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ANATOMICAL COMPARISON OF TWO OAK SPECIES (*QUERCUS AEGILOPS* AND *QUERCUS INFECTORIA* OLIV.) GROWN NATURALLY IN DISTRICT ATRUSH, IRAQ

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SUMMARY

The presented study probed to compare two naturally grown oak species in the District Atrush, Dohuk Governorate, Northern Iraq. These two species were *Quercus aegilops* L. and *Quercus infectoria* Oliv., which belonged to the genus *Quercus* L. The research employed two methods to determine the comparative anatomical characteristics, i.e., chemically separating cells (Maceration) and mechanically separating cells using the Macrotome. The results showed significant differences in the quantitative and qualitative characteristics of the wood of the two studied species**.** It helps diagnose and separate the two species, which were similar in phenotypic attributes. *Quercus infectoria* acorns appeared with lengthier vascular elements than edible oak wood. However, the eating oak was distinguishable by the vessel elements, which had a larger diameter than the gall oak. The edible oak differed by the greater thickness of the vessel wall than that of the stump oak vessel. An edible oak also illustrated greater values of the vessel element cavity diameter, the inner diameter of the inserted holes, and the fiber length and diameter. However, the acorn gall demonstrates a fiber wall of superior thickness to the edible oak fiber wall. The Runkel ratio was 0.499 and 0.937 in the species *Quercus aegilops* and *Quercus infectoria*, respectively. Thus, according to Runkel's ratio, the edible acorn was better in paper pulp manufacturing and the paper compared with the acorns**.**The qualitative traits also contributed to the diagnosis and isolation of two studied species.

Keywords: Genus oak, *Quercus aegilops* L., *Quercus infectoria* Oliv., maceration, macrotome, phenotypic traits

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Key findings: The species *Quercus aegilops* and *Quercus infectoria's* scrutiny for quantitative and qualitative differences in their wood can help distinguish the phenotypically same species. The *Quercus infectoria* acorns have lengthier vascular components than edible oak wood. The edible oak had higher ratios of the vessel element cavity diameter, the inner diameter of the inserted holes, and the fibers' length and diameter than the gall and stump oak. Acorn galls had thicker fiber walls than edible oak galls.

INTRODUCTION

Oak trees have become one of the most prominent species of vegetation that grows naturally in the Northern regions of Iraq at elevations between 600 and 1900 meters above sea level (masl). Genus *Quercus* L. has three species, i.e., *Quercus aegilops*, *Quercus infectoria* Oliv., and *Quercus libany* Oliv. These species are characteristically slow-growing, light-loving, and not resistant to extreme temperatures, water sources, and other secondary purposes in beautifying tourist places (Abdullah, 1988; Abdullah *et al*., 1990). Oak wood has benefited, for an extended period, in furniture construction and manufacture due to the available natural forests, which were its chief source. With scientific and technical development taking place worldwide, researchers found new uses for wood and, presently, it also serves in the manufacture of paper, rayon, cellulose acetate filaments, plastics, ethyl alcohol, paint materials, acetic acid, and glucose in addition to the production of charcoal and fuel wood, which increased the economic value of timber (Kassir, 1990).

In addition to the steady increase in the demand for wood of all kinds, and despite the emergence of different and competing alternatives to wood, wood is still the base material many industries rely upon. Therefore, the interest is still valid in various countries in expanding the establishment and management of forests to contribute to increasing wood production. Its fruits as a food item also contain 30% protein in dry weight. Moreover, its wood also served as a source in various industries, such as, paper, compressed wood, wood chips, matches, fiberboards, crafts, and handicrafts (Dalal, 2022).

In light of the vast similarity in phenotypical characteristics of the two oak species (*Quercus aegilops* and *Quercus infectoria*) and due to the lack of anatomical information, it is challenging to diagnose them. Also, less research has progressed related to studying the comparative diagnostic anatomical properties of oak and gall oak, particularly probing the three faces of wood (cross, tangential, and radial sections). The anatomical features' diagnostic value is vital in the species analysis (Stace, 1984). Among them, the most important characteristics have a linkage to the wood structure in terms of the distribution of vascular elements, tracheids, fibers, pulp rays, annual rings, and border pits, and these often contribute to the diagnosis process and evolutionary trends (AL-Katib, 2000).

The oak tree has immense anatomical importance due to many factors necessary for humans and the environment. It is durable and has a sturdy internal design, which makes its wood desirable for use in various industries, such as, construction and furniture. Corrosion doesn't affect oak hardwood, and it can handle harsh weather conditions very well. Improving the quality of the soil is also very important because it makes the structure stronger and makes it better at absorbing water and nutrients. Its leaves and branches also give the earth organic matter that makes it better and healthier. It's also important because it's a home for many living things, like birds, animals, and insects. Because of this, we can say that the oak tree helps to increase the variety of life in the area. The oak tree also lowers carbon dioxide levels by taking in dangerous gases and giving off oxygen (Robert *et al*., 2017). A new study on anatomy correctly identified the type of hawthorn (*Crataegus azarolus* L.) growing in the District of Aqrah in northern Iraq (Dalal, 2022). This study contributed to identifying and diagnosing the wood of hawthorn trees and setting a diagnostic key for this type of tree. Zebari (2022) used the anatomical characteristics of wood to analyze the various species and varieties of nut Juglans L. growing in Northern Iraq. Al-Taee (2023) diagnosed species within the genus *Pistacia* L. growing in Northern Iraq and isolated them apart. Their study further strengthened the phenotypic study and separated similar species phenotypically.

Anatomical studies' significance is evident in shedding light on various structural features, and the importance of oak wood, one of the finest species of wood, can help meet the diameter requirement for distinctive wood. The pertinent study results may also be highly relevant to direct the future use of oak wood in industries of economic value, providing data on its anatomical characteristics and developing a diagnostic key that helps detect various species.

MATERIALS AND METHODS

Selection of the species and study sites

The selection process for two naturally growing oak species identified *Quercus aegilops* and *Quercus infectoria* in the Atrush region, Northern Iraq. For their study, two sites became the choices, i.e., for the *Quercus aegilops* at an altitude of 1218 masl, with a longitude of 43° 20' 31.494" and latitude of 36° 54' 33.101" and for *Quercus infectoria* at an altitude of 1210 masl, with a longitude of 43° 20' 30.677" and latitude 36° 54' 31.042" (Table 1***)***.

Study samples

Three healthy and straight trees (free from diseases and insect infestations) were chosen for each species and at each site, with six trees selected for the study. The methodology comprised samples to measure the diameter breast height (dbh) for each species, as mentioned by Saribas and Yaman (2005) and Schweingruber (2007). After collecting the samples, preparation of wooden sections for the study of anatomical features ensued, with the anatomical study conducted using two methods in the laboratory of Wood Sciences, Department of Forest Sciences, College of Agriculture and Forestry, University of Mosul, Iraq***.***

Chemical separation of wood cells (Maceration)

A process of chemically separating the wood cells transpired for the trunks of the two oak species trees studied for each site, following the methodology of Franklin (1945). The procedure included the following: 1) wood samples cut into small pieces (1–2 cm) in the shape of matchsticks, 2) placing in glass vials with a metal cover, adding equal volumes of icy acetic acid CH_3COOH and hydrogen peroxide (H_2O_2) continued at a concentration of 9% in a ratio of 1:1 (based on sample size) to melt the lignin of the middle plate, 3) then the samples' placement in an electric oven had a temperature of 75 °C until the color of the wood inside the bottles changed to white, 4) the wooden parts' washing with running water followed, then adding distilled water to it, shaking the bottle well progressed to ensure obtaining the highest number of separated cells, and 5) the cells' placement on glass slides used a clean steel rod without putting a cover to prevent any distortion of the dimensions of the separated cells (Adamopoulos and Voulgaridis, 2002).

Table 1. Study locations with altitude above sea level, latitude, and longitude.

Seq.	Tree type	Tree diameter (cm)	Above sea level (ASL)	Latitude N	Longitude E
1	*Q. aegilpos*	18.8	1218	36° 54' 33.101"	43° 20' 31.494"
2	*Q. infectoria*	16.0	1210	36° 54' 31.042"	43° 20' 30.677"

The wood cells separated mechanically (Macrotome)

The wood samples, cut into cubes, had dimensions of 1 cm × 1 cm × 2 cm before being subjected to a softening process by boiling them in distilled water until they sank under their own weight. For the purpose of speeding up the dipping process, adding cold water to it continued from time to time. After that, the samples' storage in a solution of glycerin and ethyl alcohol had a ratio of 1:1 before their use, then making microscopic slides ensued with a thickness of 20 µm for the three different sections, i.e., cross, longitudinal, and longitudinal radial sections, using the microtome (rotary microtome, Indian Rotary Microtome, with a steel knife angle [15°], Indian-made type HL.207 HL. Scientific industries) (Yaman, 2007).

The microscopic analysis had the following steps: 1) wooden chips with the microtome taken and placed on a microscope slide, then covered with a slide and examined under an advanced microscope of the type Motic Image Plus 2, equipped with a camera connected to a laptop that took 20 readings; and 2) studying the quantitative and qualitative characteristics of the three aspects of wood. The studied anatomical characteristics included 38 anatomical characteristics using an objective lens with a 10× magnification to measure the cell lengths, while a lens with a 40× magnification measured the diameters and wall thickness of the separated cells.

The Runkel ratio measurement used the following equation:

$$\text{Runkel ratio} = \frac{\textit{twice the wall thickness}}{\textit{bore diameter}};$$

RESULTS AND DISCUSSION

Wood cells separation of chemicals (Maceration)

Vessel elements length

The results showed a remarkable variation between the two wood species of oak studied for the length of vessel sphincters (Table 2). The acorns of the species *Quercus infectoria* revealed a greater rate than the average length of vessel elements of the species *Quercus aegilops*. The average length of the vessel elements of the oak wood bowls was 0.349 mm, while the average length of the vessel elements of the eating oak bowls was 0.249 mm. It is an anatomical diagnostic characteristic between the two studied species, and these results also showed that the length of the vessel elements differed between the species of the same genus (Figure 1). In past studies, the length of the vessel elements also became a diagnostic characteristic of plant species (Al-Jowary, 2017; Al-Sharifi, 2020; Zebari, 2022).

Table 2. Quantitative characteristics of wood cells separated by the chemical method for the two species of oak studied.

Seq.	Tree type	Vessel elements length (mm)	Vessel elements diameter (µm)	Vessel element wall thickness (µm)	Diameter of vessel elements (µm).	Internal diameter of the border (µm)	Fiber length (mm)	Fiber diameter (µm)	Fiber wall thickness	Runkel ratio
1	*Q. aegilops*	-0.140 0.362	-208.127 424.520	62.225 -23.431	-148.432 315.940	-13.892 20.125	-0.337 1.098	-47.043 91.280	-12.042 20.518	0.499
Average		0.249	298.269	40.465	206.015	17.498	0.739	71.686	17.340	
2	*Q. infectoria*	0.239 - 0.417	77.621 - 183.469	9.481 - 26.571	60.440-136.308	12.806 - 19.866	0.548 - 0.925	34.278 - 81.049	13.416-24.042	0.937
Average		0.349	115.451	16.301	88.610	16.989	0.698	61.692	18.454	

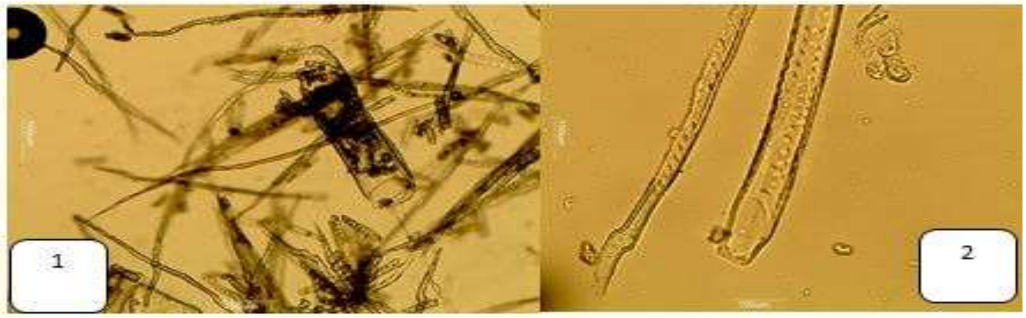


Figure 1. 1- *Q. aegilops* bowls separated by the chemical method; 2- *Q. infectoria* bladders separated by the chemical method.

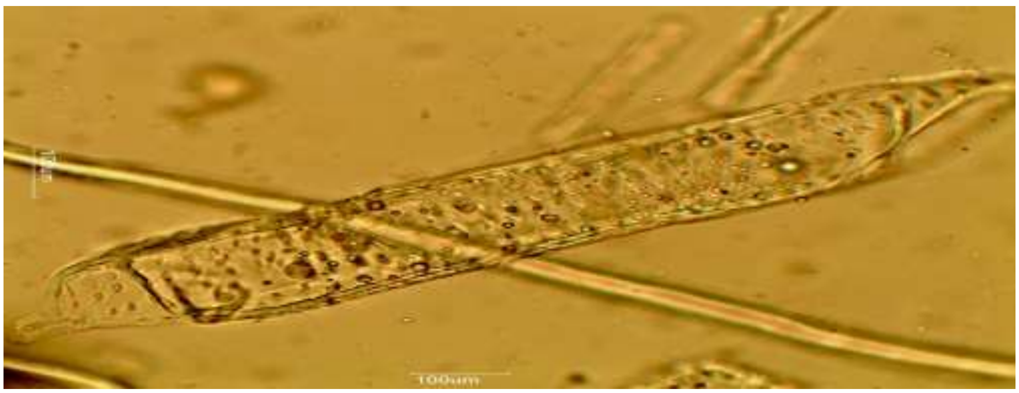


Figure 2. The diameter of the bowl element in acorn *Quercus infectoria*

Vessel elements diameter

The results revealed that there was considerable variation in the diameter of the vessel elements between the two oak-studied species (Table 2). The species *Quercus aegilops* was distinguished by the highest average diameter of vessel elements (298.269 μm) compared with the species *Quercus infectoria* (115.451 μm). Aloni *et al.* (1997) reported that trees control the diameter of the potted element to regulate physiological and waterborne stress and preserve the species. The diameter of the pot element has a close link to the auxin found in the tree, and a low concentration of auxin leads to the slow specialization of the vessel elements, and the abundance of water leads to an increase in pressure on the vessel element wall, widening the diameter before the deposition of the secondary wall (Aloni, 2000). The species *Q. aegilops* also has defused seporous stomata. Larson's (1973) findings indicated that the diameter of the vessel element may develop with the development of the crown of the tree with age due to increasing production of the IAA hormone by enlarging the size of the crown and then enhancing the cell diameter. In view of the naturally growing oak species *Quercus aegilops* in Northern Iraq, having the highest diameter of the vessel elements compared with the vessel diameter of *Quercus infectoria*, it became one of the most desirable species of trees for pulp and paper industry use. Edible acorns also showed superior use in the paper and paste industries. Carlquist (1988) stated that the diameter of the vessel element has a direct effect on the amount and volume of water running into the tree (as the diameter increases with increasing humidity and decreases with rising dryness) (Figure 2).

Vessel elements wall thickness

The wall thickness of the vessel element highly varied between the two studied species (Table 2). The species *Quercus aegilops* (40.465 µm) had a much higher ratio than the pot element wall thickness of *Quercus infectoria* (16.300 µm). Therefore, it became an essential diagnostic characteristic for classifying the two oak species, specifically the wood of both types (Figure 2***)***. The cause of the variation in the wall thickness of vessel elements may be due to a genetic variation among species and the inverse relationship between wall thickness and vessel element diameter (Zebari, 2022).

Diameter of vessel element Alveolus

Both species also showed a broad variation in the diameter of the cavity of the vessel element (Table 2). The eating oak (*Quercus aegilops*) had a much higher rate than that of the species *Quercus infectoria* (206.015 and 88.610 µm, respectively). This characteristic also appeared as an influential diagnostic characteristic to differentiate the vessel component of the two studied oak species (Figure 2). Explaining this variation may refer to the disparity in growing conditions and the difference in heights above sea level. Waleock *et al.* (2000) found that the diameter of the vessel element depends mainly on the abundance of water and decreases with its scarcity. Aloni (2000) also showed that trees that adapt to the environment in which they grow control the vessel elements' diameter. It regulated the physiological and water efforts, thus preserving the plant type. The vessel element diameter also indicated an association with the amount of auxin in the tree. A low auxin concentration leads to slow growth and specialization of vessel elements, causing a vast expansion of the cell diameter. The abundance of water also increases pressure on the inner wall of the vessel element, expanding the diameter before the process of secondary wall deposition (Aloni, 2001), in addition to the genetic differences between species (Al-Jowary,2017).

Internal diameter of the bordered pits

The results revealed a little difference between the two oak species for the average internal diameter of bordered pits (17.498 and 16.989 µm, respectively) (Table 2 and Figure 3). This result was consistent with the findings of Dalal (2022) in her study of hawthorn trees, which showed that the largest cavity diameter was in the first location with a lower sea level. The second, with a higher location, gave a lower average cavity diameter.

Fiber length

The dimensions of the fibers varied for the two concerned species. On fiber lengths, the oak species *Quercus aegilops* and *Quercus infectoria* provided average values of 0.689 and 0.698, respectively (Figure 4). Thus, it proves that there is an effect of locations, interfaces, and environmental influences on the fiber length. The higher the height, the shorter the fiber length, and vice versa. These results aligned with those of Nazari *et al.* (2021), which showed the effect of heights on fiber length, indicating that the lowest height had longer fibers than the rest heights. It also agreed with the results of Dalal (2022), who studied the effect of sites on the anatomical characteristics of hawthorn growing in northern Iraq and found that the site located at an altitude of 895 masl had longer fibers than the fibers of trees maturing in the site with the highest elevation.

Fiber diameter

A significant difference also occurred between the edible oak and the oak gall in the fiber diameter (Table 2***.*** The species *Quercus aegilops* possessed fibers with larger diameters (71.686 µm) than *Quercus infectoria* fibers (61.692 µm) (Figure 5). These results were consistent with the findings of Nazari *et al.* (2021), who showed that lower altitudes were indicative of broader fiber diameters than higher altitudes with smaller fiber diameters. It also supported the results of Dalal (2022),

Figure 3. 1- The inner diameter of the grooves in the *Quercus eagilops*; 2- The inner diameter of the holes placed in the *Quercus infectoria.*

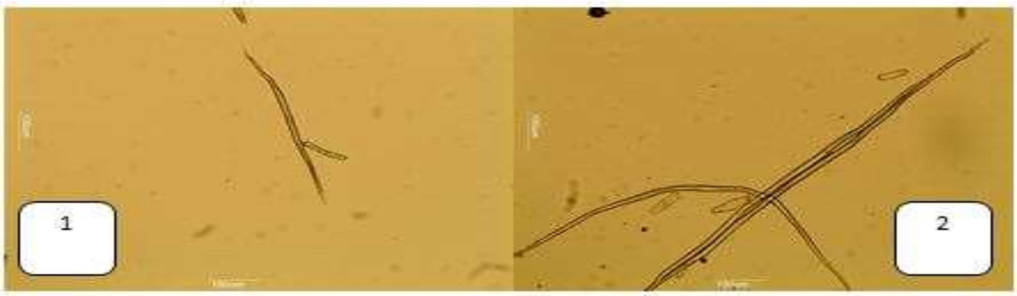


Figure 4. 1- The length of the fiber in the *Quercus aegilops;* 2- The length of the fiber in the Quercus *infectoria.*

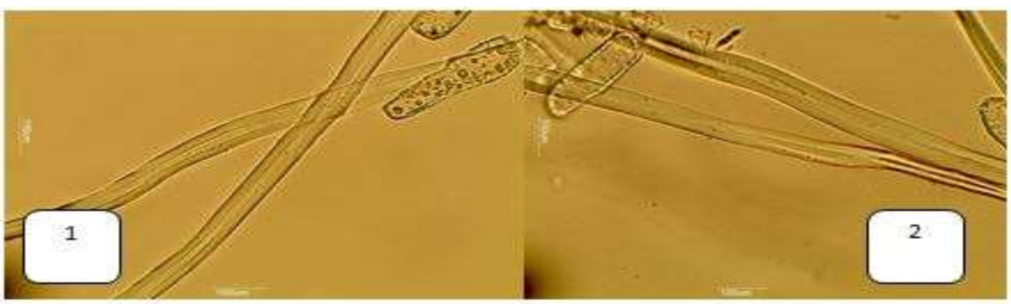


Figure 5. 1- The diameter of the fiber in the *Quercus aegilops*; 2- The diameter of the fiber in the *Quercus infectoria.*

which found an effect of locations on the diameter of hawthorn fibers growing in northern Iraq. Higher altitudes had smaller fiber diameters than the fiber diameters of trees growing at lower altitudes.

Fiber wall thickness

In light of the results, a slight difference emerged in the fiber wall thickness between the two studied oak species (Table 2). The average fiber wall thickness of the oak species *Quercus aegilops* and *Quercus infectoria* was 17.340 and 18.454 µm, respectively (Figure 5). The thickness of the fiber walls and the increase in their formation depend primarily on the accumulation of metabolic products (cellulose, hemicellulose, lignin, and waxes), which increase with growing cell maturity. Raising the diameter of the fiber cavity leads to a decrease in wall thickness (Ververis, 2004; Martínez *et al.,* 2009). It is because fewer substances to deposit on the fiber wall can cause its thickness to decrease. Many studies and research proved a significant impact of cell wall thickness on the wood's mechanical and physical properties and the quality and quantity of pulp, paper, and other industries that rely primarily on chemically converting wood to benefit from it (Swelam and Al-Maarouf ,1981) . This result was consistent with the mechanism of cell wall formation: as the cell diameter increases, the wall thickness decreases, and vice versa.

Runkel ratio

For the Runkel ratio and its ranges, the results showed that the rate of the Runkel ratios for both oak species, *Quercus aegilops,* and *Quercus infectoria,* were the same at 0.937. For use in other wood industries, the research has proven that the acceptable range for the Runkel ratio when using it in the paper and pulp industry ranges between 0.25 and 1.5. Outcomes further revealed the validity of using the species *Quercus aegilops* in pulp and paper manufacturing was better than using the species *Quercus infectoria* due to a low ratio of Runkel compared with *Quercus aegilops* (Shashikala and Rao, 2009).

Qualitative traits

The presence of crystal regions

The presence of crystal regions is a distinguishing characteristic of these two species growing naturally in Iraq (Figure 6). However, it is not permanent in most plant genera, as it may differ in its presence, size, relative abundance, and the type of cells it contains, and it is also evident in some species of Rosaceae families (Zhang and Pieter, 1992).

Number of border pits

The results showed a discrepancy in the number of pecking rows in the wood fibers of the species *Quercus aegilops* and *Quercus infectoria* (Table 3). Outcomes also greatly aided the species diagnosis, and in *Quercus aegilops,* it was a biseriate type, while in *Quercus infectoria*, it was in a row form (Figure 7). The reason is due to the difference in genetic characteristics between the two species studied and the variance in growth according to altitudes above sea level (Al-Taee, 2023).

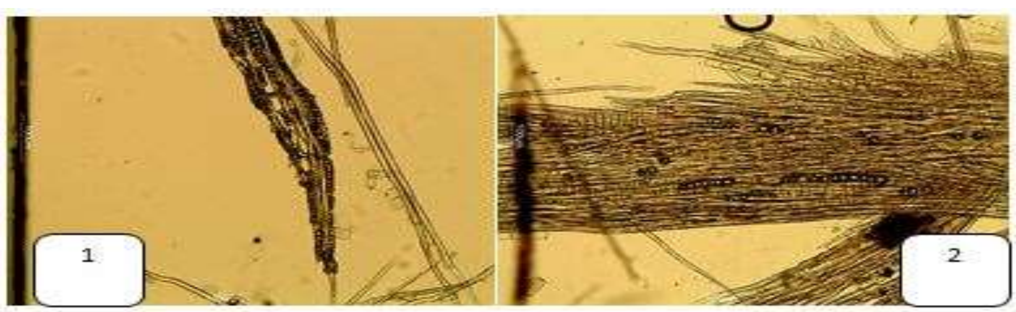


Figure 6. 1- The crystalline regions in the *Quercus aegilops;* 2- crystalline regions in *Quercus infectoria.*

Table 3. Qualitative characteristics of wood cells separated by the chemical method of the two studied oak species.

Spesies	Crystal region	Number of bordered pit rows in the fiber	Type of pitting in the vessels	Helickal thickening	Dentate wall	Type of perforated plate
Quercus aegilops	+	Biseriate	Alternate	+	+	Simple
Quercus infectoria	+	Uniseriate	Alternate	+	+	Pitting

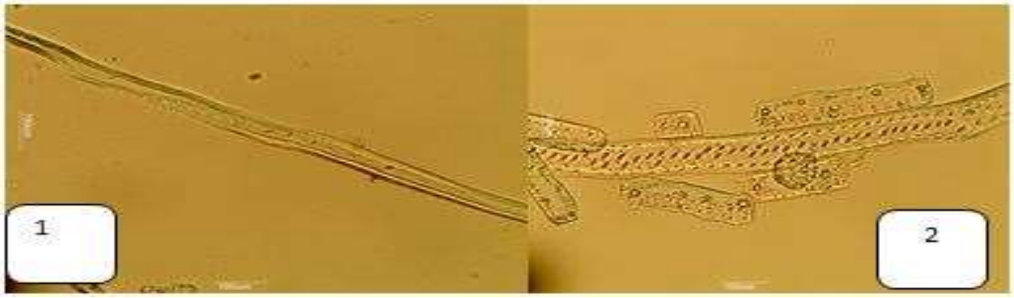


Figure 7. 1- The number of mortise rows in *Quercus aegilops* fibers; 2- The number of pecking rows in the *Quercus infectoria* fibers.

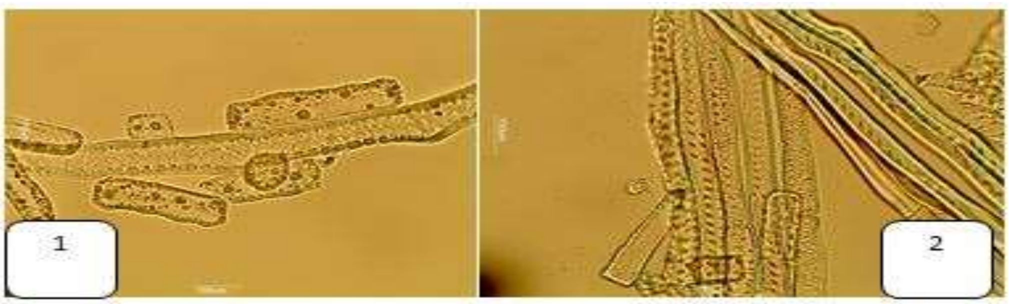


Figure 8. 1- Spiral indentations in the *Quercus aegilops*; 2- Spiral thickenings in *Quercus infectoria.*

The presence of helical thickening

The findings showed the presence of helical thickenings in the fibers of the two species of wood studied; however, they differed in the type of spiralization (Table 3). The types were of two directions in the species *Quercus aegilops* wood, but in *Quercus infectoria*, it gave a one-sided direction (Figure 8). Research indicated that the presence of spiral thickenings increases fiber strength and, thus, increases support and reinforcement (Vazquez-Cooz and Meyer, 2006).

Dentate walls

The results signified the presence of serrated walls for both species of oak scrutinized, and these jagged walls give support and strength to the wood (Table 3). Hence, the strength of oak wood and its preference in the wood industry is due to the strength of its wood. Figure 9 shows the dentate in the studied oak wood cell walls. Sectioned fibers are an imperative diagnostic characteristic for distinguishing species, as Dalal (2022) also pointed out.

Type of perforated plate

This characteristic was of high diagnostic value in distinguishing the two oak species. *Quercus aegilops* had a simple perforated plate, while *Quercus infectoria* had a pitting plate, substantially isolating and identifying the edible oak from the gall oak (Figure 10). Dalal (2022) stressed the importance of this characteristic in species diagnosis. Zebari (2022) confirmed the diagnostic worth of this feature in diagnosing walnut species grown in Iraq.

The wood cells separated mechanically (Macrotome)

Both oak species showed a variation in the quantitative characteristics of the cells separated by the mechanical method (Table 4), and the same also occurred in the following

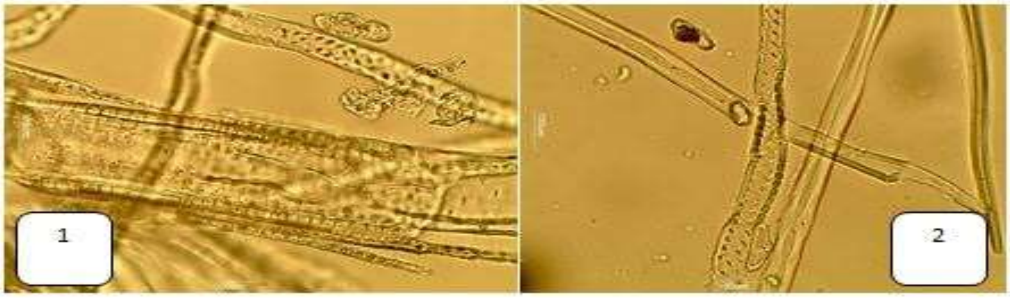


Figure 9. 1- The toothed walls of the *Quercus aegilops;* 2- The dentate walls made in *Quercus infectoria.*

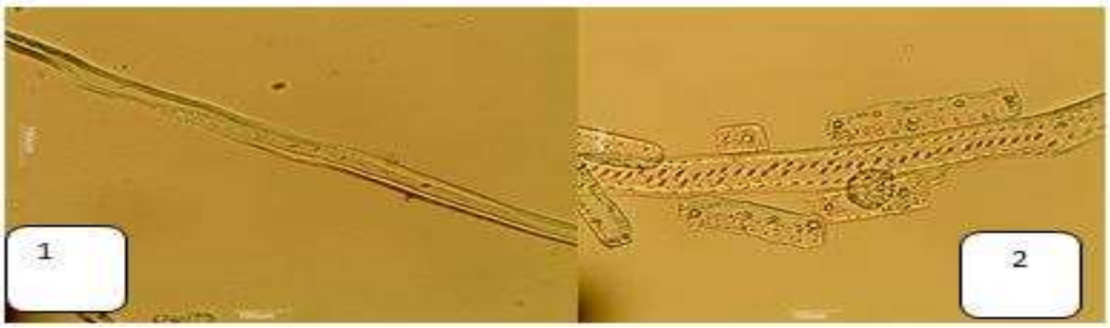
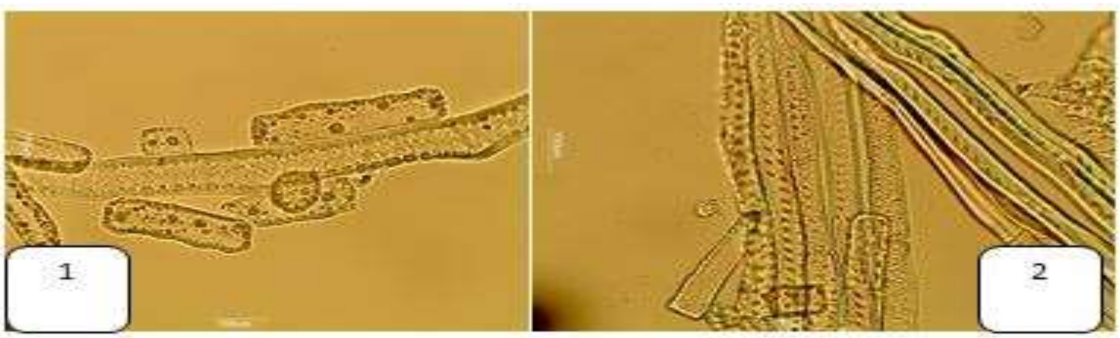


Figure 7. 1- The number of mortise rows in *Quercus aegilops* fibers; 2- The number of pecking rows in the *Quercus infectoria* fibers.



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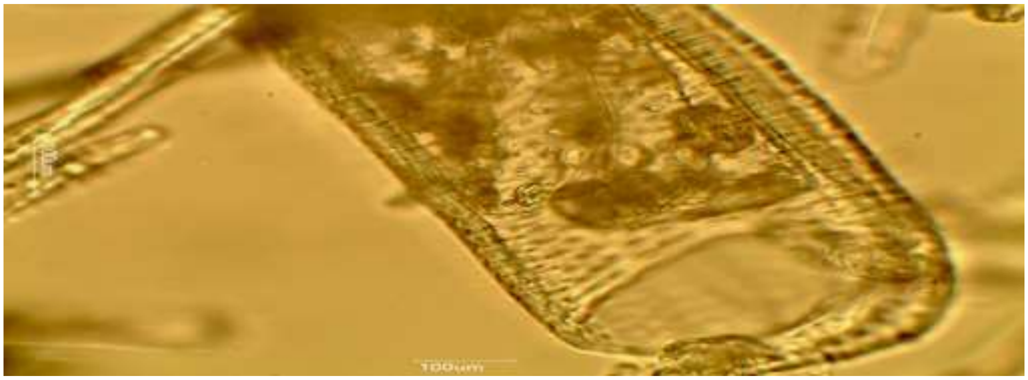

Figure 10. The perforated plate in the *Quercus aegilops.*

Table 4. Quantitative characteristics of the cells separated by the mechanical method for the two species of oak wood studied.

Seq.	Species	Monolayer Ryes per tangential section in height (mm**)**	Height of the rays cells in the tangential face (µm**)**	Height of fusiform rays in tangential section (µm)	Height of the transverse rays cells in the radial face (µm)	Number of stomata per mm^2
1	*Q. aegilops*	85-57	180.645-113.432	168.431 -72.625	290.845-47.043	17-10
Average		71.600	151.065	96.012	140.020	14.714
2	*Q. infectoria*	80-40	266.503-134.530	111.534-69.643	195.023-63.323	62-17
Average		60.400	194.831	88.700	125.566	39.000

paragraphs***.*** Separated fibers are an influential diagnostic characteristic for distinguishing species, as Al-Taee (2023) validated.

Monolayer rays per tangential section

The number of monolayer rays varied in the two species of oak probed (Table 4). *Quercus aegilops* was notable for wider single-layer rays than *Quercus infectoria*, as the rates were 71.600–60.400 mm***.*** The discrepancy is due to the genetic characteristic differences between the two species.

Height of ray cells in the tangential face

This characteristic contributed to differentiating the wood of the two oak species (Table 4). The gall oak (194.831 µm) was distinct by having a higher cell elevation than *Quercus aegilops* (151.065 µm)***.*** Such discrepancy is due to the difference in genetic characteristics between the two species (Alaaddin Ahmed *et al.,* 2022).

Height of fusiform rays in the tangential section

The fusiform rays exhibited varying heights between the two oak species (Table 4). The species *Quercus aegilops* had a higher height (96.012 µm), while the fusiform rays of the *Quercus infectoria* species reached 88.700 µm. Specific traits, as diagnostic characteristics, supported isolating and diagnosing the two oak trees***.*** Said disparity between the two species may be due to the nature of the genetic makeup of each and the extent to which each variety incurred effects from environmental conditions (Saltan *et al.,* 2019).

Height of the transverse ray cells in the radial face

A discrepancy in the height of the cells of the cross-sectional rays appeared between the two oak species studied (Table 4). The species *Quercus aegilops* had a higher height at

140.020 µm, and it was 125.566 µm in *Quercus infectoria*, differentiating the two species from each other***.*** An explanation could be due to the nature of their genetic makeup (Al-Rousan, 2013).

Number of stomata per mm²

Table 4 shows a broad distinction between the oak species in their number of stomata per mm^2. The oak species *Quercus infectoria* manifested with the widest stomata per mm^2 (39.000), whereas the stomata was 14.714 per mm^2 in the species *Quercus aegilops*.

CONCLUSIONS

According to the results of the anatomical study and based on two chief methods of wood cell separation (chemical and mechanical), a wider variation in the chemically identified anatomical features and mechanically separated characteristics for the three sections (transverse, radial, and tangential) emerged. The wood of the oak species *Quercus aegilops* was better for making paper and pulp than the oak species *Quercus infectoria* due to the Runkel ratio. Their broader usage in various industries encourages the cultivation of both species and prevents illegal cutting.

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