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COMPARATIVE BOTANICAL STUDIES ON SOME SPECIES OF CUPRESSACEAE AND TAXODIACEAE IN EGYPT

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Abstract

Morphological aspects of five taxa of Cupressaceae and three taxa of Taxodiaceae namely: *Chamaecyparis lawsoniana*, *Cupressus arizonica, Cupressus lusitanica*, *Cupressus sempervirens, Platycladus orientalis*, *Sequoia sempervirens, Taxodium distichum* and *Taxodium distichum* cv. imbricatum have been investigated. Micro-morphological studies by Scanning electron microscopy (SEM) on female cone were carried out. These diagnostic characters appeared to be significant in differentiation between the studied taxa. Short, opposite, scale like leaves, yellow, ovoid-oblong male cone and peltate, not ridged scales were characteristic bmorphological features of Cupressaceae. Long, alternate, linear leaves, greenish, ovoid male cone and obliquely shield-shaped, ridged scales were characteristic morphological features of Taxodiaceae. Surface sculpturing pattern presented three different types: Tuberculate, glebulate and sulcate. The ultrastructure of female cone cuticles, epicuticular wax crystals were described. The data recorded from the morphological and SEM characters were used in numerical analysis.

Keywords: Cupressaceae, Taxodiaceae, macro-morphology, micro-morphology, SEM, numerical analysis.

Introduction

Cupressaceae is a conifer family, the cypress family, which includes 19 genera with about 125 species. It is the most important conifer family in modern horticulture. Several species of *Chamaecyparis, Cupressus* and *Juniperus* are of major importance in horticultural trade, accounting for about 99.9% of all conifers sold for garden planting in Britain; many thousands of cultivars have been named (Farjon, 1998).

The plants are trees or shrubs, evergreen, monoecious or dioecious. Leaves decussate or in whorls of 3, scale like, flattened facial leaves and keeled lateral leaves, often with an abaxial resin gland. Pollen cones terminal or axillary, solitary, maturing and shed annually; microsporophyll 6–16, decussate or whorled, each bearing (2 or) 3–6 (–9) pollen sacs; pollen wingless. Seed cones usually terminal, solitary, globose, ovoid, or oblong, dehiscent or indehiscent when mature in 1st or 2nd year; cone scales developing after ovules originate in bract axils; bracts almost completely enveloped by cone scales, free only at apex; ovules 1–numerous per bract axil, erect; cone scales of mature cones 3–16, flat or peltate, woody, ± leathery, or succulent, 1– 20 seeded. Seeds winged or not; wings derived from seed coat. Cotyledons usually 2, rarely 3– 6. Germination is epigeal (Fu *et al.,* 1999 and Mao *et al.,* 2012).

Taxodiaceae is a conifer family, the swamp cypress family, includes 9 genera with about 12 species. The plants are trees, evergreen, semi evergreen, or deciduous, monoecious; trunk straight; main branches ± whorled. Leaves spirally arranged or scattered (decussate in *Metasequoia*), monomorphic, dimorphic, or trimorphic on the same tree, lanceolate, subulate, scalelike, or linear. Microsporophylls and cone scales spirally arranged (decussate in *Metasequoia*). Pollen cones borne in panicles, or solitary or clustered at branch apices, or axillary, small; microsporangia with (2- 3) or (4–9) pollen sacs; pollen nonsaccate. Seed cones terminal or borne near apex of previous year's growth, ripening in 1st year, persistent or late deciduous; cone scales developing after ovules originate in bract axils; bracts and cone scales usually spirally aranged (decussate in *Metasequoia*), sessile, opening when ripe (falling in *Taxodium*), semiconnate and free only at apex, or completely united; bracts occasionally rudimentary (in *Taiwania*); ovules 2–9 per bract axil, erect or pendulous; cone scales of mature cones flattened or shield-shaped, woody or leathery, 2–9-seeded on abaxial side. Seeds flat or triangular, wingless (in *Taxodium*), narrowly winged all round or on 2 sides, or with a long wing on proximal part Cotyledons 2–9 (Fu *et al.,* 1999 and Mao *et al.,* 2012).

Both of the two families are distributed in temperate and subtropical regions, in both the Old and New Worlds. Only one genus is represented in the Southern Hemisphere. Most of these are fast growing trees which can achieve a large size and an impressive age. Some members of this group have been known to be 3,000 years old (Kubitzki *et al.,* 1990; Vidakovic 1991 and Welch and Haddow 1993).

Female cones in conifers consist of numerous overlapping structures that each represents an integrated complex combining a bract and an ovuliferous scale; here termed the bract/scale complex (Coulter & Chamberlain 1917; Florin, 1951; Wilde, 1975 and Rudall *et al.,* 2011). The bract is a modified leaf that bears in its axil an ovulate short shoot (the seed scale or ovuliferous scale). Thus, the seed cone represents a polyaxial, branched structure (Schuhmann, 1902; Herzfeld, 1914; Pilger, 1926; Florin, 1951 and 1954; Schweitzer, 1963; Mundry, 2000 and Rudall *et al.,* 2011).

The characteristics of the female cones of Cupressaceae and Taxodiaceae are that they are generally larger than the male cones of the same species. They are usually solitary and globular. The female cones of Cupressaceae can be woody, leathery or semi-fleshy. The scales are woody or fleshy and in few opposing pair. The scales meet at the edges and are partially or wholly adnate (one side attached to the stem), decussate or whorled. There are a small number of fertile scales, with one or more ovules on each fertile scale. The bract scale of the female Cupressaceae cone is not externally visible. The female cones of the Taxodiaceae plants are

terminal on shoots. These cones open when ripe. The scales originate from the seed bulge and are woody when ripe. The fertile scales are more or less adnate to the bract scale. At first, the fertile scale is bulging but then it enlarges and surpasses the bract scale. There are generally two to nine erect or inverted ovules for each fertile scale (Vidakovic 1991).

Many Cupressaceae are treated with very high regard by traditional societies. In Japan, *Cryptomeria japonica* is the national tree, and of the 'five sacred trees of Kiso', four are in this family (*Chamaecyparis obtusa*, *Ch. pisifera*, *Thuja standishii*, *Thujopsis dolabrata*). *Thuja plicata* is highly revered among the tribes of the Northwest Coast of North America, who made their houses, canoes, baskets, boxes and even clothing from its bark, wood and roots, while *Sequoia dendron* have inspired a deep sense of reverence among people from western cultures ever since their discovery (Farjon, 1998).

Pilger (1926) separated Cupressaceae into two families; Cupressaceae sensu stricto (genera with leaves opposite in four ranks or whorled) and Taxodiaceae (leaves mostly alternate). This classification has been widely utilized (Florine, 1951; Sporne, 1965; Dallimore and Jackson, 1966 & Fu *et al.*, 1999). However, Pilger (1926) recognition has been challenged on the basis of several evidences. Many studies have further confirmed the close relationship between the Cupressaceae s. str. and Taxodiaceae where considered Cupressaceae includes Taxodiaceae (Eckenwalder, 1976; Hart, 1987; Price and Lowenstein, 1989; Hart and Price, 1990 and Gadek and Quinn, 1993).

Scanning electron microscopy (SEM) have been invaluable tools for the study of the micromorphology of plant cuticles. It has been principally used to study the distribution of epicuticular waxes (Edith *et al.,* 2004; Dragota and Riederer, 2007. Koch and Ensikat 2008; Nadiminti *et al.,* 2013; Go *et al.,* 2014 and Nadiminti *et al.,* 2015).

This work aimed to use the macro and micro morphological characters to support any of the two opinions; if Taxodiaceae must be included in Cupressaceae or it is a distinct family.

Materials and Methods

This study was carried out during seasons 2018- 2019 on five taxa of Cupressaceae and three taxa of Taxodiaceae. The studied specimens were collected fresh from El-Orman Garden (O.G.) and Agriculture Museum Garden (A.M.G.) as shown in (Table 1). The taxa were identified by means of comparison with authentic specimens kept in the herbarium of the Flora and Phytotaxonomy Researches Department (CAIM). In addition to botanical keys of Bailey (1949), Zohary (1966), Rehder (1967), Davis (1975), Humphries (1981) and Thompson (1992).

Table 1: List of the collected species for the present study:

	Taxa	Locations
Family: Cupressaceae		
1	*Chamaecyparis lawsoniana* (A.Murray bis) Parl.	O.G.
2	*Cupressus arizonica* Greene	O.G.
3	*Cupressus lusitanica* Mill.	O.G.
4	*Cupressus sempervirens* L.	A.M.G.
5	*Platycladus orientalis* (L.) Franco.	A.M.G.
Family: Taxodiaceae		
6	*Sequoia sempervirens* (D.Don) Endl	O.G.
7	*Taxodium distichum* (L.) Rich.	A.M.G.
8	*Taxodium distichum* v. imbricatum (Nutt.) Croom.	O.G.

The detailed surface scan features were examined by Scanning electron microscopy (SEM) with different magnifications. Scanning was don using JEOL-JSM T 100 Model Scanning electron microscope of the Central Laboratory, National Information and Documentation Center (NIDC), Dokki, Giza, Egypt. The terminology of (Barthlott 1981 & 1990 and Stearn 1992) was adopted to describe the SEM aspects of the female cone. The relationship among the studied taxa has been analyzed using Average Linkage (Between Groups) of SPSS program version 22.

Results & Discussion

(I) Morphological Characters

(a) Macro- morphological characters

The different macro- morphological characters of studied taxa were investigated to indicate the importance of these characters. Variations in these aspects among the species are listed in (Table 2) and illustrated in Plates 1, 2, 3, 4 & 5.

The morphological characters were found diagnostic to the investigated taxa. All studied taxa were trees varied in length and width. Bark was thin in *Sequoia sempervirens* and *Taxodium distichum var. imbricatum* while in the rest was thick. Bark was brown in *Taxodium distichum* and *Taxodium distichum var. imbricatum* and reddish brown in the reminders. Blade outline was subulate in *Taxodium distichum var. imbricatum*, linear in *Taxodium distichum* and *Sequoia sempervirens* and scale like in the remainders. Blade color was bluish-green in *Cupressus arizonica*, *Cupressus lusitanica* and *Cupressus sempervirens* while bright green in the remainders. Staminate flower was ovoid and greenish in *Sequoia sempervirens, Taxodium distichum* and *Taxodium distichum var. imbricatum* while ovoid-oblong and yellow in the rest. Female cone was ovoid in *Sequoia sempervirens, Taxodium distichum* and *Taxodium distichum var. imbricatum,* oblong in *Platycladus orientalis* and globose in the remainders. Female cone scales are obliquely-shield from15 to 20 scales per cone in *Sequoia sempervirens, Taxodium distichum* and *Taxodium distichum var. imbricatum.* However, scales are peltate from 6 to 12 scales per cone in the remainders. Number of cotolydones from 4 to 9 in *Taxodium distichum* and *Taxodium distichum var. imbricatum,* from 3 to 4 in *Cupressus arizonica*, *Cupressus lusitanica* and *Cupressus sempervirens* and 2 in the rest. Number of seeds per scale from 8 to 10 in *Cupressus arizonica*, *Cupressus lusitanica* and *Cupressus sempervirens* while from 2 to 4 in the remainders. Seed wing absent in *Platycladus orientalis,* two-spongy in *Sequoia sempervirens,* three-thick in *Taxodium distichum* and *Taxodium distichum var. imbricatum* and two-narrow in the remainders. Seed shape elliptic-oblong in *Platycladus orientalis,* triangle in *Taxodium distichum* and *Taxodium distichum var. imbricatum* and flattened in the rest.

Table 2 : Data coding of macro- morphological characters of the studied taxa.

Taxodium distichum var. imbricatum	*Taxodium distichum*	*Sequoia sempervirens*	*Platycladus orientalis*	*Cupressus sempervirens*	*Cupressus lusitanica*	*Cupressus arizonica*	*Chamaecyparis lawsoniana*	**Taxa►** / **Characters ▼**
1	2	1	3	3	3	3	2	1- Height: Very tall (more than 100 m) [1], tall (up to 50 m) [2]/ short (less than 26 m) [3].
2	2	1	2	2	2	2	2	2-Diameter: Very wide (25 m) [1] / wide (less than 25 m) [2].
1	2	1	2	2	2	2	2	3- Bark thickness: Thick [1]/ thin [2].
2	2	2	1	1	1	2	1	4-Bark shape: Scaly [1]/ fissured [2].
2	2	1	1	1	1	1	1	5-Bark color: Reddish-brown [1]/ brown [2].
1	1	1	2	2	2	2	2	6- Leaf arrangement: Alternate [1]/ opposite [2].
3	2	2	1	1	1	1	1	7- Blade outline: Scale-like [1]/ linear [2]/ subulate [3].
3	1	3	2	2	4	4	4	8- Blade apex: Apiculate [1]/ obtuse [2]/ incurved [3]/ acute [4].
1	1	1	1	2	2	2	2	9- Blade margin: Entire [1]/ toothed [2].
2	2	2	2	1	1	1	2	10- Blade color: Bluish-green [1]/ bright green [2]
2	2	2	1	1	1	1	1	11- Blade length: Short (0.2-0.3 cm) [1]/ long (1.5-2 cm) [2].
1	1	1	1	1	2	2	2	12- Male cone length: Short (1- 1.5 mm) [1]/ long (3- 5 mm) [2].
2	2	2	1	1	1	1	1	13- Male cone shape: Ovoid-oblong [1]/ ovoid [2].
2	2	2	1	1	1	1	1	14- Male cone color: Yellow [1]/ greenish [2].
2	2	2	1	4	2	2	2	15- Female cone length: Short (1- 1.5 cm) [1]/ long (1.5-2 cm) [2]/ very long (up to 4 cm) [3].
3	3	3	2	1	1	1	1	16- Female cone shape: Globose [1]/ oblong [2]/ ovoid [3].
2	2	2	1	1	1	1	1	17- Female cone scales: 6- 12scales [1]/ 15-20 scales [2].
2	2	2	1	1	1	1	1	18- Scales shape: Peltate [1]/ obliquely shield-shaped [2].
2	2	2	1	1	1	1	1	19- Scales: Not ridged [1]/ ridged [2].
4	4	4	3	2	2	2	1	20- Scales mucro: Acute reflexed [1]/ small piont [2]/ hooked [3]/ absent [4].
3	3	1	1	2	2	2	1	21- No. of cotolydones: 2 [1]/ 3- 4 [2]/ 4- 9 [3].
1	1	1	1	2	2	2	1	22- No. of seeds/scale: 2- 4 [1]/ 8- 10 [2].
3	3	2	4	1	1	1	1	23- Seed wing: 2-narrow [1]/ 2-spongy [2]/ 3-thick [3]/ absent [4].
2	2	2	1	1	1	1	1	24- Seed length: Short (3-5 mm) [1]/ long (5-10 mm) [2].
3	3	2	2	1	1	1	1	25- Seed shape: Flattened [1]/ elliptic-oblong [2]/ triangle [3].

Plate 1 : Habit of the studied taxa

(b) Micro-morphological Aspect

The examination by scanning electron microscope was carried out on female cone of the studied taxa. The examination showed differences in sculpture pattern, outerpericlinal walls, anticlinal walls, gonal numbers and epicuticular wax crystals. These differences in micro-morphological characters of female cones were summarized in Table 3 and Plates 6, 7 & 8.

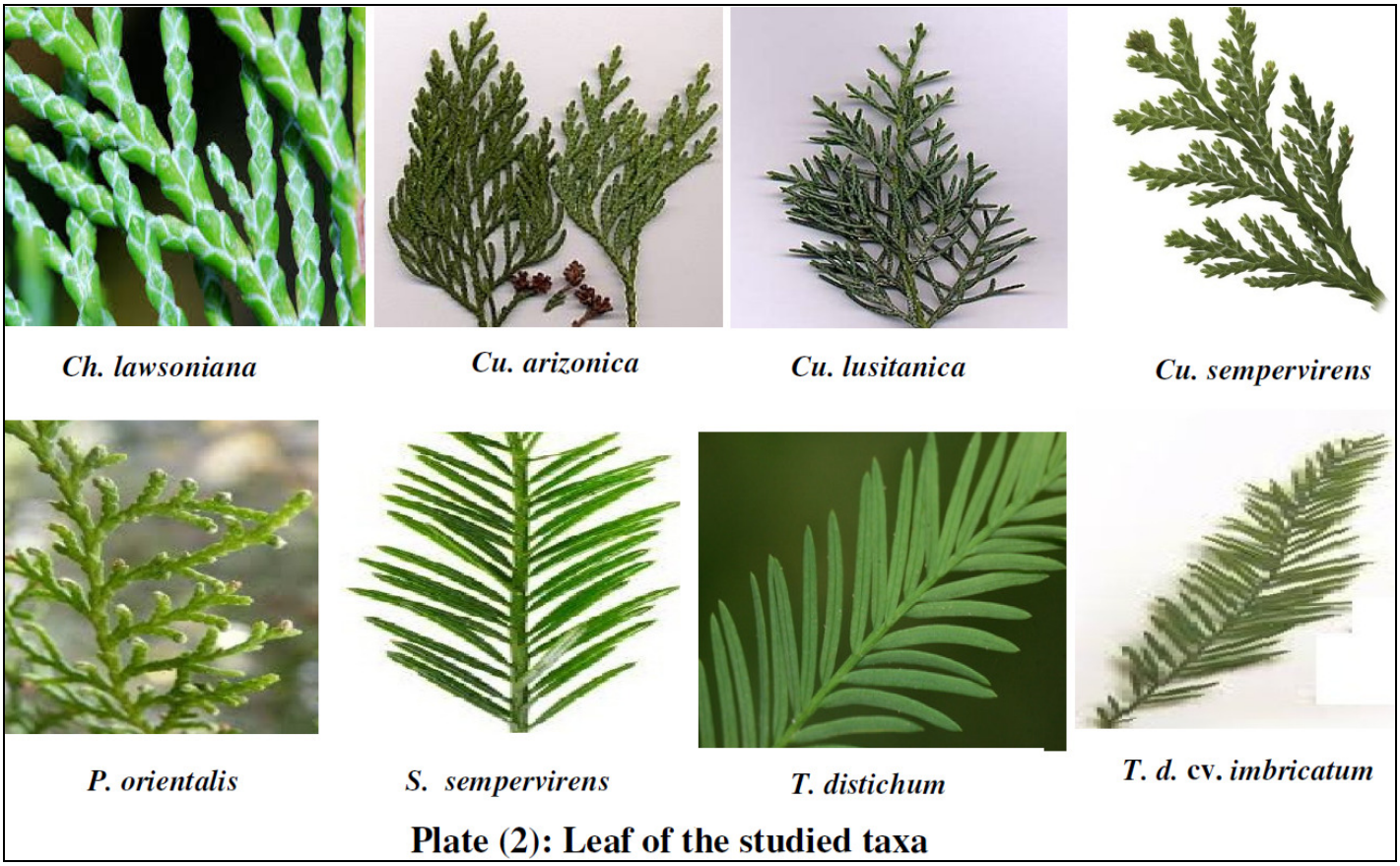


Plate (2): Leaf of the studied taxa

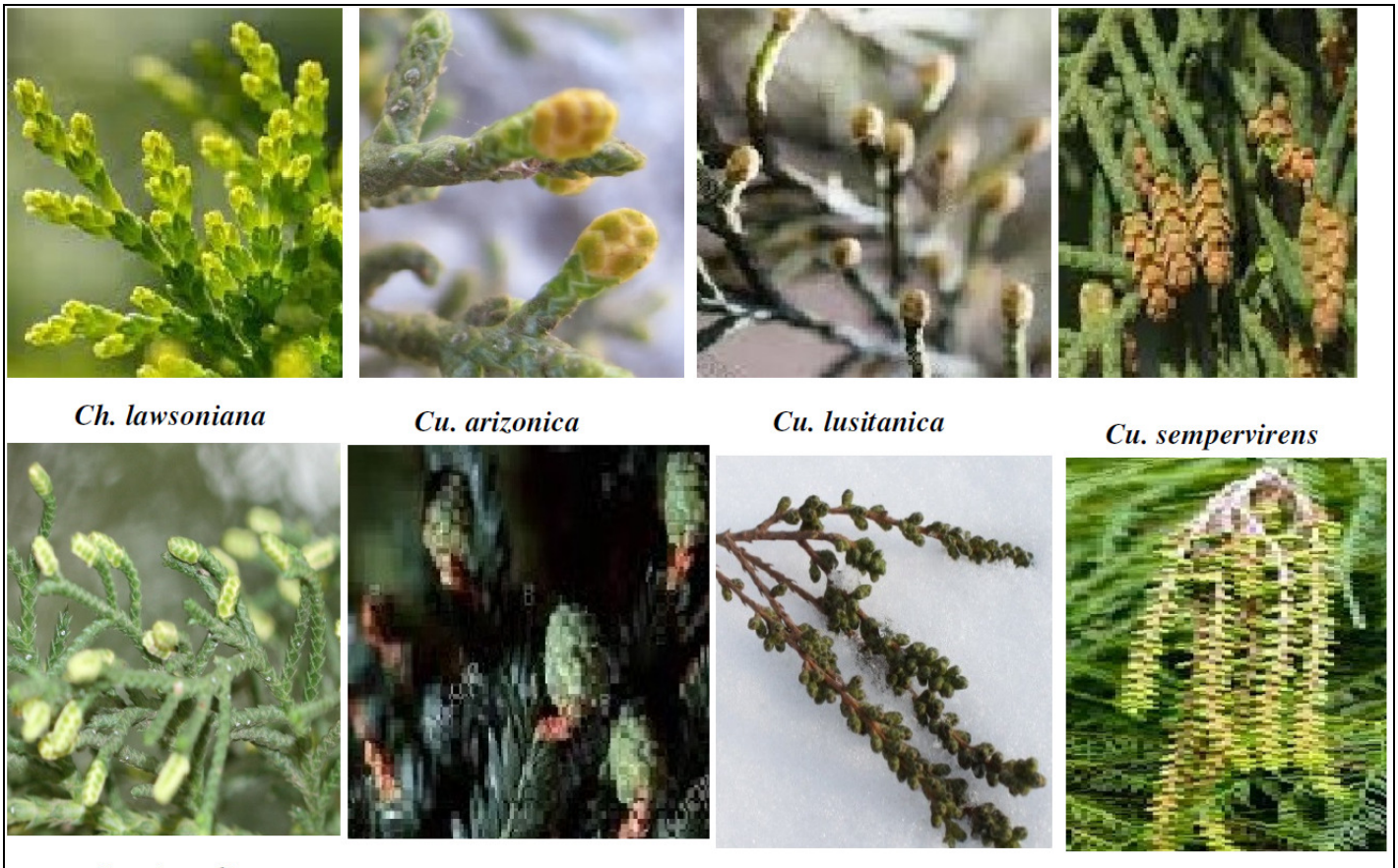


Plate 3 : Pollen cone of the studied species

Chamaecyparis lawsoniana *Cupressus arizonica* *Cupressus lusitanica* *Cupressus sempervirens*

P. orientalis *S. sempervirens* *T. distichum* *T. d.* cv. imbricatum

Plate 4 : Immature female cone of the studied species

Ch. lawsoniana *Cu. arizonica* *Cu. lusitanica* *Cu. sempervirens*

P. orientalis *S. sempervirens* *T. distichum* *T. d.* cv,*imbricatum*

Plate 5 : Mature female cone of the studied species

As shown in table 3 sculpture pattern was varied from tuberculate in (*Chamaecyparis lawsoniana, Taxodium distichum* and *Taxodium distichum var. imbricatum*), glebulate in (*Cupressus arizonica*, *Cupressus lusitanica* and *Cupressus sempervirens*) and sulcate in the rest. Outerpericlinal walls recorded five types. Concave in *Chamaecyparis lawsoniana.* Superficial, raised and flat in *Cupressus arizonica* and *Cupressus lusitanica.* Superficial disintegrated forming holes in *Cupressus sempervirens*. Raised- convex in *Platycladus orientalis* and *Sequoia sempervirens*. Superficial- semi raised in *Taxodium distichum* and *Taxodium distichum var. imbricatum.*

Anticlinal walls ranged between depressed- elongated in *Sequoia sempervirens, Taxodium distichum* and *Taxodium distichum var. imbricatum* and depressed in the reminders. SEM of the ultrastructure of cuticles of female cone showed differences in epicuticular wax crystals characters. Most of the crystals were arranged perpendicular to the underlying wax layer while some were arranged at an angle. Epicuticular wax crystals were branched in *Cupressus lusitanica, Sequoia sempervirens* and *Taxodium distichum var. imbricatum* but in the reminders were unbranched. The crystals were short in *Cupressus lusitanica, Platycladus orientalis, Taxodium distichum* and *Taxodium distichum var. imbricatum* while long in the reminders. They were thick (290- 450 nm) in *Chamaecyparis lawsoniana, Cupressus arizonica* and *Sequoia sempervirens* and were thin (90- 190 nm) in the restEpicuticular wax crystals apex were concave.

Table 3 : Data coding of micro- morphological characters of the Studied taxa

Taxodium distichum cv. imbricatum	***Taxodium distichum***	***Sequoia sempervirens***	***Platycladus orientalis***	***Cupressus sempervirens***	***Cupressus lusitanica***	***Cupressus arizonica***	***Chamaecyparis awsoniana***	**Species** / **Characters**
1	1	3	3	2	2	2	1	1- Sculpture pattern: Tuberculate [1], glebulate [2]/ sulcate [3].
5	5	4	4	3	2	2	1	2- Outerpericlinal walls: Concave [1]/ superficial, raised and flat [2]/ superficial disintegrated forming holes [3]/ raised- convex [4]/ superficial- semi raised [5].
2	2	2	1	1	1	1	1	3- Anticlinal walls: Depressed [1]/ depressed-elongated [2].
3	3	3	3	2	2	2	1	4-Gonal No.: 4- 5 [1]/ indistinct [2]/ 5- 6 [3].
1	2	1	2	2	1	2	2	5- Epicuticular wax crystals: Branched [1]/ unbranched [2].
2	2	1	2	1	2	1	1	6- Epicuticular wax crystals: Short [1]/ long [2].
2	2	1	2	2	2	1	1	7- Epicuticular wax crystals: Thick (290- 450 nm) [1]/ thin (90- 190 nm) [2].
2	1	1	2	2	2	1	1	8- Epicuticular wax crystals apex: Concave [1]/ blunt [2].

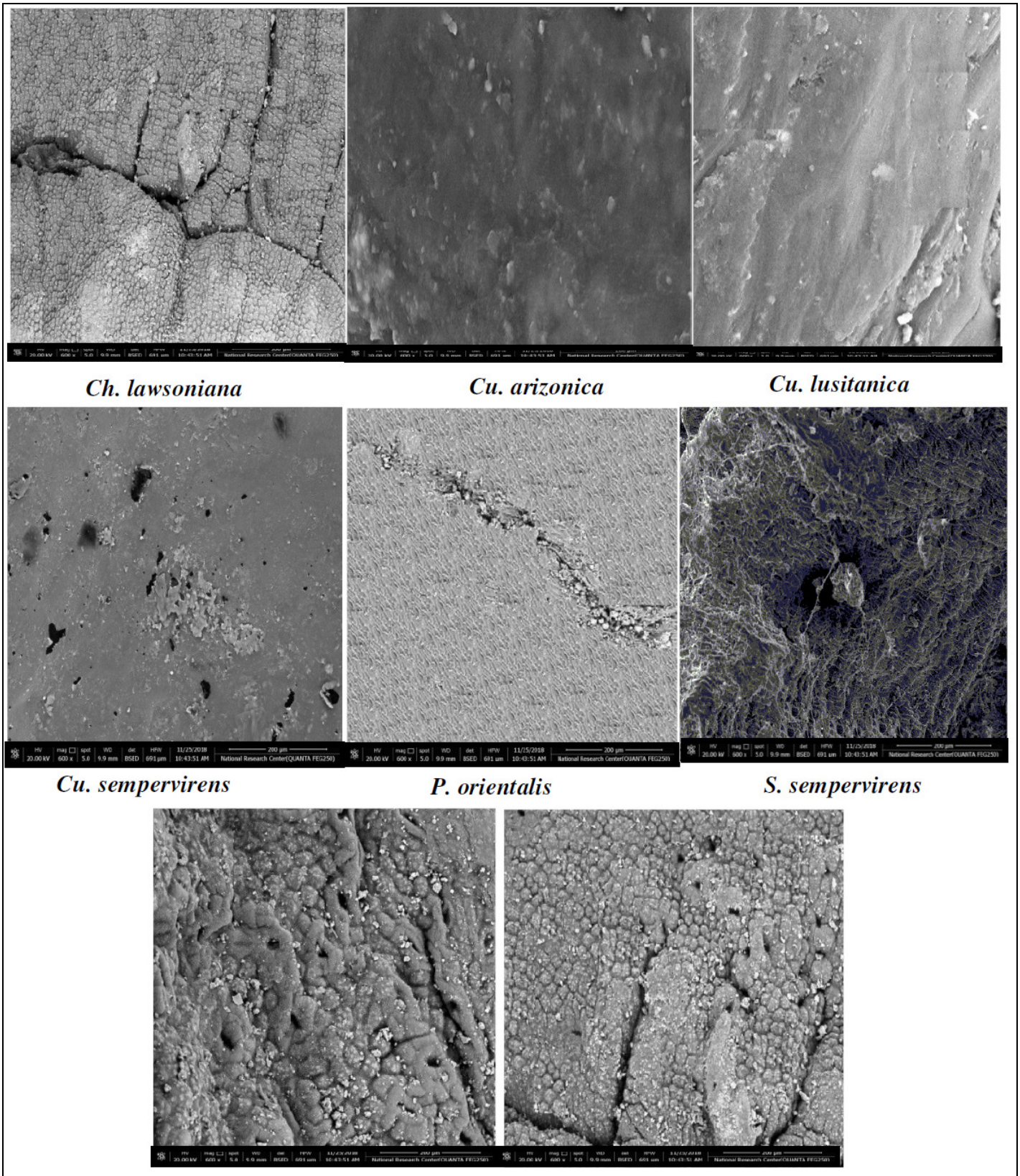
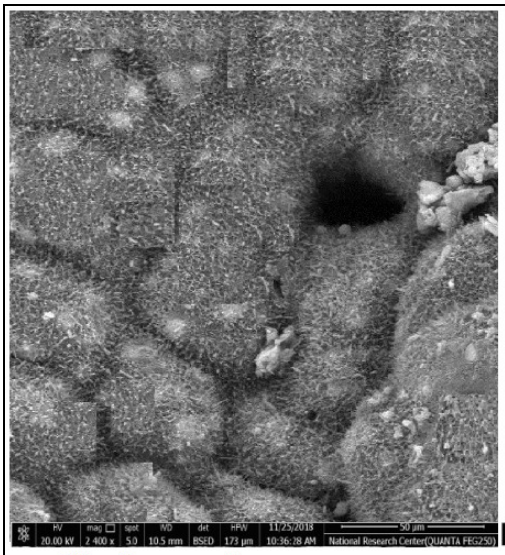
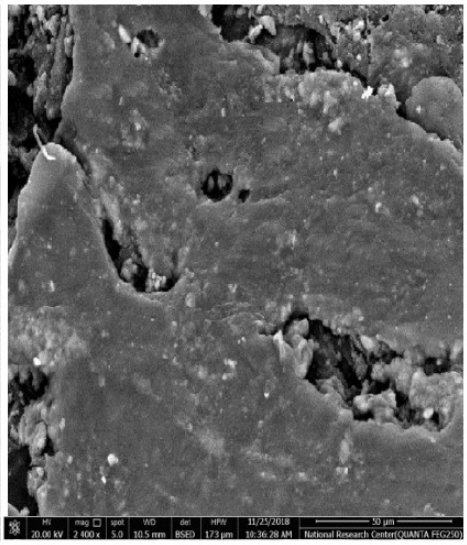


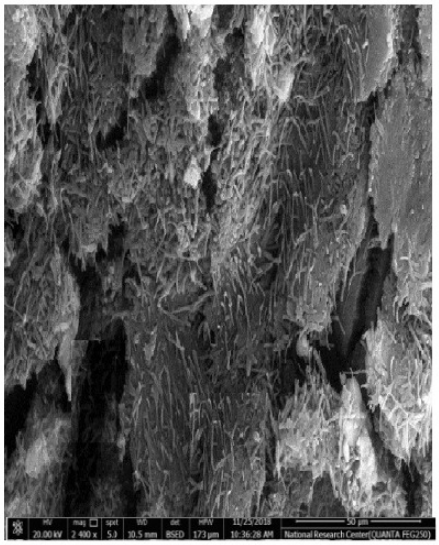
Plate (6): SEM showing the surface texture of mature female cone of the studied taxa



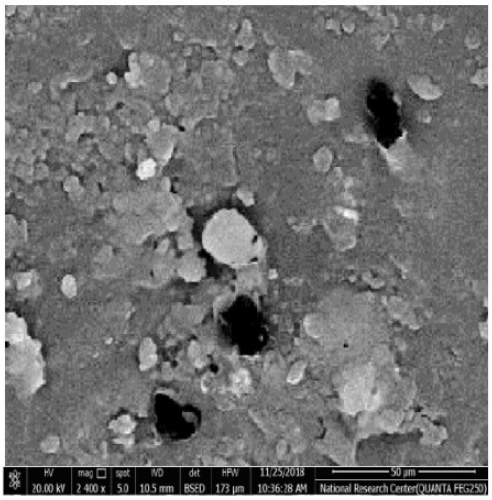
Ch. lawsoniana



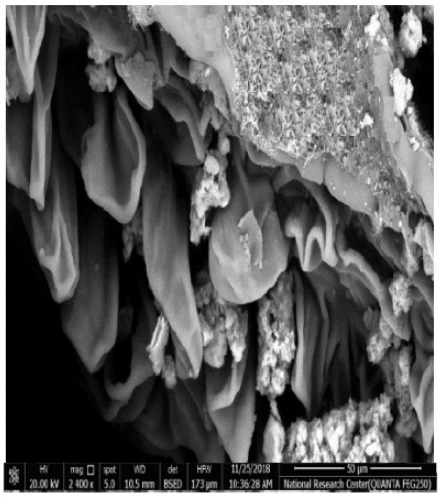
Cu. arizonica



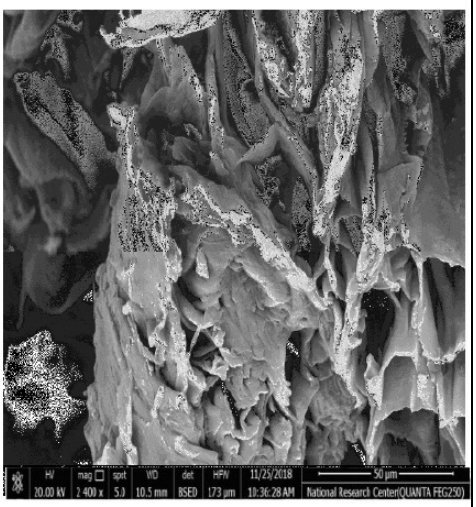
Cu. lusitanica



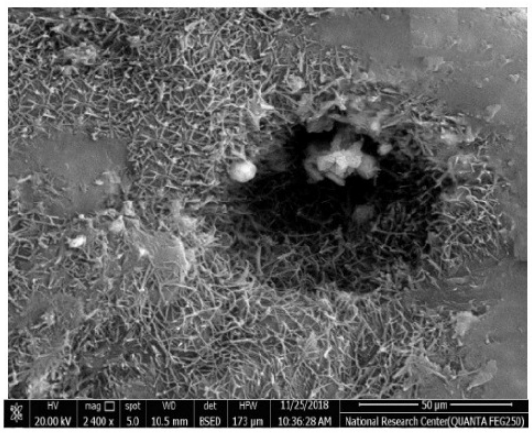
Cu. sempervirens



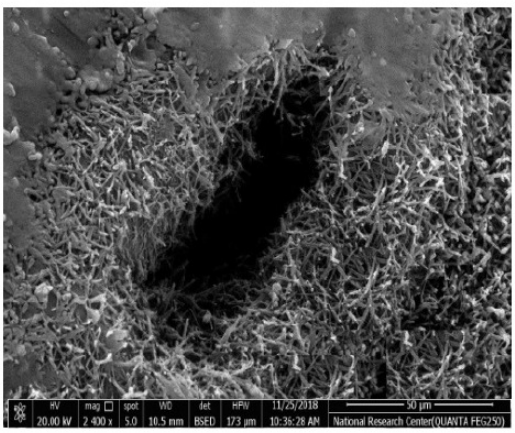
P. orientalis



S. sempervirens

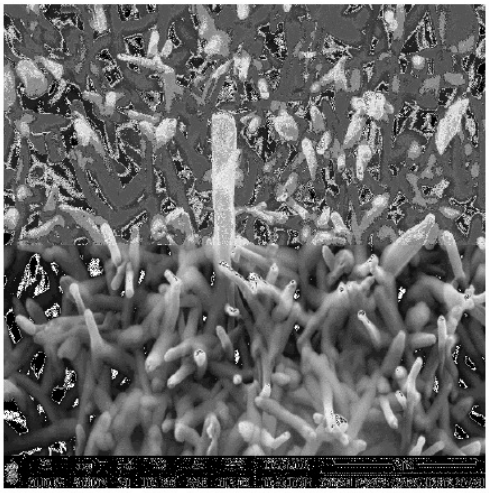


T. distichum

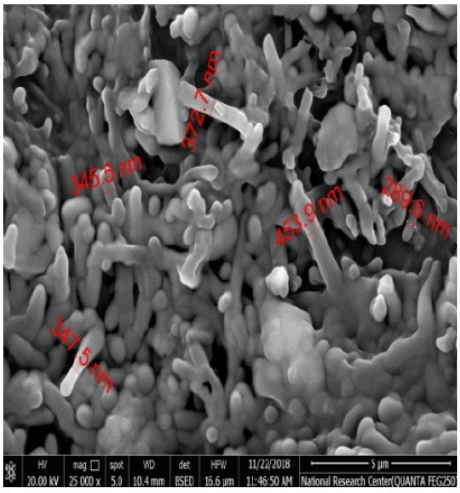


T. d. **cv. imbricatum**

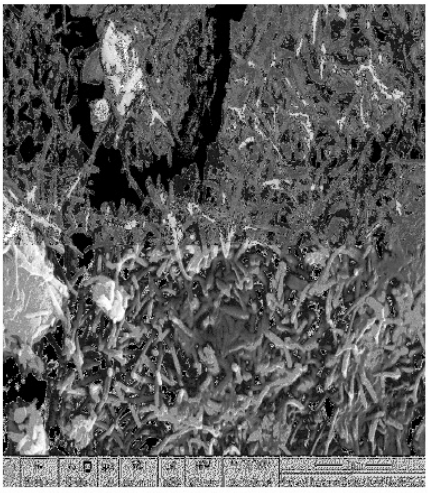
Plate (7): SEM showing surface structure of mature female cone of the studied taxa



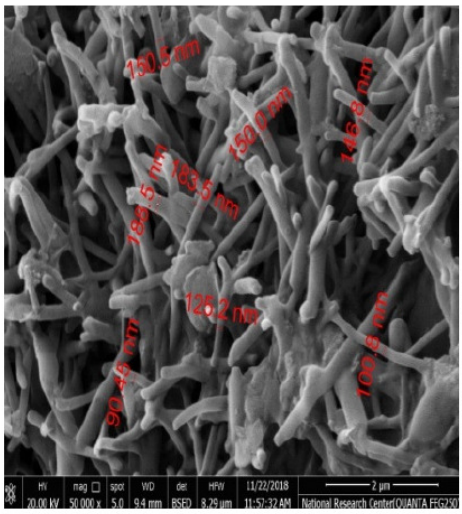
Ch. lawsoniana



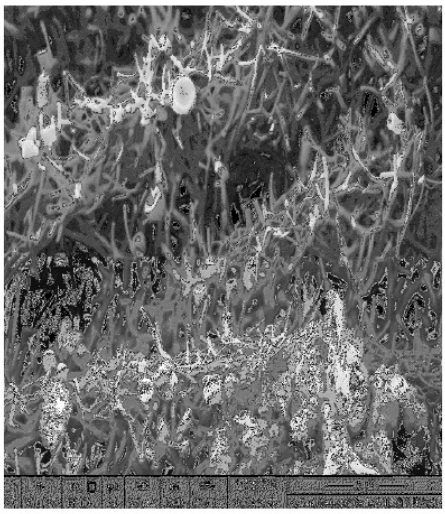
Cu. arizonica



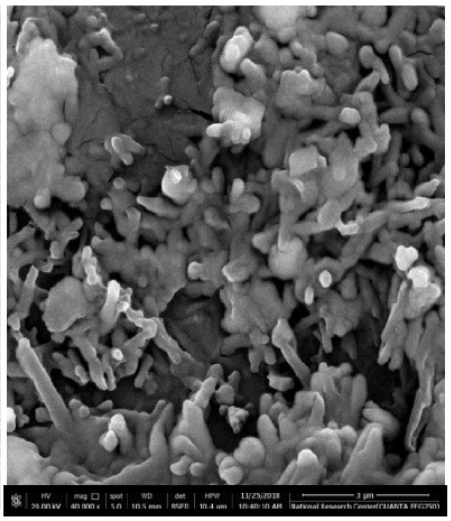
Cu. lusitanica



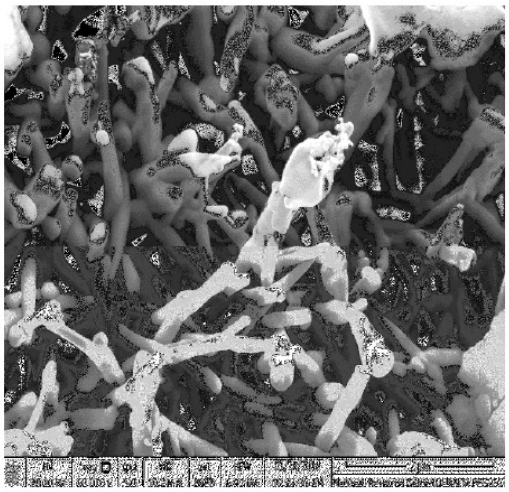
Cu. sempervirens



P. orientalis



S. sempervirens



T. distichum

T. d. cv. **imbricatum**

Plate (8): SEM showing epicuticular wax crystals of the mature female cone of studied taxa

(C) Numerical analysis

The characters from macro and micro-morphological investigations for 5 taxa of Cupressaceae and three taxa of Taxodiaceae were used for numerical analysis by using the method of clustering analysis. Such analysis was used as a tool in the identification of the studied taxa and in distinguishing the taxonomic relationships among the studied taxa. The results of clustering analyzed by the agglomeration of Schedule measure distance and similarity using average linkage between groups by SPSS program.

The results of numerical analysis showed that the studied taxa were grouped into two major clusters as shown in Fig. (1). The first cluster consisted of five taxa of Cupressaceae while the second cluster included the three taxa of Taxodiaceae. The first cluster is divided into two groups. The first group included *Platycladus orientalis* while the second group contained the other taxa of Cupressaceae. The second cluster is divided into two groups. The first group included *Sequoia sempervirens* while the second group contained *Taxodium distichum* and *Taxodium distichum* cv. *Imbricatum.*

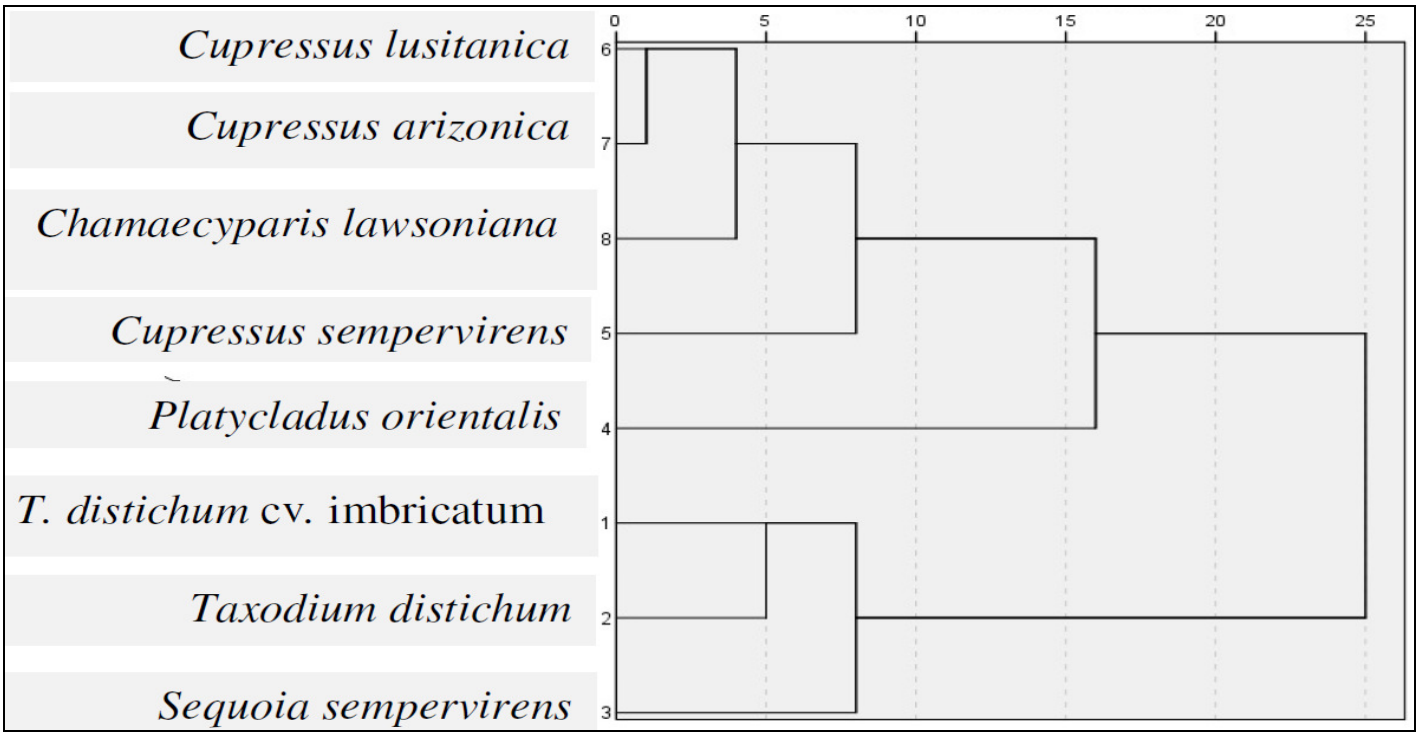


Fig. 1 : Dendrogram Using Average Linkage between Groups Related Distance Cluster combine

Degree of similarity among the studied taxa

The data presented in (Table 4) showed that the highest degree of similarity ratio was 96.6 % between *Cupressus arizonica* and *Cupressus lusitanica.*

Table 4 : Proximity matrix showed similarity value of the studied taxa based on macro and micro-morphological characters

Proximity Matrix

	Absolute Correlation between Vectors of Values							
	Taxodium_distichum_var._imbricatum	Taxodium_distichum	Sequoia_sempervirens	Platycladus_orientalis	Cupressus_sempervirens	Cupressus_lusitanica	Cupressus_arizonica	Chamaecyparis_lawsoniana
Taxodium_distichum_var._imbricatum	1.000	.762	.766	.305	.202	.142	.148	.208
Taxodium_distichum	.762	1.000	.535	.445	.112	.337	.342	.513
Sequoia_sempervirens	.766	.535	1.000	.253	.170	.085	.071	.061
Platycladus_orientalis	.305	.445	.253	1.000	.179	.275	.239	.203
Cupressus_sempervirens	.202	.112	.170	.179	1.000	.685	.652	.477
Cupressus_lusitanica	.142	.337	.085	.275	.685	1.000	.966	.826
Cupressus_arizonica	.148	.342	.071	.239	.652	.966	1.000	.797
Chamaecyparis_lawsoniana	.208	.513	.061	.203	.477	.826	.797	1.000

This is an absolute similarity matrix

Conclusion

The results of macro- and micro- morphological characters considered Cupressaceae and Taxodiaceae as two separate families. This study supported the systems of Florine, 1951; Sporne, 1965; Dallimore and Jackson 1966 & Fu *et al.*, 1999 while was not agreed with the systems of Eckenwalder 1976; Hart, 1987; Price and Lowenstein 1989; Hart and Price, 1990 and Gadek and Quinn 1993.

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