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RESEARCH ARTICLE

THE COMPARATIVE ANATOMY AND MORPHOLOGY OF THREE XEROPHYTIC LEAVES NAMELY *CYCAS REVOLUTA, NERIUM OLEANDER AND PINUS CARIBEA*

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ABSTRACT

Naturally physical environment largelly determines which plant lives in a particular climate, plants growing in different ecological conditions; consequently they are referred to as xerophytes, hydrophytes, and epiphytes etc. Thus, shows certain adaptive features which enables them to survive under such conditions, these are phylogenetic characters. In the course of time, plants were able to cope with a range of conditions through the physiological, morphological and behavioral abilities they possessed. The anatomy and morphological comparative study of leaves of Pinus caribaea Morelet, Cycas revoluta Thunb. and Nerium oleander (L) were conducted through fixation process were the leaves are fixed in FAA (formalin acetic acid). After fixation, pieces of leaves were dehydrated in varying concentrations of alcohol i.e 30%, 50%, 70%, 95% and absolute alcohol for 2 hours each. After dehydration, the leaves were then infiltrated with paraffin wax. The inner surfaces of the watch glasses were smear with 10% glycerine to allow the solidified blocks of paraffin wax slip out of the watch glass easily. Molten wax was poured into the glasses and warm forceps were used to orient the impregnated leaves at their cutting angles. The paraffin wax blocks with the leaves were trimmed in equal sizes, so as to obtain straight ribbon during sectioning. The ribbons obtained were mounted on slides smeared with egg albumen. The slides were dewaxed in two changes of xylene for 5 minutes each. The slides were then treated in series of alcohol starting from absolute alcohol two changes, 95%, 70%, 50% and 30% alcohol for 2minutes each. The specimens were stained in safranin for 20minutes and in fast green for 5minutes. The sections of each material were observed under light microscope, to study the following parameters: Cuticle, Epidermis, Sclerenchyma and The location and number of stomata. In each case, the thickness was studied with the help of already calibrated microscope. In addition, the location and numbers of stomata were also studied. In conclusion, location actually affects the anatomical features of the leaf but it depends on what ecological zone the plant is found and the climatic diversity experienced by the plant.

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INTRODUCTION

The nature of the physical environment is largelly determines which plant lives in a particular climate, plants growing in different ecological conditions; consequently they are referred to xerophytes, hydrophytes, epiphytes etc, the plant which are growing in such conditions, shows certain adaptive features which enables them to survive under such conditions, these are phylogenetic characters In the course of time, plants were able to cope with a range of conditions through the physiological, morphological and behavioral abilities they possess. Due to many Agricultural practices and Industrial development, many plants have been taken to areas away from their own habitats and many have been able to adapt to their new habitat and are well growing such as *Pinus caribaea* Morelet, *Cycas revoluta* Thunb. and *Nerium oleander L.*

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Pinus caribaea Morelet belongs to the family Pinaceae. It is found in India, and is confined to the north east and north east of Himalaya regions. It is mostly known as Caribbean pine. It is found growing in hilly regions. The foliage leaves are green and needle-like produced mostly in clusters of 2-5. The leaves have thick cuticle and sunken stomata which represents an environmental adaptation for retarding water loss. *Pinus caribae*a Morelet is often 20-30m tall, with a diameter of 50-80cm and occasionally up to 1m. The needle-like nature of the foliage leaves indicates xerophytic adaptation of the plant. This species grows best in areas up to about 700m in more fertile sites with good subsoil drainage and annual rainfall of 2000-3000mm. Generally at elevations of 600-800m is grown. *Cycas revoluta* Thunb. is a distinct species of the Cycadaceae family mostly established in the subtropics. It is a native of China and Southern Japan. It is widely distributed through the Ryukyu island of Southern Japan *Cycas revoluta* Thunb. has pinnate leaves.

The leaflets are sessile and elongated with revolute margins. It is fairly drought tolerant and tolerant to mild cold temperatures, provided the ground is dry. *Nerium oleander L.* is an evergreen shrub in the family Apocyanaceae. It is planted in many subtropical and tropical areas of the world. It typically occurs around dry stream beds. The leaves are in whorls, thick, leathery, dark green, narrow and lanceolate. The leaf has multiple epidermis and the leaf has stomatal crypts. It is drought tolerant and will tolerate light frost, down to -10^{o}C.The leaves have entire margin and the flower grow in cluster at the end of each branch. The stomata are in groups and confined to depressions found in the leaf surface. Apparently, there seem to be no difference in the morphology of these xerophytes growing in different areas. However, there may be some variations that may exist in their anatomical characters which can be studied.

MATERIALS AND METHODS

The study of the leaves of *Pinus caribaea* Morelet*, Cycas revoluta* Thunb and *Nerium oleander L.* was carried out in the department of Biological Science, Ahmadu Bello University, Samaru, Zaria Nigeria. They were obtained from two localities namely Kaduna and Zaria.

Fixation

The leaves of *Pinus caribaea* Morelet*, Cycas revoluta* Thunb and *Nerium oleander L*. obtained from Kaduna and Zaria were fixed in FAA (formalin acetic acid). These were put inside a hollow glass tube; one end of the glass tube was covered with muslin cloth. The tube containing the leaves was hanged in a specimen jar containing FAA, for different specimens, different tubes were used. They were well labeled.

Dehydration

After fixation, pieces of the leaves were dehydrated in varying concentrations of alcohol i.e 30%, 50%, 70%, 95% and absolute alcohol for 2 hours each. Later the specimens were passed through different ratios of alcohol: chloroform mixtures for 2 hours each i.e

75% alcohol: 25% chloroform
50% alcohol: 50% chloroform
25% alcohol: 75% chloroform

Then it was cleared in only chloroform for 2 hours.

Infiltration

After dehydration, the leaves were then infiltrated with paraffin wax. Some chips of the wax were added into the container carrying the chloroform and the leaves. It was placed in a hot air oven at 60^{0}C. It was left until the chloroform had bubbled off completely and the paraffin wax started to gel. The leaves were left for 24 hours in the oven so that it will be impregnated by the paraffin wax.

Embedding

The inner surfaces of the watch glasses were smear with 10% glycerine to allow the solidified blocks of paraffin wax slip out of the watch glass easily. After the glasses have been smear with glycerine, molten wax was poured into the glasses and warm forceps were used to orient the impregnated leaves at their cutting angles. Each glass was labeled accordingly to avoid any mix up. After a thin film had formed on the top of the wax, the glasses were submerged inside water for the blocks to harden. The blocks were then removed and placed in cool, dry and dust free atmosphere for future use.

Sectioning

The paraffin wax blocks with the leaves were trimmed in equal sizes, so as to obtain straight ribbon during sectioning. The trimmed blocks were mounted on a microtome specimen holder and it was mounted on a rotary microtome. Sections of different thickness ranging from 12-18 microns thick were cut with a microtome knife.

Mounting of Sections on the Slides

The ribbons obtained were mounted on slides smeared with egg albumen. The specimens were stretched and dried on a slide drier. The slides were packed in slide racks and were well labeled.

Staining Procedure

The slides were dewaxed in two changes of xylene for 5minutes each. The slides were then treated in series of alcohol starting from absolute alcohol two changes, 95%, 70%, 50% and 30% alcohol for 2minutes each. The specimens were stained in safranin for 20minutes and in fast green for 5minutes. It was then rinsed in tap water twice. The specimens were again put through series of alcohol concentration that is from 30%, 50%, 70%, 95% and absolute alcohol. Specimens were cleared in xylene for a minute and in 0.5M of HCL for some seconds. Sections were then mounted in Canada balsam which is used as an adhesive and covered with cover slip. It was dried on a slide drier and the slides were well labeled.

Study of different parameters

The sections of each material were observed under light microscope, to study the following parameters:

Cuticle
Epidermis
Sclerenchyma
The location and number of stomata

In each case, the thickness was studied with the help of already calibrated microscope. In addition, the location and numbers of stomata were also studied.

Statistical Analysis

The data collected were statistically analyzed using the T-Test analysis.

RESULTS

It is apparent from the table above, that there was no significant difference in the measurement of different parameters of the leaf of *Pinus caribaea* Morelet collected from Kaduna and Zaria.

Table 4.1. Measurement of different parameters of *Pinus caribaea* Morelet leaf collected from Kaduna and Zaria

Parameter	Location	N	Mean (x)	SD	Std error mean	t-value	DF	P
Upper cuticle	Kaduna	10	30.10	5.763	1.822	-1.769	18	0.094
	Zaria	10	35.00	6.600	2.087			
Lower cuticle	Kaduna	10	29.40	6.433	2.034	-1.460	18	0.162
	Zaria	10	33.60	6.433	2.034			
Upper epidermis	Kaduna	10	30.80	5.903	1.867	-1.028	18	0.318
	Zaria	10	34.30	9.007	2.848			
Lower epidermis	Kaduna	10	31.50	6.803	2.151	-0.967	18	0.346
	Zaria	10	34.30	6.127	1.938			
Sclerenchyma	Kaduna	10	30.80	5.903	1.867	-0.805	18	0.431
	Zaria	10	32.90	5.763	1.822			

There is no significance difference, when P>0.05 and there is significant difference when P≤0.05.

Keys

N= the number of measurement taken
X= Mean (in microns)
STD= Standard deviation
STD Error mean= Standard error mean
T-value= Tabulated value
DF= Degree of freedom
P= Level of Significance.

Table 4.2. Measurement of different parameters of Cycas revoluta Thunb. leaf collected from Kaduna and Zaria

Parameter	Location	N	Mean (x)	Standard deviation	Std error mean	t-value	DF	P
Upper cuticle	Kaduna	10	40.20	7.569	2.394	-0.748	18	0.464
	Zaria	10	37.80	6.763	2.139			
Lower cuticle	Kaduna	10	35.70	7.704	2.436	0.460	18	0.651
	Zaria	10	37.10	5.763	1.822			
Upper Epidermis	Kaduna	10	45.50	5.949	1.881	-2.377	18	0.029
	Zaria	10	39.20	5.903	1.867			
Lower epidermis	Kaduna	10	44.20	16.123	5.099	-1.049	18	0.308
	Zaria	10	38.50	5.949	1.881			
Sclerenchyma	Kaduna	10	39.90	6.641	2.100	-0.943	18	0.358
	Zaria	10	37.10	6.641	2.100			

There is no significance difference, when P>0.05 and there is significant difference when P≤0.05.

Keys

N= the number of measurement taken
X= Mean (in microns)
STD= Standard deviation
STD Error mean= Standard error mean
T-value= Tabulated value
DF= Degree of freedom
P= Level of Significance.

Table 4.3. Measurement of different parameters of Nerium oleander L. leaf collected from Kaduna and Zaria

Parameter	Location	N	Mean (x)	Standard deviation	Std error mean	t-value	DF	P
Upper cuticle	Kaduna	10	25.90	4.725	1.494	-0.342	18	0.736
	Zaria	10	26.60	4.427	1.400			
Lower cuticle	Kaduna	10	28.70	5.165	1.633	0.976	18	0.342
	Zaria	10	26.60	4.427	1.400			
Upper epidermis	Kaduna	10	43.40	5.522	1.746	1.523	18	0.145
	Zaria	10	39.90	4.725	1.494			
Lower Epidermis	Kaduna	10	38.50	6.803	2.151	-1.863	18	0.079
	Zaria	10	44.10	6.641	2.100			
Sclerenchyma	Kaduna	10	32.20	4.894	1.548	0.318	18	0.754
	Zaria	10	31.50	4.950	1.565			

There is no significance difference, when P>0.05 and there is significant difference when P≤0.05.

Keys

N= the number of measurement taken
X= Mean (in microns)
STD= Standard deviation
STD Error mean= Standard error mean
T-value= Tabulated value
DF= Degree of freedom
P= Level of Significance.

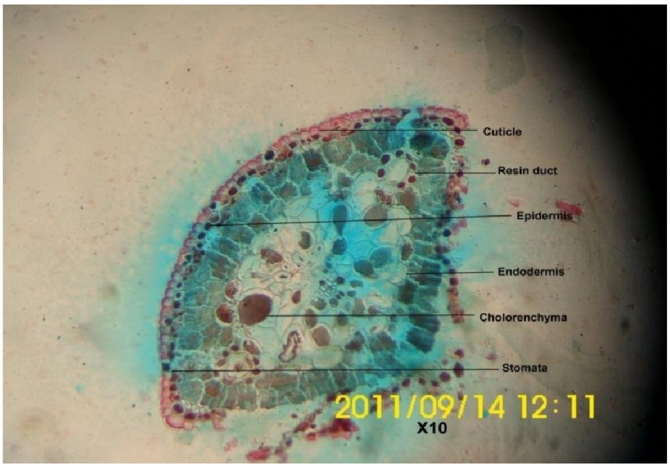

Plate 4.1. T.S of Pinus caribaea leaf from Kaduna

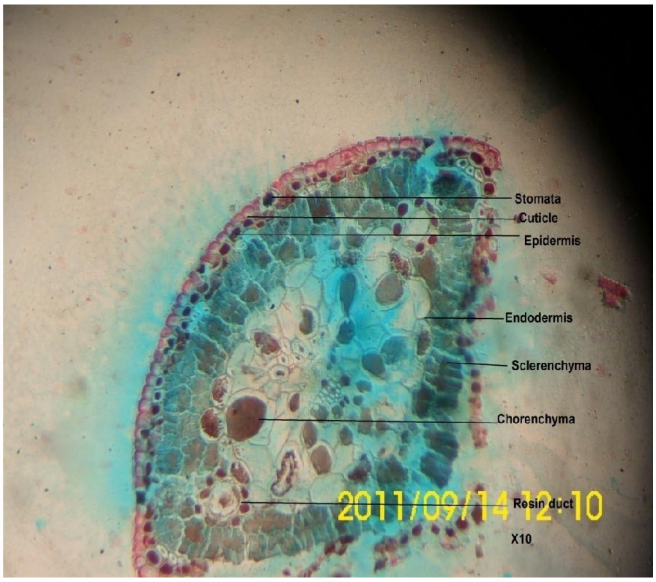

Plate 4.2. T.S of Pinus caribaea leaf from Zaria

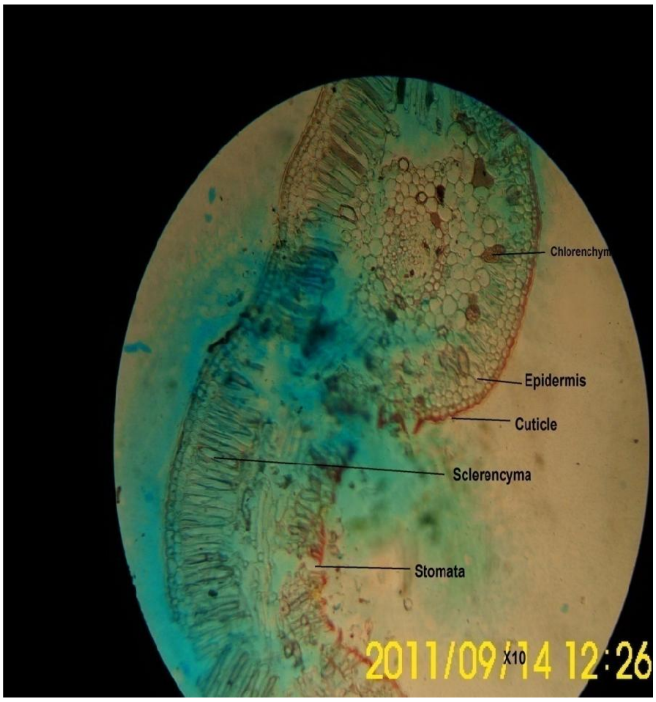

Plate 4.3. T.S of Cycas revoluta leaf from Kaduna

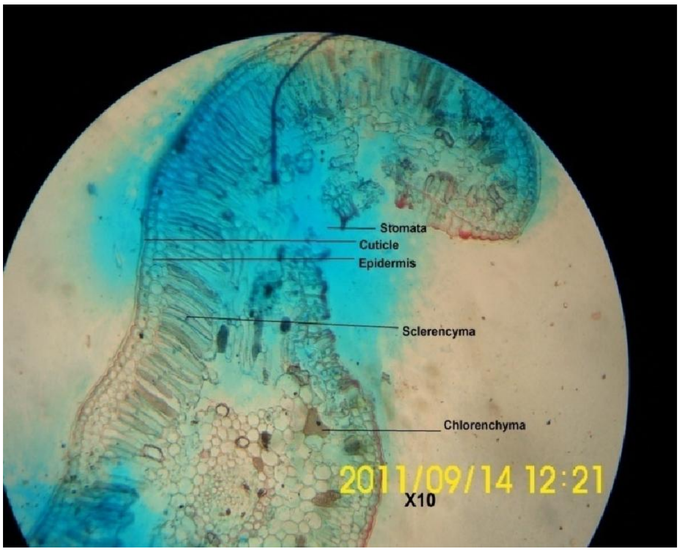

Plate 4.4. T.S of Cycas revoluta leaf from Zaria

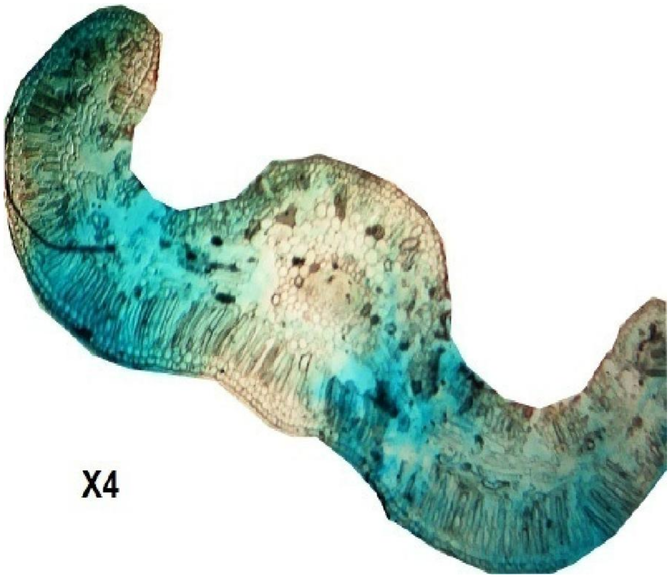

Plate 4.5. T.S of Cycas revoluta leaf

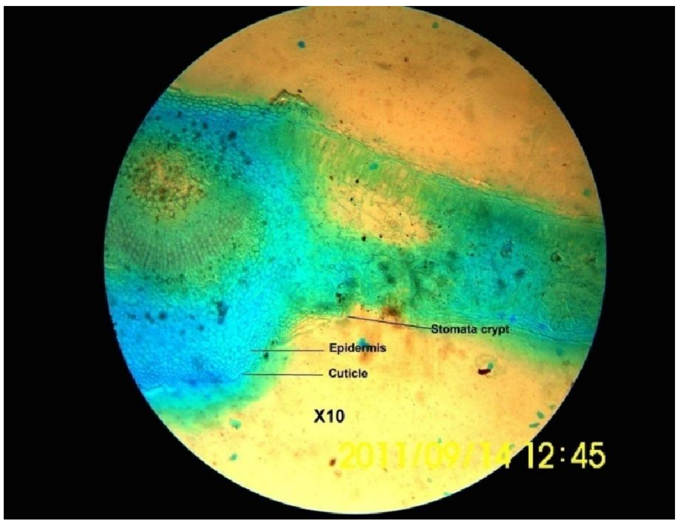

Plate 4.6, T.S of Nerium oleander leaf from Kaduna

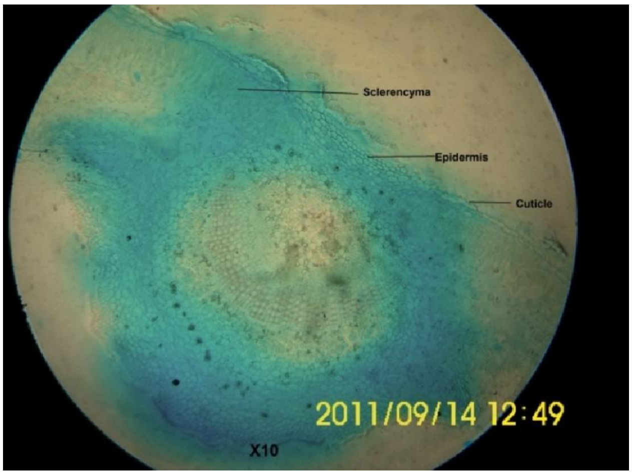

Plate 4.7, T.S of Nerium oleander leaf from Zaria

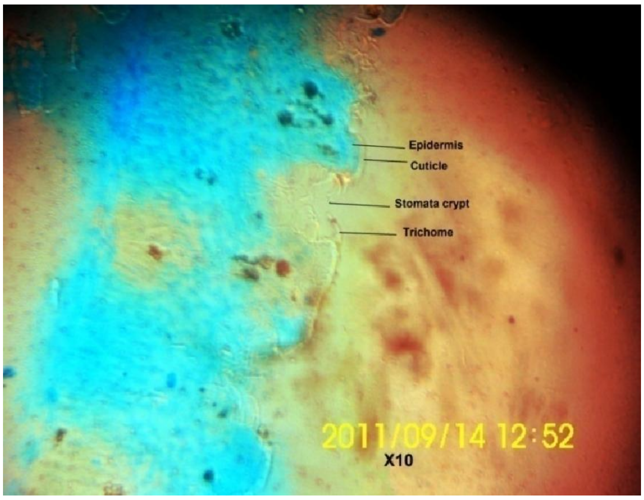

Plate 4.8. T.S of Stomata Crypt in Nerium oleander

It is apparent from the table above, that there was no significant difference in the measurement of different parameters of the leaf of *Nerium oleander* L. collected from Kaduna and Zaria.

The locations of the stomata were all sunken. The number was the same in the leaf of *Pinus caribaea* Morelet and *Nerium oleander* L. collected from Kaduna and Zaria but the number was different in the leaf of *Cycas revoluta* Thunb. collected from Kaduna and Zaria.

DISCUSSION AND CONCLUSION

In the leaf of *Pinus caribaea* Morelet found in Kaduna, the upper cuticle and the lower cuticle had no difference with the upper and lower cuticle of the same *Pinus caribaea* Morelet found in Zaria. Also the upper epidermis, the lower epidermis and the sclerenchyma of the same *Pinus caribaea* Morelet had no difference when found in Kaduna or Zaria. The number of stomata counted was the same in both locations. It can therefore be deduced from these studies that the difference in the location does not affect the anatomy. It may be due to the fact that Kaduna and Zaria are falling under the same ecological zone (Guinea savanna) and experiencing the same climate. The structure of leaves depends to a large extent on the environment in which the plant is growing particularly, the availability of water in that environment (Graham, 2006).

In *Cycas revoluta* Thunb. the upper cuticle, the lower cuticle, the lower epidermis and the sclerenchyma had no difference when found in Kaduna or Zaria but the upper epidermis of the same leaf found in Kaduna was significantly different from the *Cycas revoluta* Thunb. found in Zaria. The number of stomata counted from both locations was different. It is shown from the studies that the difference in the location affected the anatomy of the leaf. Leaf anatomy of xerophytic plants is characterized by various adaptive strategies which enable the plant to survive in the ecological environment (Raven, 2008). In the leaf of *Nerium oleander* L., the upper cuticle, the lower cuticle, the upper epidermis, the lower epidermis and the sclerenchyma had no significant difference when found in Kaduna or Zaria. The number of stomata counted from both locations was the same. It may be due to the fact that Kaduna and Zaria are falling under the same ecological zone (Guinea savanna) and experiencing the same climate. The nature of the physical environment in large measure determines which plant survives in a particular climate (Raven, 2008).

Table 4.4. Parameters for the length and width ratio of Pinus caribaea Morelet leaf collected from Kaduna and Zaria

Parameter	Location	N	Mean (x)	Standard deviation	Std error mean	t-value	DF	P
Length	Kaduna	10	786.80	24.517	7.753	-0.205	18	0.840
	Zaria	10	791.00	59.854	18.927			
Width	Kaduna	10	553.00	38.056	12.034	1.715	18	0.103
	Zaria	10	523.60	38.595	12.205			
Length/ Width Ratio	Kaduna	10	1.428	0.100	0.032	-1.486	18	0.155
	Zaria	10	1.518	0.165	0.052			

There is no significance difference, when $P>0.05$ and there is significant difference when $P\leq0.05$.

Keys

N= the number of measurement taken
X= Mean (in microns)
STD= Standard deviation
STD Error mean= Standard error mean
T-value= Tabulated value
DF= Degree of freedom
P= Level of Significance.

Table 4.5. Study on the number and location of stomata of the leaves collected from Kaduna and Zaria

Location	Number of stomata	Location of stomata
Pinus caribaea Kaduna	Three	Sunken stomata
Pinus caribaea Zaria	Three	Sunken stomata
Cycas revoluta Kaduna	Five	Sunken stomata
Cycas revoluta Zaria	Six	Sunken stomata
Nerium oleander Kaduna	Five	Sunken stomata
Nerium oleander Zaria	Five	Sunken stomata

In conclusion, location actually affects the anatomical features of the leaf but it depends on what ecological zone the plant is found and the climatic diversity experienced by the plant. Kaduna and Zaria falls under the same agro-ecological zone that is the northwest Zone. Kaduna and Zaria are under Guinea savanna and the climatic conditions are the same. The soil elevation of Kaduna and Zaria is between the ranges of 652.6m-660m above sea level. In the case of *Pinus caribaea Morelet* and *Nerium oleander* L. the thickness was not affected by the location, but in the *Cycas revoluta* Thunb. The thickness was affected by the two locations. Plant are able to conform to the environment in which they find themselves, they are able to adopt the temperature, water availability and other physical aspects of the environment (Raven, 2008).

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