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LEAF SURFACE FEATURES AS TAXONOMIC EVIDENCES TO IDENTIFY SOME SPECIES OF GENUS *EUCALYPTUS* IN EGYPT BY USING SCANNING ELECTRON MICROSCOPE

Hanan S. Abd–El Maksoud [1] ***; M. M. E. Abd El-Kader*** [2] ***and Nermeen T. Shanan*** [3]

1. *Flora and Phyto-taxonomy Department, Research Hort., Research Institute, A .R.C., Giza, Egypt.*
2. *Forestry Department, Research Hort., Research Institute, A .R.C., Giza, Egypt.*
3. *Ornamental Department Hort., Faculty of Agriculture, Cairo University, Giza, Egypt.*

ABSTRACT

Morphological characters and ultrastructural leaf variations of nine species belong to genus Eucalyptus were studied. These species are; Eucalyptus camaledulensis, E. cinerea, E. citriodora , E. ficifolia , E. globules, E. gomphocephala, E. kruseana, E. resinfera and E. rostrata .

The macro-morphological characters revealed that, there are different leaf shapes; each characterized more than one species. The micro-morphological results of upper epidermis clarified that there are five sharply distinct leaf sculpture patterns; Pusticulate, Ruminate, Glebulate, Colliculate and Favulariate. While that of the lower epidermis had six leaf sculpture patterns; Ruminate, Verrucate, Verculate, Rugose, Favulariate and Clliculate, except one species, where the leaf has sculpture pattern overlaphing between Ruminate and Favularlate.

The artificial key has been designed to identify the studied species. However, this study can be serve as a limited taxonomic knowledge about the studied and may be useful in other future studies.

Conclusively, *from the pervious results of such research it could be conclude that the more close species to each other were; E. ficifolia and E.globules; E.resinfera and E.rostrata. Contrary, the most far species from each other were E.cinerea and E.krusean.*

Key words: Macro & micro morphological characters, SEM, *Eucalyptus*

INTRODUCTION

The *Eucalyptus*, a widespread genus of the family *Myrtaceae*, covers more than 50% of Australian territory (Pryor and Johnson 197l).
Eucalyptus genus includes 900 species and its subspecies spread considerably in many countries (Brooker and Kleingi, 2004). *Eucalyptus* species are believed to be introduced in Egypt during the 1800's, (El-Lakny *et. al*., 1980) . They are highly adapted to local environmental condations and grow very fast. These species are traditionally planted as windbreaks, for shade and to supply wood for lumber, particleboard and charcoal production as well as to produce of medical and aromatic components . A mature *Eucalyptus* may take the form of a low shrub or a very large tree. There are three main habit and four size categories that species can be divided into. *Eucalypts* are called "sclerophyllous" which means 'hard leaves'. They hang vertically so they are not exposed to the midday sun which reduces water loss through transpiration.

Tree size based on the following criteria; small (10 m), medium(10–30m), tall (30–60 m) and very tall (over 60 m). The bark, leaves and reproductive structures are greatly varied in different species (Brooker and Kleingi, 1996). The bark is of three kinds; 1. Soft and brittle (shedding in short irregular flakes, lacking in fibre), 2. Hard and long-fibred (shedding in long broad, thick flakes or strips and 3. Very thin and finely fibred (shedding in ribbons). The leaves are of 3 kinds; passing through juvenile, intermediate and mature stages (Humphries *et al.*, 1981).

Nearly all *Eucalyptus* species are evergreen but some tropical species lose their leaves at the end of the dry season. As in other members of the myrtle family, *Eucalyptus* leaves are covered with oil glands. Many *Eucalyptus* species are heteroblastic; producing juvenile and adult leaves differing markedly in morphology and anatomy (Johnson 1976; Pryor ,1976). Juvenile leaves are commonly sessile, decussate, glaucous, oriented horizontally, discolorous (dorsiventral) and often cordate, orbicular or ovate in shape. Size and shape of juvenile leaves in particular are important taxonomic characters in *Eucalyptus* (Pryor, 1976; Phillips and Reid ,1980; Potts and Reid ,1965 and Wiltshire *et al*., 1991) . Adult leaves tend to be petiolate, alternate, glabrous, pendulous, lanceolate and concolorous (isobilateral) (Coppen, 1996).

Many micro-characters have diagnostic value only when characterizing lowest taxonomic categories (*e.g.* many types of cuticular striations, most of the frequent types of cell shapes, many types of widely distributed epicuticular crystalloids).There are many characters could be used to characterize groups of related species, genera or taxonomic categories up to the sub-family levels. Some characters of the micro-morphology and orientation of epicuticular wax crystalloid are surprisingly high systematic significance. Wax platelets may

have parallel orientation patterns resembling electromagnetic field lines around the stomata. This pattern called Convallaria-type (Cole and Benhnke, 1975 and Barthlott, 1981). Dhalgren (1975) indicated the distribution of wax type (hatched) in the revised classification of angiosperms.

Carr and Carr (1979) found that closure line of stoma in fully grown adult leaves of certain eucalyptus is formed by special ridges of cuticle called 'stomatal bars' developed at the line of closure itself or from up growth of the cuticle of the lower surface of the guard cells. Stomatal bars were previously discovered in three members of the informal group 'Bisectae' and were shown to be restricted to certain species in that group. Possession of stomatal bars may affect stomatal performance but does not appear to be a general adaptive response to the habitats of the species which possess them. Mrobably, the possession of stomatal bars is an inherited character with taxonomic value. Barclay and Watson (1998) revised species of genera *Carum* and *Trachyspermum* (umbelliferae) by using SEM and the classical morphology . Results showed that these endemic species should be treated as one variable species; trachyspermum pimpinelloides. Ostroumove (1990) studied the stomata types on leaves of some species belong to tribes coriandreae and scandiceae (umbelliferae) in relation to taxonomy. Szujko-lacza (1994) studied the leaf characters of *coriandrum sativum*.

El-Khanagry (2003) purposed a key to identify 49 species of grasses belong to 33 genera by using vegetative of leaves as well as trichomes and cuticular ornamentations. However, leaf morphological characters have been used for identification purposes. With increased sophistication of classification systems, it has become increasingly important to have more elaborative means for identification. The leaf has not lost its importance as a taxonomic tool but rather has proved to be more useful when a fuller understanding of all its characteristics are known and appreciated.

The objective of such research was to find out remarkable micro and macro-morphological leaf features which could be used by further studies as taxonomic evidences reflecting the taxonomic relationship between the studied species.

MATERIALS AND METHODS

This study was carried out during 2010 – 2011 seasons on fresh juvenile and adult leaves of nine species of genus *Eucalyptus* (Table 1). Taxonomical evidences and characters which will explore the relationships between the studied species were gathered from morphological descriptions for each species and from the taxonomic of leaf surface using Scanning

Table 1. The studied species and the collection regions.

No.	Scientific name	English name	Regions
1.	*Eucalyptus camaledulensis* Dehnh.	Red Gum, Murray Red Gum , River Red Gum	Experimental field of Faculty of Agriculture Cairo University, Giza Zoo and Experimental Field of El-Kassasin Horticulture Research Institute, Agricultural Research Center
2.	*Eucalyptus cinerea*	Argyle Apple **,** Mealy Stringybark, Silver Dolar	Experimental Field of Faculty of Agriculture Cairo University
3.	*Eucalyptus citriodora* Hooker	Lemon Gum	Experimental Field of Faculty of Agriculture Cairo University and Experimental Field of El-Kassasin Horticulture Research Institute, Agricultural Research Center
4.	*Eucalyptus ficifolia*	Red Flowering Gum	Al Salhiah Algadida
5	*Eucalyptus globules* Labill.	Tasmanian Blue Gum, Eurabbie	Giza Zoo
6	*Eucalyptus gomphocephala*	Tuart	Experimental Field of El-Kassasin Horticulture Research Institute, Agricultural Research Center
7	*Eucalyptus kruseana*	Book-leaf Mallee	Al Salhiah Algadida
8	*Eucalyptus resinfera*	Red Mahogany, Red Messmate	Giza Zoo
9	*Eucalyptus rostrata*		Giza Zoo

Electron Microscope (SEM) on leaf surface. All data were subjected to using numerical analysis technique called NTSYS-PC.", version1.5 program (Rohlf, 1993) which resulted in a form of dendrogram representing the relationships among the studied species. Also, artificial keys using the posterior characters were designed.

- Preparing the leaf surface samples for SEM exam

Upper and lower surfaces of adult leaves of each species were examined by using Scanning Electron Microscope (SEM). Leaf samples were prepared before examination at National Research Center, Giza. as follows:

- Fixation and Dehydration . The specimens were mounted on copper stubs with double-sides adhesive tape and coated with gold using Sputter Coater S 150 A Edwards-England. The specimens were examined under JXA- 840A Electron Probe Microanalyzer- JEOL-JAPAN. Terminology concerning description leaf surface pattern was adopted after Stearn (1983) and Barthlott and Frolich (1984).

Obtained data of micro and macro morphological studies on the leaf surfaces were analyzed by using a Single Linkage Clustering analysis technique (Sneath and Sokal, 1973). Final results of analysis were represented in a form of dendrogram.

RESULTS AND DISCUSSION

Data of species under consideration were gathered from the two main sources*; i.e*., macro-morphological description of leaves (juvenile and adult) and micro-morphological description of adult leaf surfaces (upper and lower surfaces) and were represented in Tables 2 and 3.

1- Macro-morphological descriptions of leaves.

Results in Table 2 revealed that there are five different shapes and three sizes of leaves among the nine studies eucalyptus species as follows :

Shape 1- Lanceolate shape characterized the leaves of four species; *E. camaledulensis , E. citriodora, E. globules* and *E. resinfera*. The leaf lengths of these species were up to 17cm, with acute apex and symmetrical base, except *E. globules* with asymmetrical base.

Shape 2- Oval lanceolate shape characterized leaves of *E. ficifolia*. Leaf length was ranged between 6-17cm, with acute and symmetrical leaf apex and base.

Shape 3- Ovate lanceolate shape in *E. gomphocephala*. The leaf length reached up to 17cm, with acute apex and very asymmetrical base.

Shape 4- Ovate to widely ovate shape was restricted in two species; *E. cinerea* and *E.rostrata*. The leaf length and shape of apex and base of the first species were less than 6 cm, acuminate and cordate. While in the second species were between 6-17cm, apiculate and symmetrical.

Shape 5- Raniformis leaf shape distinguished only in *E. krusean* with leaf length less than 6 cm, with obtuse apex and reniform base.

Moreover leaves of all species were petiolate, alternate and leathery texture leaves, except those of *E. krusean* which were sessile, opposite and not leathery in texture.

Based on the leaf thickness, the studied species split into two categories; thick leaves in four species; i.e., *E. citriodora, E. ficifolia, E. globules* and *E. gomphocephala* and thin leaves in five species; i.e., *E. camaledulensis, E. cinerea, E. kruseana, E.resinfera* and *E.rostrata*.

2- Microrphological description of leaves

A- Upper epidermis:

There are five sculpture patterns of the leaf upper epidermal surface of the nine studied species (Table 3 and Plates 1and 2):

Pattern 1- Pusticulate as in *E.camaledulensis, E. ficifolia* and *E. rostrata*

Pattern 2- Ruminate as in *E. cinerea* and *E. citriodora.*

Pattern 3- Colliculate as in *E. gomphocephala* and *E. resinfera.*

Pattern 4- Glebulate as in *E. globules*

Pattern 5- Favulariate as in *E. krusean*

B- Lower epidermis:

There are six sculpture patterns of the leaf lower epidermal surface of the studied species (Table 3):

Pattern 1- Verruculate as in *E. camaledulensis, E. resinfera* and *E. rostrata.*

Pattern 2- Favulariate as in *E. cinerea* and *E. krusean.*

Pattern 3- Ruminate - favulariate as in *E. ficifolia.*

Pattern 4- Ruminate as in *E. globules.*

Pattern 5- Rugose as in *E. citriodora.*

Pattern 6- Colliculate as in *E. gomphocephala.*

Numerical analysis

Illustrated dendrogram in Figure (1) shows level of similarity in which the studied species have been shared, in other words, determining the similarity or dissimilarity distance between these species. However, it could

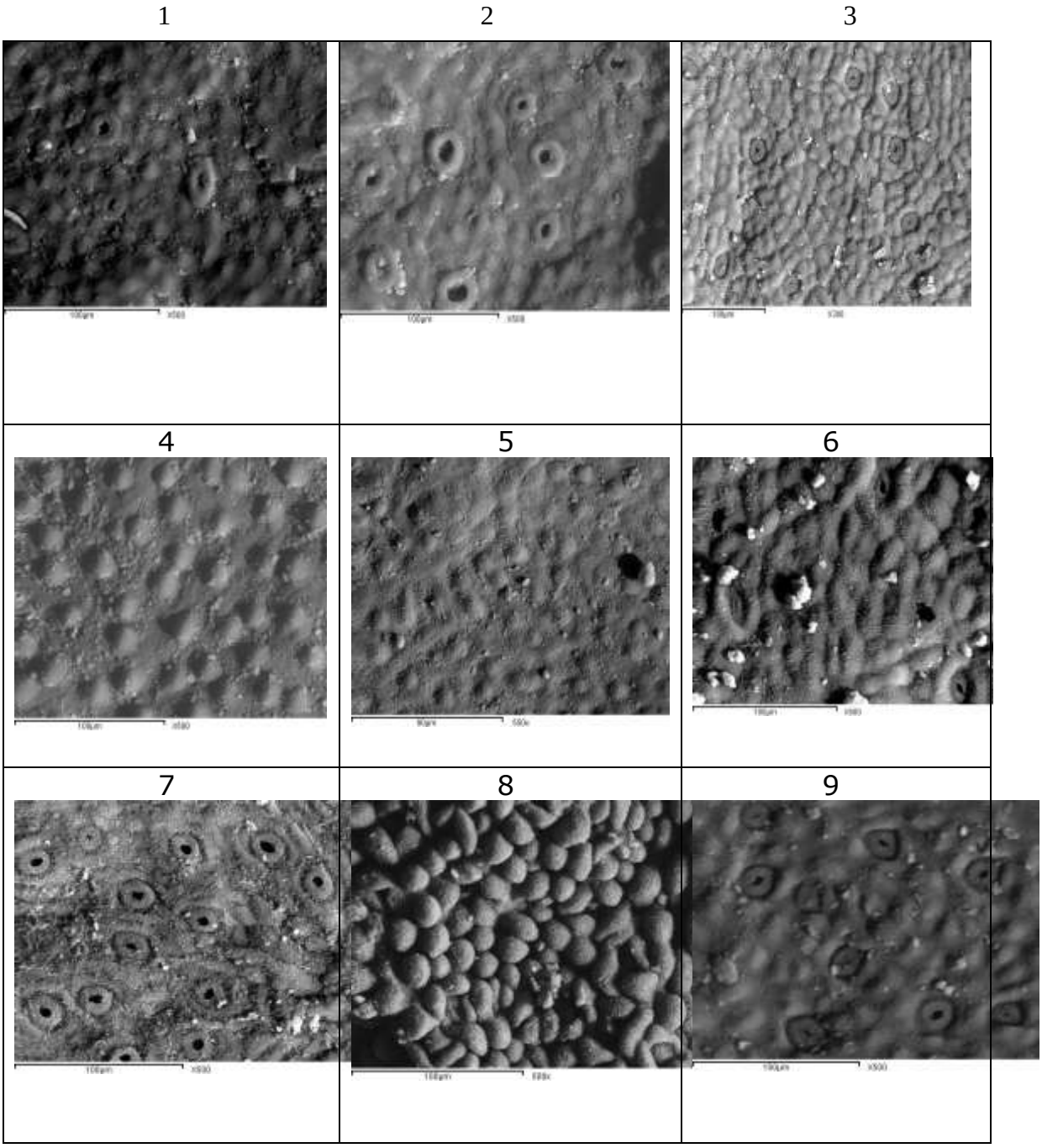

Plate.1: Leaf upper epidermal surface as shown under the SEM

Key: 1) *E.camaledulensis* 2) *E.cinerea* 3) *E.citriodora*
4) *E. ficifolia* 5) *E.globules* 6) *E.gomphocephala*
7) *E.krusean* 8) *E.resinfera* 9) *E.rostrata*

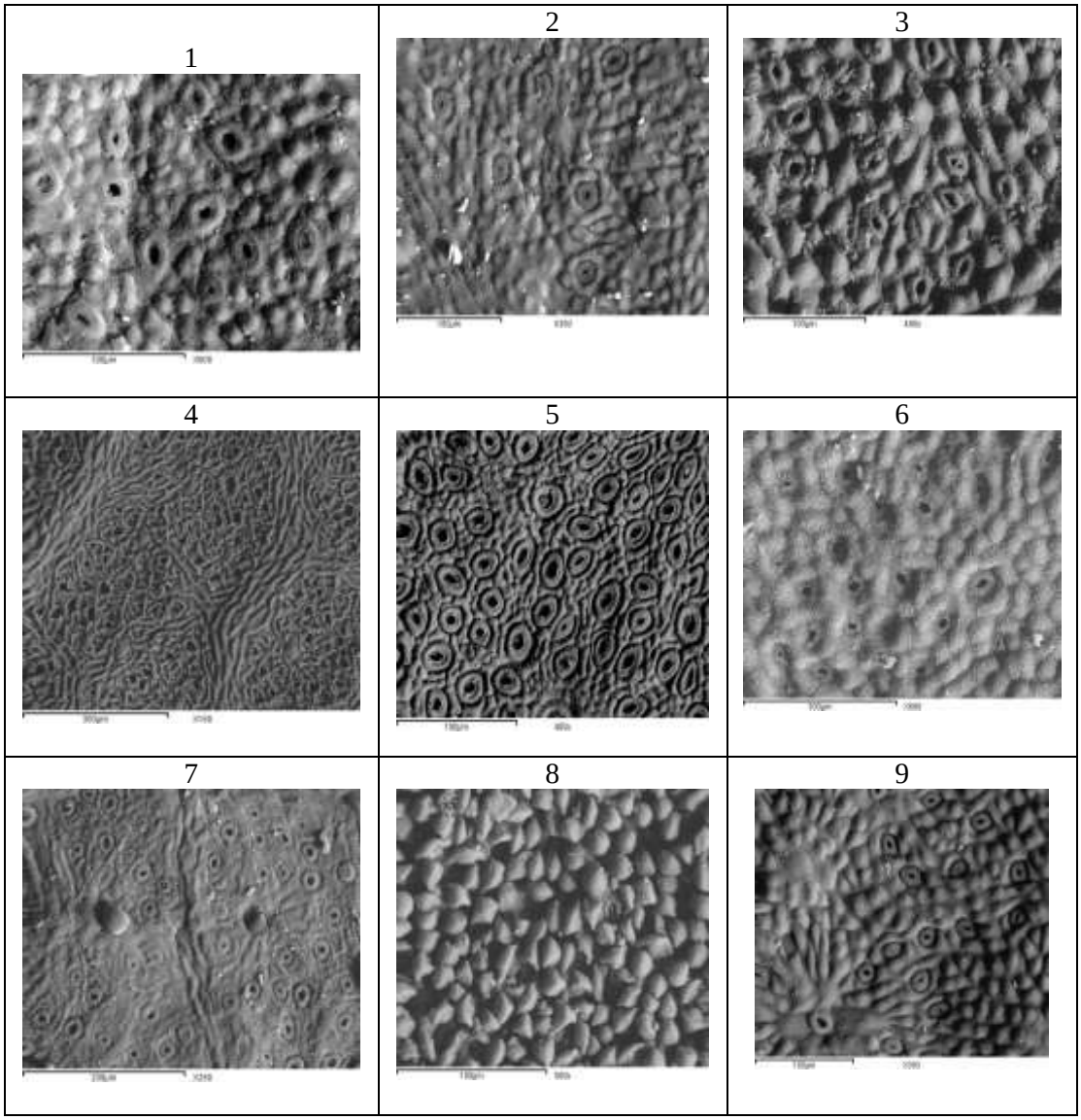

Plate2: Leaf lower epidermal surface as shown under the SEM

Key: 1) *E.camaledulensis* 2) *E.cinerea* 3) *E.citriodora*
4) *E. ficifolia* 5) *E.globules* 6) *E.gomphocephala*
7) *E.krusean* 8) *E.resinfera* 9) *E.rostrata*

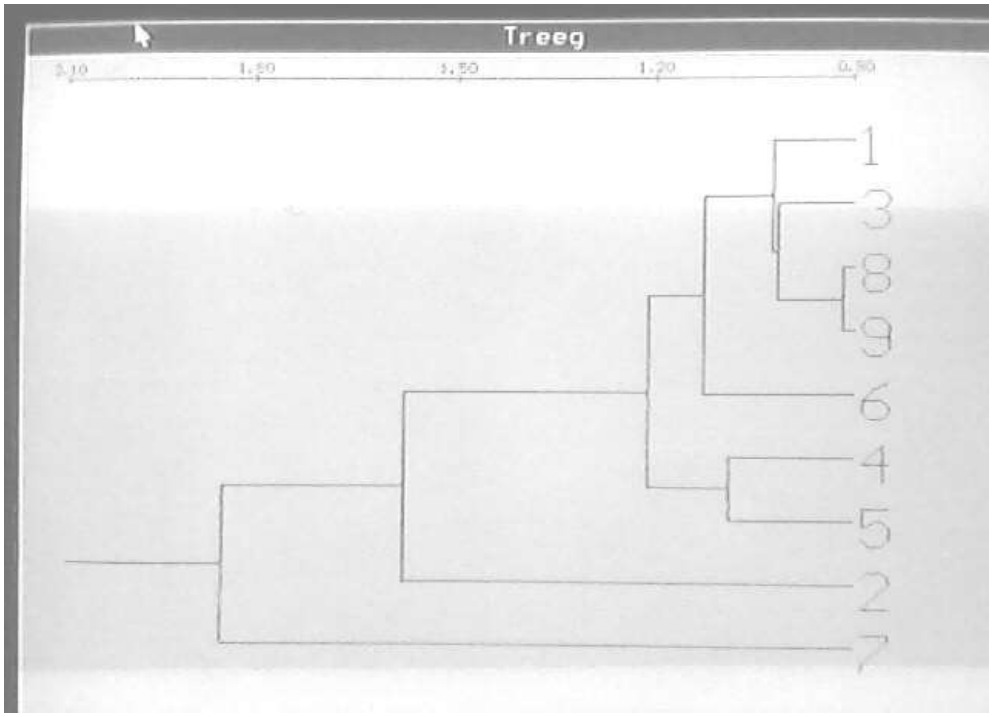

Figure 1. Dendrogram of operational taxonomic units (otus) based on macro- and micromorphological attributes using numerical analysis

Key : The following key was proposed based on the macro and micro morphological characters of leaves to distinguish the studied species**.**

A) Juvenile and adult leaves are the same in outline shape.

b) Leaf reniformis, opposite, sessile, obtuse apex, reniform base. Upper surface of epidermis weak favulariate, stomata depressed. Lower surface of epidermis favulariate, stomata superficial……*E. kruseana*

bb) Leaf ovate, petiolate. Juvenile leaf opposite. Adult leaf alternate, apiculate apex, symmetrical base. Upper surface of epidermis pusticulate, stomata semidepressed. Lower surface of epidermis verrucate, stomata depressed……*E. eostrata*

AA) Juvenile and adult leaves are different in shape.

c) Juvenile and adult leaves are alternate, petiolate, apex acute.

d) Very asymmetrical base. Upper and lower surfaces of epidermis colliculate, stomata depressed……...…….*E.gomphocephala*.

dd) Symmetrical base, with light veins. Upper surface of epidermis pusticulate, stomata absent. Lower surface of epidermis ruminate- favulariate, stomata superficial……..…….*E. ficifolia*

cc) Juvenile leaf opposite and adult leaf alternate.

e) Petiolate, apex acute symmetrical base.

f) Upper surface of epidermis colliculate, stomata absent. Lower surface of epidermis verrucate, stomata very depressed…….*E. resinfera*

ff) Upper surface of epidermis ruminate, stomata depressed. Lower surface of epidermis rugose, stomata depressed…….*E. citriodora*

ee) Petiole absent in juvenile leaf but adult leaf petiolate,

g) Apex acuminate, cordate base. Upper surface of epidermis weak ruminate, stomata very raissed. Lower surface of epidermis favulariate, stomata smiraised…….……...….*E. cinerea* .

gg) Apex acute,

h) Asymmetrical base, light green. Upper surface of epidermis glebulate, stomata absent. Lower surface of epidermis ruminate, stomata ……*E. globulus*

hh) Symmetrical base, dull and bluish green. Upper surface of epidermis weak pusticulate, stomata . Lower surface of epidermis verruculate, stomata raised in two surface ……*E. camaledulensis*

be stated that the studied species, according to the similarity or dissimilarity distance, split into three main clusters. The very far cluster which started at the highest similarity level (1.70) included *E.krusean.* The second cluster, which started at similarity level quit closer to the previous cluster, included *E.cinerea***.** while the third cluster split into three sub clusters. The first included two species; *E. ficifolia* and *E.globules* where they shared at 1.10 similarity level. The second subcluster included only one species; *E.gomphocephala* where it joined with the third subcluster at 1.17 similarity level. The latter subcluster included four species; *E.resinfera* and *E.rostrata* joined together at 0.93 similarity level, while *E.citriodora* joined with the previous two species at 1.03. The fourth species; *E.camaledulensis* joined with *E.citriodora*, *E.resinfera* and *E.rostrata* at 1.06 similarity level. The third cluster with all subclusters linked with the second cluster at 1.57, then all linked with the first cluster at 1.70 similarity level as they all species belong to the same genus and family.

Conclusively, from the pervious results of such research it could be conclude that the more close species to each other were; *E. ficifolia* and *E.globules; E.resinfera* and *E.rostrata.* Contrary, the most far species from each other were *E.cinerea* and *E.krusean.*

Appendices

Appendix 1. Characters and characters states list for numerical analysis of the studied species.

1) Juvenile and Adult leaves in outline shape 0. the same 1. different **Juvenile leaves macromorphology** 2) Reniformis shape 0. present 1. absent 3) Cordate shape 0. present 1. absent 4) Blue grey color 0. present 1. absent 5) Petiole 0. present 1. absent 6) Leaf **Arrangement** 0. Opposite 1. Alternate **Adult leaves macromorphology** 7) Reniformis shape 0. present 1. absent 8) Leaf length 0.less than 6cm. 1.more than 6m. 9) Leaf colour 0. Silvery white 1.not so 10) Veins 0. light Veins 1. not so 11) Petiole 0. present 1. absent 12) Leaf **Arrangement** 0. Opposite 1. Alternate 13) Leaf **Thickness** 0.Thin 1. Thick 14) Leaf texture 0. Leathery 1. not so 15) Leaf acut apex 0. . present 1. absent 16) Leaf acuminatet apex 0. . present 1. absent 17) Leaf obtuse apex 0. present 1. obsent 18) Leaf apicular apex 0. present 1. obsent 19) Leaf symmetrical base 0. present 1. absent	20) Leaf asymmetrical base 0. present 1. absent 21) Leaf reniform base 0. present 1. absent 22) Leaf cordate base 0. present 1. absent **Adult leaves micromorphology** **-Upper epidermis** 23) Surface pattern Pusticulate 0. present 1. absent 24) Surface pattern Ruminate 0. present 1. absent 25) Surface pattern Glebulate 0. present 1. absent 26) Surface pattern Colliculate 0. present 1. absent 27) Surface pattern Favulariate 0. present 1. absent 28) Stomata 0. present 1. absent **-Lower epidermis** 29) Surface pattern Verruculate 0. present 1. absent 30) Surface pattern Favulariate 0. present 1. absent 31) Surface pattern Rugose 0. present 1. absent 32) Surface pattern Ruminate 0. present 1. absent 33) Surface pattern Colliculate 0. present 1. absent 34) Surface pattern Verrucate 0. present 1. absent 35) Somata leveling raised 0.present 1. absent 36) Somata leveling depressed 0.present 1. absent 37) Somata leveling Superficial 0.present 1. absent

Appendix 2. The characters descriptions, character states and codes for the Numerical analysis.

Species Characters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	1	1	1	1	1	1	0	1	0
2	1	1	1	1	1	1	0	1	1
3	1	0	1	1	1	1	1	1	1
4	1	0	1	1	1	1	0	1	1
5	0	1	0	0	1	0	1	0	0
6	0	0	0	1	0	1	0	0	0
7	1	1	1	1	1	1	0	1	1
8	1	0	1	1	1	1	1	1	1
9	1	0	1	1	1	1	0	1	1
10	1	1	1	0	1	1	1	1	1
11	0	0	0	0	0	0	1	0	0
12	1	1	1	1	1	1	0	1	1
13	0	0	1	1	1	1	0	0	0
14	0	0	0	0	0	0	1	0	0
15	0	1	0	0	0	0	1	0	1
16	1	0	1	1	1	1	1	1	1
17	1	1	1	1	1	1	0	1	1
18	1	1	1	1	1	1	1	1	0
19	0	1	0	0	1	1	1	0	0
20	1	1	1	1	0	0	1	1	1
21	1	1	1	1	1	1	0	1	1
22	1	0	1	1	1	1	1	1	1
23	0	1	1	0	1	1	1	1	0
24	1	0	0	1	1	1	1	1	1
25	1	1	1	1	0	1	1	1	1
26	1	1	1	1	1	0	1	0	1
27	1	1	1	1	1	1	0	1	1
28	0	0	0	1	1	0	0	1	0
29	0	1	1	1	1	1	1	1	1
30	1	0	1	0	1	1	0	1	1
31	1	1	0	1	1	1	1	1	1
32	1	1	1	0	0	1	1	1	1
33	1	1	1	1	1	0	1	1	1
34	1	1	1	1	1	1	1	0	0
35	0	0	1	1	1	1	1	1	1
36	1	1	0	1	1	0	1	0	0
37	1	1	1	0	0	1	0	1	1

Key: 1) *E.camaledulensis* 2) *E.cinerea* 3) *E.citriodora*
4) *E. ficifolia* 5) *E.globules* 6) *E.gomphocephala*
7) *E.krusean* 8) *E.resinfera* 9) *E.rostrata*

GLOSSARY

Sculpture of surface:

Colliculate:With rounded broad elevations closely spaced covering the seed – coat.

Favulariate: With the surface finaly ribbed , the ribs separated by zigzag furrows

Glebulate: With small clumps of irregularly placed granules. Lineate : marked with fine lines.

Pusticulate: With small broad slight elevations not so high or a bundant as on a colliculate surface and not having as a brupt elevations as a minutely tuberculate surface.

Rugose: Wrinkled, the irregular elevation making up the wrinkles and running mostly in one direction.

Ruminate: Penetrated by irregular channels giving an eroded appearance and running in different directions.

Verrucate: With irregular projections or knobs.

Verruculate: With closely spaced tiny irregular projections.

Type of stomata:

Actinocytic type : The stomata is surrounded by epidermial cells which are uniformally arranged along the radii of a circle.

Anomocytic type: The stomal pores surrounded by unmodified epidermal cells of varying size which are irregularly arranged and can not be differentiated from the other epidermal cells.

Type of Trichomes (Glandular):

Belladonna : It contains uniseriate multicellular stalk and head.

Mentha: It contains short stalk with multicellular headand unicellular stalk

Vasaka: It contains unicellular glandular trichomes in which stalk is absent.

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وصف سطح الورقة كدلائل تصنيفية لتمييز بعض أنواع جنس الكافور فى مصر باستخدام المجهر الألكترونى الماسح

حنان سلامة عبدالمقصود*- محمود محمد السيد عبدالقادر– نرمين طه شنن*****

*قسم بحوث الفلورة وتصنيف النباتات – معهد بحوث البساتين – مركز البحوث الزراعية - مصر.

**قسم بحوث الغابات – معهد بحوث البساتين – مركز البحوث الزراعية –مصر.

**قسم الزينة – كلية الزراعة – جامعة القاهرة – مصر.

درست الصفات المورفولوجية والاختلاف البنائى الدقيق لسطح الورقة لتسعة أنواع من جنس الكافور، كانت هذه الأنواع هى:

Eucalyptus camaledulensis, *Eucalyptus cinerea*, *Eucalyptus citriodora*, *Eucalyptus ficifolia* , *Eucalyptus globules*, *Eucalyptus gomphocephala*, *Eucalyptus kruseana* , *Eucalyptus resinfera* and *Eucalyptus rostrata*.

أظهرت الصفات المكرومورفولوجية وجود اختلافات فى شكل الورقة وأختص كل نوع بكثير من الصفات. كما أظهرت نتائج الفحص الميكرومورفولوجى للطبقة العلوية لسطح الورقة أن هناك خمسة أشكال زخرفية مميزة للورقة وهى: Pusticulate, Ruminate , Glebulate, Colliculate , Favulariate، بينما وجد ست أشكال زخرفية مختلفة لسطح الورقة السفلى وهى: Ruminate, Verrucate, Verculate, Rugose, Favulariate , Clliculate، أستثنى من ذلك نوع واحد جمعت ورقتة بين شكلين زخرفيين هما : Ruminate – Favularlate

وقد صمم مفتاح اصطناعى للفصل بين الأنواع المدروسة.

التوصية : تم التنويه بامكانية ان تخدم هذه الدراسة كمعلومات تقسيمية للبحوث المستقبلية.