone failed to consistently and satisfactorily reveal variation between accessions in terms of agronomic performance. Consequently, in their study a character discard resulted in the selection of only eight determinant characters which included: growth habit, days to 50% flowering, extent of branching, leaflet length, leaf yield, plant height or length of the principal stem, leaf percentage and canopy spread measured at the widest point. These were regarded as the core attributes for *Indigofera* germplasm characterisation, which can be used for the identification of suitable breeding material for specific purposes.

In present study, numerical taxonomy with its quantitative feature provided greater discrimination along the spectrum of taxonomic differences and was also more sensitive in the delimitation of the taxa. In addition to demonstrating the relative value of morphometric methods in the taxonomy of the genus *Indigofera,* the study presents greater and more detailed information on the level of relationship within the genus. Conclusively, it is important to note that, morphometric analysis is not

enough in delimiting taxa though it has benefited systematics. Other methods which include anatomical, palynological, cytotaxonomic and chemotaxonomic differences should be investigated together with morphometry in order to confirm or change the existing classification based on morphology in the genus.

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