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A COMPARATIVE ANATOMICAL STUDY OF THREE TYPES OF GYMNOSPERM PLANTS *CYCAS REVOLUTA*, *THUJA OREINTALIS* AND *CUPRESSUS SEMPERVIENS*

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Abstract

The three types of *Cycas revolute*, *Thuja orientalis* and *Cupressus semperviens* are among the most important Gymnospermplants, studied the anatomy of the petiole and leaf for all studied species by light microscope. The fact that the cultivated plants in Iraq are subject to the same environmental conditions and few anatomical studies of the plants of the Gymnosperm plants, therefore this research study was conducted to learn the anatomical study of the plants of the Gymnosperm as well as their assistance to distinguish between the three types, which gave them a taxonomic value and by knowing the anatomical qualities of this research. The three types were distinguished and find out the tissues that are configured for them in a detailed and scientific way.

Key words : Anatomical characteristics, *Cycas revolute*, *Cupressus semperviens*, *Thuja orientalis*.

Introduction

The Gymnosperm plants have high important cannot be ignored, they help to reduce the speed of the wind carrying dust and dirt, as contribute to the reduction of air pollution as well as the role that play in reducing the presence of carbon dioxide and oxygen production. The Gymnosperm plants also have economic importance as their woods are used in the furniture industry and are included in the formation of perfumes and ointments (Al-Kilani, 2011).

Cycas revoluta plant belong to the order Cycadales and it is one of the most expensive and long-lived ornamental plants. Australia is the original home of this plant and its adaptation in most regions of the world and *Cycas revoluta* plant is like a palm-shaped cylindrical trunk, it is smooth sleek and ends up in a pointy fork like a palm fork (Linnacus, 1753a).

The *Cupressus semperviens* plant, which belong to the order of Coniferales and the family Cupressaccae, also it is ornamental plants and their original home, Turkey, is characterized as leafy and its buds are covered with the opposite leaves and they are burned on four rows, an unisexual plant and thorns are coherent range of 6-14 with small and winged seeds. The length of *Cupressus semperviens* plant up to 30 meters, which is slow-growing and fragrant wood (Linnacus, 1753b). While, *Thuja orientalis*, which also belong to Cupressaccae family, is no less important than its predecessors, because of it is also an ornamental tree whose home is the continent of Asia and from which oil is extracted Cedar oil used in the polishing of ivory wood as well as a source of resin substances enters the work of dyes. The *Thuja orientalis* its length (3-5) meters with branched-erect stem and brown coloured, the leaves are small and flatferns and unisexual flowers (https://or.m.wikipedia.org.wiki).

Materials and Methods

Sample collection

Samples were collected from scattered areas through field tours of several gardens from Baghdad governorate, specifically in the Al-Adhamiyah region.

Preparation of transferase permanent sections for the petiole and vertical sections of the leaves

a. **Fixation:** a soft part of the middle of the petioles and leaves were took and put in a small glass vials with a capacity of 30 ml and placed in each bottle 20 milliliters of FAA solution with 90% alcohol 70%, 5 ml formaldehyde and 5 ml of glacial acetic acid for 24 hours at room temperature and then washed the samples with alcohol at 70% concentration to remove the traces of the fixation and saved in alcohol 70%. Then small portions of both leaf and petiole were cut by a sharp scalpel with length of the piece between 1-2 mm and the severed parts were passed in an ascending chain of ethyl alcohol concentration (80%, 90%, 96%) for three hours in each concentration and then in absolute alcohol for two hours.

b. Clearing and infiltration: The sampling was successively passed in a mixture of ethyl alcohol and xylene in volumetric proportions (3:1, 1:1, 3:1) then the pure xylene at two-hour for each of them (Sass, 1968). Then the half of the xylene in which the samples were poured and instead added a quantity of liquid paraffin in the oven at 55-60°C for 48 hours to replacement of the paraffin by the fumigated xylene. After that, the paraffin was poured from the sample receptacles, and instead a pure liquid paraffin was added and the samples were left in the oven for two hours (this process was repeated 5-6 times) and in the last time, the oven was left in the overnight.

c. **Embedding and amount :** Paper molds of reinforced satin paper and various sizes, poured from them a quantity of molten wax and put in each of them a special model and left in a cool place to a whole day to ensure that it is sufficiently hardened and then to install the wax molds on the samples on a special wooden cut for bearer after they have melted all of their bass using a special blade until the shape of the heart becomes regular rectangles of wax mediated by the sample required to be ready to be picked by the rotary microtome. The samples were cut with a thickness of 2-12 µm and the appropriate thickness for almost all of the samples were 10-20 micrometer, and the sections were brushed in shaped with ribbons on clean glass slices that were already painted with a thin swab of glycerin-albumin and put slices on a hot plate 40-45 for 12 hours for the purpose of installing ribbons of sections and removing their wrinkles.

d. **Dewax and staining :** The glass slides passed which contain the plant sections during the solutions :

1. Xylene 2-4 hours at 50°C.
2. Xylene to absolute alcohol 1:1 for 5 minutes.
3. Descending series of ethyl alcohol (30%, 70%, 80%, 96%) 5 minutes each.
4. The safranin stain is at concentration 1/2% dissolved in alcohol with a concentration of 50% for 2-24 hours.
5. Ascending series of ethyl alcohol (30%, 50%, 70%, 80%, 96%) 5 minutes each.
6. Fast green, 1% concentration in an absolute ethyle alcohol.
7. Absolute alcohol for 5 minutes.
8. Xylene to Cedar oil with a size ratio of 1:1 for 5 minutes.
9. Xylene for twice time.
10. The slides were cleaned from the excess dye with a cloth, then permanent mounting by placing droplets of D.P.X adhesive material on the sections and then putting cover slide gently. The sections on the slides were transported to a hot plate with a temperature 40-45°C and for a full day to be dried, then studied and photographed under the Meijtechno microscope.

Results and Discussion

1. **Petioles :** All samples relating to the petioles of the studied species are listed in table 1 and plates (1, 2, 3), the study showed different in the form of the petioles, the shape of circle solid in the species *Cycas revolute*. As it appeared alatus in the middle and the two sides and a solid in the species *Cupressus semperviens*. The species *T. oreintalis* was semi oval, alatus in the middle and the two sides and this convergence in form between the last two species is due to the fact that they are of the same family, which gave importance to the study of the distinction among species. The study of the tissue layers from the outside to the inside of the studied species is composed of the following :

   1. **Epidermis :** It was made from one layer of rectangular cells interspersed with the stomatal complex type sunken, it is topped by a smooth thick cuticle but species *C. revoluta* was a dentate and this is in accordance with Laah *et al.* (2015). Following the epidermis tissue of sclerenchyma (fibres) and shown a clear difference between them, ranging number of

Table 1 : Qualitative and quantitative characteristics related to the micrometre.

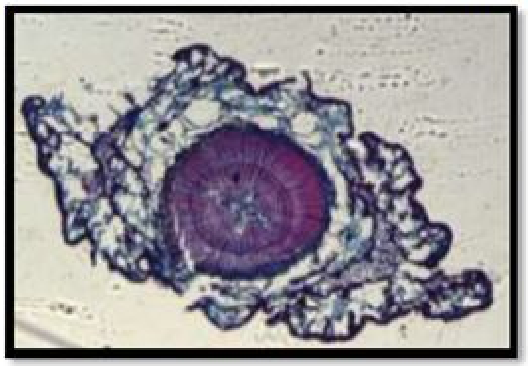
Characteristic / Plants	Shape of stem	Cuticle thickness	Epidermis thickness	Sclerenchyma tissue thickness	Shape of phloem	Number of sieve cell units in one row	Shape of xylem	Number of tracheid in one row	Number of Sclerenchyma tissue rows
1. *C. revoluta*	Solid circular	12-11(11.5)	72-78(75)	90-85(87.5)	Oval	(7-5)	Semi-spherical	(5-3)	(12-11)
2. *C. semperviens*	Oval and in the middle and upper and lower end of the ellipse featuring a semi-oval part	10-9(9.5)	67-65(66)	81-79(80)	Circular	(6-4)	Circular	(22-26)	(4-3)
3. *T. orientalis*	Semi-spherical parts of 3 ellipse in the middle and two large and two small	11.5-10.5(11)	62-59(60.5)	79-75(77)	Circular	(6-5)	Circular	(18-14)	(3-2)

Table 2 : The quantitative and qualitative characteristics of the leaves vertical sections of the studied species measured in micrometre.

Characteristic / Plants	Leaf vertical Section shape	Cuticle thickness	Upper Epidermis thickness	Lower Epidermis thickness	The number of layers of palisade	Shape of phloem	Shape of xylem	Number of sieve cell units in one row	Number of xylem intracheid units in one row
1. *C. revoluta*	The midrib region Semi-oval and blade area rectangular	11-10(10.5)	72-70(71)	78-74(76)	1	Mass of cells as an arch	Semi-oval	(6-3)	(8-7)
2. *C. semperviens*	Part of the middle big oval and at the upper and lower end of the ellipse includes every other semi-oval	10-8(9)	71-69(70)	76-72(74)	1	Semi-oval	Semi-oval	(8-7)	(8-4)
3. *T. orientalis*	Part of meddle semi-spherical consists of three parts, a large part and a small part	11-8.5(9.7)	68-66(67)	71-69(10)	2	Archs	Semi-oval	(7-5)	(5-3)

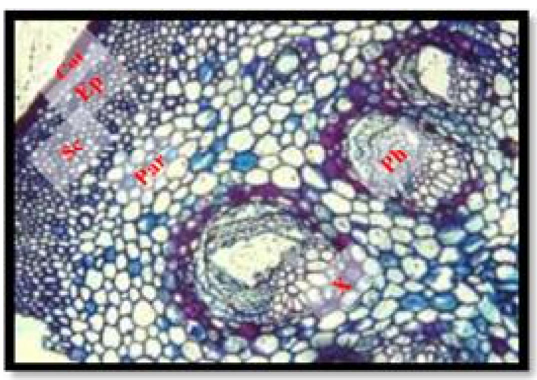
C. revoluta 4X

C. semperviens 4X

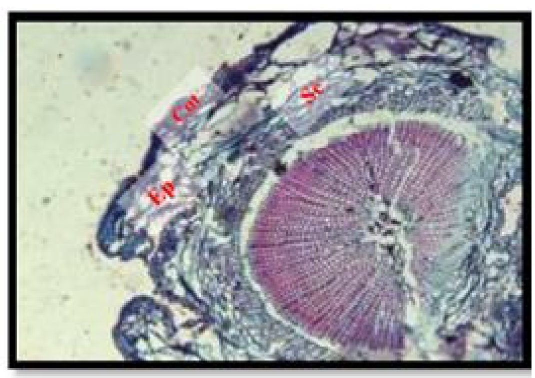


T. orientalis 4X

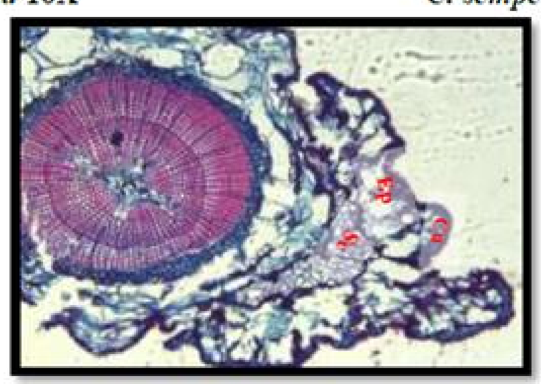
Plates 1 : The characteristics of the transferase section of petioles inspecies under study.



C. revoluta 10X

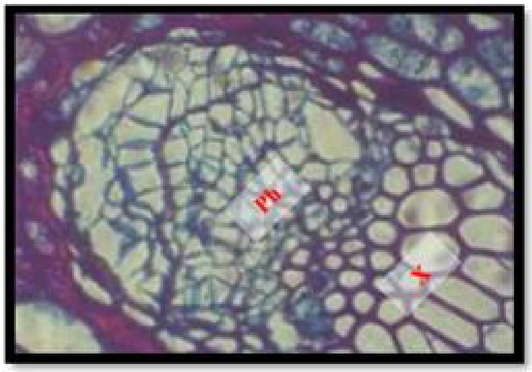


C. semperviens 10X

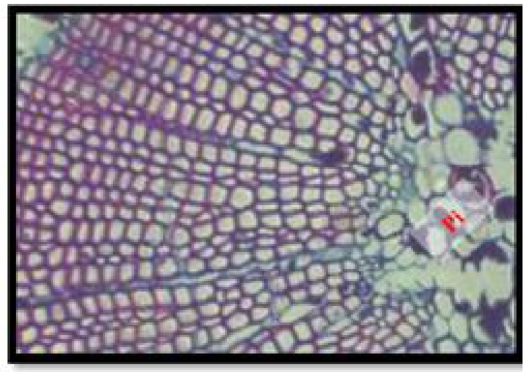


T. orientalis 10X

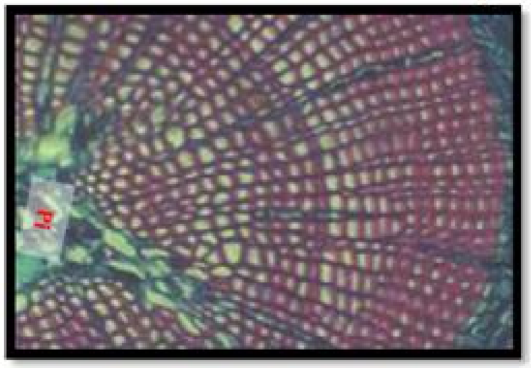
Plates 2 : The characteristics of the transferase section of petioles in species under study, Xylem: X, Sclerenchyma: Sc, Phloem: Ph, Cuticle: Cu, Par: Parenchyma, Ep: Epidermis.



C. revoluta **40X**

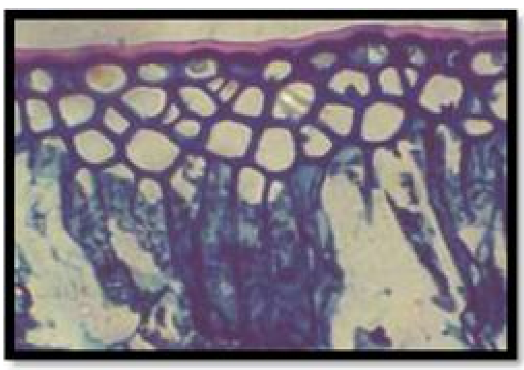


C. semperviens **40X**

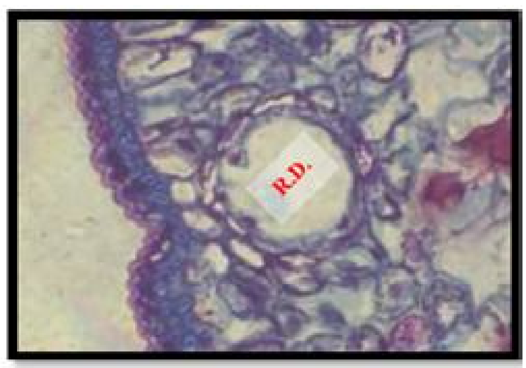


T. orientalis **40X**

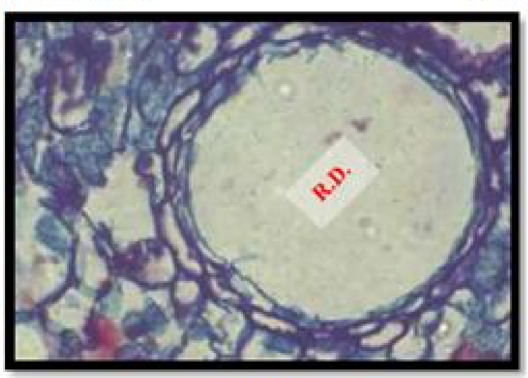
Plates 3 : The characteristics of the transferase section of petioles in species under study, Xylem: X, Phloem: Ph, Pi: Pith.



C. revoluta **40X**

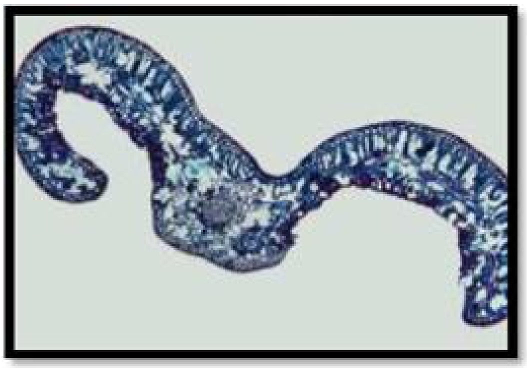


C. semperviens **40X**

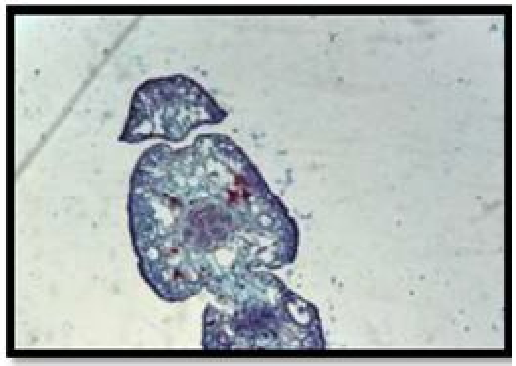


T. orientalis **40X**

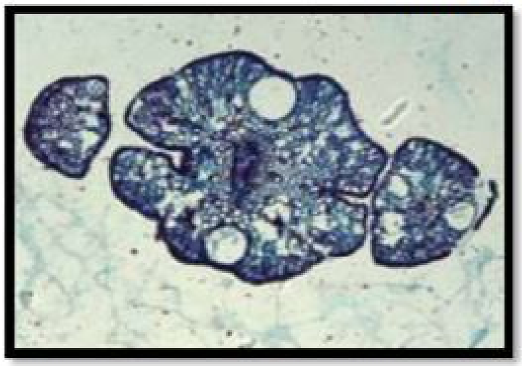
Plates 4 : The characteristics of the vertical section of leaves in species under study. Resin duct: R.D.



C. revoluta 4X

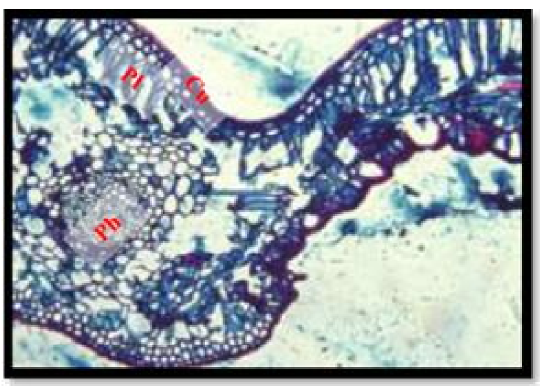


C. semperviens 4X

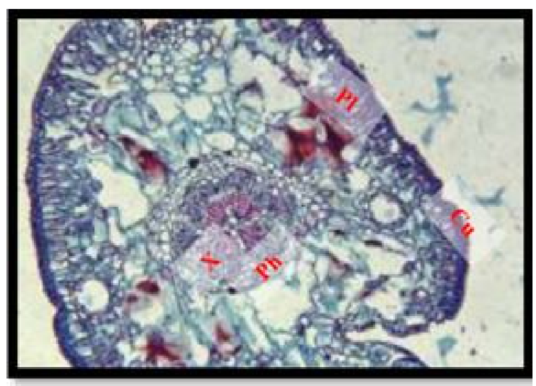


T. orientalis 4X

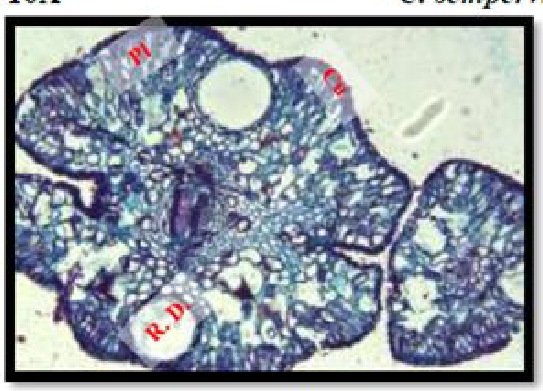
Plates 5 : The characteristics of the vertical section of leaves in species under study.



C. revoluta 10X

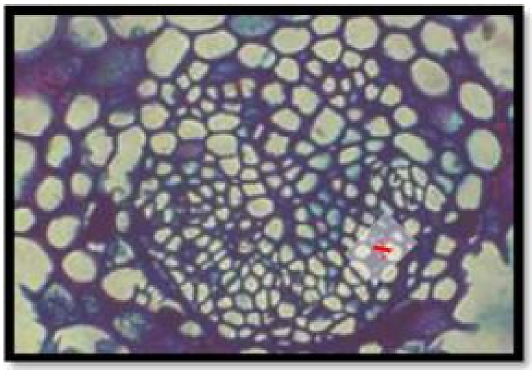


C. semperviens 10X

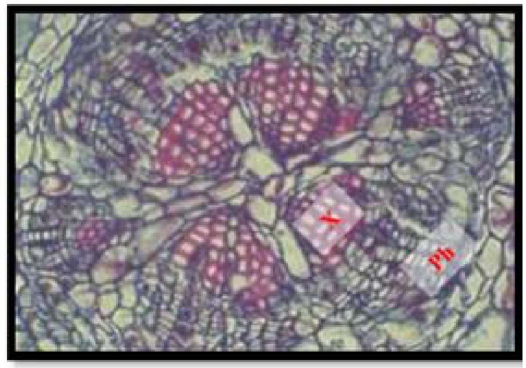


T. orientalis 10X

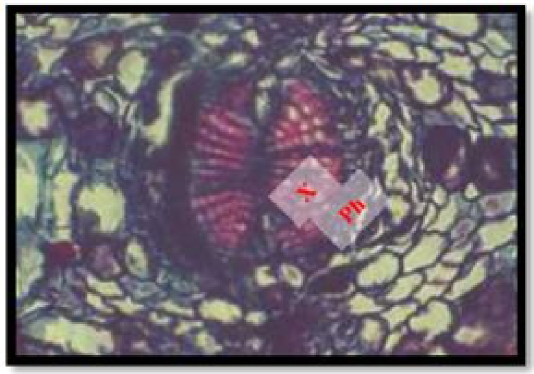
Plate 6 : The characteristics of the vertical section of leaves in species under study. Resin duct: R.D., Xylem: X, Phloem: Ph, Cuticle: Cu, Pl: Palsied.



***C. revoluta* 40X**



***C. semperviens* 40X**



***T. orientalis* 40X**

Plate 6 : The characteristics of the vertical section of leaves in species under study. Xylem: X, Phloem: Ph.

rows (11-12) in species *C. revoluta* and (2-4) *C. semperviens* and (2-3) row in *T. oreintalis*. The sclerenchyma tissue also showed a variation in the thickness of the species studied and as shown in table 1 and this is consistent with (Suleiman, 2003). The sclerenchyma tissue was following parenchyma tissue from polygonal cells differentiated in size, interspersed with the parenchyma tissue of the vascular bundles each vascular bundle surrounded by the sclerenchyma tissue of fibres are from (2-3) a row of the studied species but the vascular bundles differed in the xylem and phloem. Where the last tissue was oval, it consisted of a mass of sieve cells, with a row of 5-7 rows separated by the cells of the parenchyma, represented by the phloem rays, while the xylem tissue appeared to be a semi-spherical form of tracheid with a range of rows (3-5) separated by the parenchyma cells, represented by the xylem rays and the xylem tissue is finished by the pith as we have stated before that the petiole is solid (Kour, 2006). In the species *C. semperviens* the phloem tissue shows a circular form consisting of (4-6) rows of sieve cells interspersed with the phloem rays of the parenchyma cells, while the xylem tissue a circular form consisting of tracheid a number of rows (20-22) separated by the xylem rays. The tracheid and rays emerged axial form and this is consistent with Farjon (2005).

While species *T. oreintalis*, the phloem tissue appear in a circular form is consist of sieve cells with a number of rows (5-6) with interspersed by phloem rays of the parenchyma cells. The xylem tissue is also appear composed of the tracheid of (14-18) a row separated by the xylem rays, also appear as axial form and finished the xylem tissue by the pith. The pith in the last two species have been showed four arm of parenchyma cells.

2. **Leaves :** All data on the leaves are included in table 2 and plates 4, 5, 6, 7. The study showed a clear difference in the form of the leaves vertical section of the studied species, which appeared in species *C. revoluta* consisting of an oval midrib and on the side of the area of the leaf blade rectangular. While the *C. semperviens* appeared in the semi-oval in the middle and upper and lower end and it was scraped as the leaf separated into 3 parts. Species *T. oreintalis* the same shape for the previous species but the semi-spherical and when tracing tissues from the outside to the inside of the vertical section of the studied

species appeared as follows :

1. The upper and lower epidermis were from of rectangular cells interspersed with the sunken stomata, the upper layer is thick and smooth cuticle, except species *C. revoluta* was odonata and the studied species showed difference in the thickness of the epidermis and cuticle as in table 2 and only appeared in *C. revolute* under epidermis 3 small rows of sclerenchyma cells (fibres) followed by this tissue of the studied species, a layer of longitudinal palisade and showed a difference in the number of layers was one layer in *C. revoluta* and *C. semperviens* and the species *T. oreintalis* was of two layers of these cells, which corresponds to Ivansecu *et al.* (2007).

The palisade layer is followed by the spongy tissue of a disjointed, wide interstitial space parenchyma cells mediated by this tissue of the vascular bundle surrounded by 2-3 layer of fiber vascular bundle tissue (Hameed *et al.*, 2012). It was surrounded by 2-3 a layer of fiber sclerenchyma tissue for studied species. The vascular bundle for the *C. revolute* and *C. Semperviens* are separated by four arms of the parenchyma cells of the pith. In general, the vascular bundle of the phloem tissue which follows the fibers of the sclerenchyma and showed a difference in the form of a mass with arch in species *C.revoluta* and semi-oval in species *C. semperviens* and in a form arch in species *T. oreintalis*.

The tissue of the phloem is made up of sieves cells and parenchyma cells. Phloem is presented by phloem rays, followed by the phloem tissue of the xylem tissue, as shown in its shape, it was semi-oval in the *C. revoluta* and *C. semperviens* and an inverted triangle in *T. oreintalis* in general, the xylem tissue of the tracheid has shown a difference in the number of units in one row, reaching (7-8) unit in species *C. revoluta* and (4-8) in species *C. semperviens* and (5-8) unit in species *T. oreintalis*.

The tracheid permeates the parenchyma cells, which are mainly xylem rays, and this is consistent with Hameed *et al.* (2012). This heterogeneity in the vascular bundle gave prominence to the anatomical study where it gave important taxonomic qualities. It should be noted that the resin duct has emerged from the spongy tissue in the studied species and appears to be composed of secretary epithelial cells are surrounded by sheath cell and showed a variation in their number, in *C. revoluta* 3 and 6 in species *C. semperviens* and 8 in species *T. oreintalis* (Hamidipour and Zarrei, 2011).

The resin channel is one of the most well-situated present in Gymnosperm plants, and the heterogeneity in its number has helped to distinguish species from one hand and to know their numbers in the studied species of the Gymnosperm plants.

As well as the thickness of the cuticle layer and the thickness of the sclerenchyma tissue has given prominence to a study showing plant adaptation to environmental conditions and this is consistent with Raven and Losos (2008).

The anatomical study of this research has given prominence as it has helped to distinguish species on the one hand and has also indicated the convergence and similarity of the species of family to two species *C. semperviens* and *T. oreintalis* as the histological characteristics of this family as well as the knowledge of the histological layers for the three species studied from Gymnosperm plants are lack of studies on them in our country.

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