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Comparative Histological Study on the Algerian Thymus Ciliatus at Different Phenological Stages

COMPARATIVE HISTOLOGICAL STUDY ON THE ALGERIAN *THYMUS CILIATUS* AT DIFFERENT PHENOLOGICAL STAGES

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ABSTRACT

The aim of the current study was to compare the histological observations of stems, leaves, and different secretory structures of *Thymus ciliatus* at three phenological stages. The anatomical observations of stems, leaves, and glandular trichomes were carried out by light microscopy. The results showed that phenological stages do not affect significantly the anatomical, whereas there was a significant increase in secretory trichomes. The glandular trichomes are distributed on the surface of the stems and the leaves. There are only two distinct types of secretory trichomes, Peltate consisting of a basal cell, a stalk cell, and an 8-celled head type, which are distributed more diffusely on the leaf than on the stem, while *capitate trichomes* were present only on the surface stem samples. Accumulation of essential oils varies according to the seasons with high sizes of trichomes secretory at the flowering period.

Keywords: Anatomy, *Thymus ciliatus*, phenological stages, trichomes.

INTRODUCTION

Algeria is known for its richness of medicinal plants considering surface area and bioclimatic diversity. The genus Thymus belongs to the Lamiaceae family includes eleven species distributed on the entire littoral and even in the internal regions until the arid zones of Algeria (Quezel and Santa, 1963). *Thymus ciliatus* locally called "Zaâteur in Arabic" is one of the thymus plants with great virtues. It is well known for its aromatic and medicinal properties and is often used in Algerian folk medicine for their antitussive, antiseptic, expectorant, antihelmintic and antidiabetic, antihypertensive (Boudjelal et al., 2013).

The leaves of thyme contain essential oils with high therapeutic value. The biological actions of essential oils have been previously determined (Hazzit et al., 2009; Sadou et al., 2018). In plants, essential oils are among the secondary metabolites that provide various functional roles. They are elements implied in chemical communication, in the defense against external aggressions, and they assure the role of attractant agents by appealing to insects for pollination. In addition, they have a role in stabilizing and protecting plant membranes against high temperatures (Hazzoumi et al., 2017). The amount of essential oils is significantly influenced by the climatic and geographical conditions, the stage of growth, and the harvested period which can affect their composition and their biological properties (Goyal et al., 2020 ; Ložienė et al., 2021).

The storage of essential oils in plants is usually limited to specialized secretory structures such as glandular trichomes, which are two types of glandular hairs, peltate and capitate, that present differences in the histological

structure and the secretion process (Guesmi et al., 2019), essential oil is released when the cuticle ruptures due to mechanical action or hygrometric variations. Recent works have reported that the micromorphology of trichomes is useful in taxonomy for many groups of plants, especially Lamiaceae (Jia et al., 2013). The determination does not lend itself easily because of its variability and higher frequency of hybridization (Horwath et al., 2008). The anatomical structure of *T. Ciliatus* had not yet been studied. The purpose of the present work was to compare the histo-anatomical properties of the stems, leaves, and trichomes between different phenological stages by means of light microscopy.

MATERIALS AND METHODS

Plant Material

Freshly harvested leaves and stem materials of *Thymus ciliatus* subsp. *coloratus* were collected during, before, and after-flowering phases, from the Peninsula of Edough-Annaba in Algeria. The botanical identification of the species was made according to the description reported in Algerian flora (Quezel and Santa, 1963).

Anatomical Observations

The fresh stems and leaves batch were fixed in 70 % alcohol, for light microscope observations ; Histological investigations were carried out on the cross-sections of stems and leaves obtained manually using a botanical razor and colored with double stained, methyl green and Congo red (Zamfirache, 2021). The best sections were chosen and fixed in glycerol and photographed using a light microscope type Leica DM LS2, with a Canon digital camera at magnifications of 10x, and 40x.

Statistical Analysis

All measurements are based on ten sections and represented as mean standard deviation (SD). Statistical analysis of the difference between different stages of growth of sample means was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple tests. P-values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Stem Anatomy

The stem is highly branched and woody in its lower part. The transversal sections of the stem revealed two regions: the cortex and the central cylinder. The rod is made up of a layer of epidermal tissue, with a single layer of rectangular joint cells, with cellulosic walls, colored purple-red. Which are covered with cuticula. On the surface, both secretory and tector hairs appeared. In the cortex, there are parenchyma cells called cortex parenchyma, colored in purple-red. At the corner of the stem was distinguished a collenchyma bundle at the four angles, given the rectangular shape of the stem. It was a support tissue type collenchymatous made up of rounded cells with cellulosic walls. From the endodermis stratum begins the cortex and corresponds to the innermost part of the cortex (bark) in young stems consisting of a single cell layer. The older the plant is, the more the endodermis is lignified.

The central cylinder was formed by vascular bundle and pith. The phloem is colored in purple-red, and the xylem is colored in green. There was a slight disproportion between the phloem and xylem, which were arranged in a single circle located just above the pith with small angular and circular cells, characterized by centrifugal differentiation. The existence of secondary tissues in the form of a ring the cambium

was found, between the xylem and phloem, consisting of xylem II and a little phloem II. Whereas, the cork was not distinguished, and a slight overall lignification are all arguments to conclude that this stem is herbaceous. Finally, the pith was presented at the middle of the stem, which was composed of oval-shaped parenchymatic cells, but the pith region was filled with xylem elements because the phloem and xylem were well-developed.

Trichomes appeared on the epidermis and were glandular and eglandular. Trichomes are characterized by 1-5-celled and multicellular hairs that are more common and covered with a thin cuticle. Secretory trichomes (Figure1) revealed 2 types of structures : the first one was represented by a short unicellular stalk and an ovoid unicellular secretory head, capitate type (Figure1), and the second one was represented by a rare distribution of peltate trichomes (Figure2).

The results of anatomical measurements of stem structures of *T. ciliatus* at the different stages are grouped in Table 1. There is a difference in tissues thickness from one stage to another. The dimension of tissues provides information on the physiological state of the plant and on the condition in which the plant evolves (water stress, anthropogenic action, pollution, etc.).

Table 1: Anatomical measurements of *T. ciliatus* ssp *coloratus* stems *(µm)*

Tissues	Before flowering	Flowering	After flowering
Epidermal	10 ± 00^{a}	11 ± 3,16^{a}	11 ± 3,16^{a}
Tector hairs	58 ± 10.25^{a}	42.7 ± 11.47^{b}	53 ± 12.51^{ab}
Cortical Parenchyma	148 ± 34.25^{a}	152.5 ± 30.57^{a}	156 ± 25.90^{a}
Endodermis	19 ± 5.67^{a}	12.5 ± 2.63^{b}	16 ± 5.16^{ab}
Phloem	29 ± 7.37^{a}	31 ± 6.14^{a}	29 ± 8.75^{a}
Xylem	116.5 ± 14.91^{a}	131.1 ± 39.37^{a}	133 ± 24.96^{a}
Pith	145.2 ± 36.38^{a}	170.5 ± 29.19^{a}	168 ± 32.59^{a}

Each value is expressed as mean ± standard error (SD). *Different letters within a line showing the significant difference using Tukey test ($p < 0.05$).*

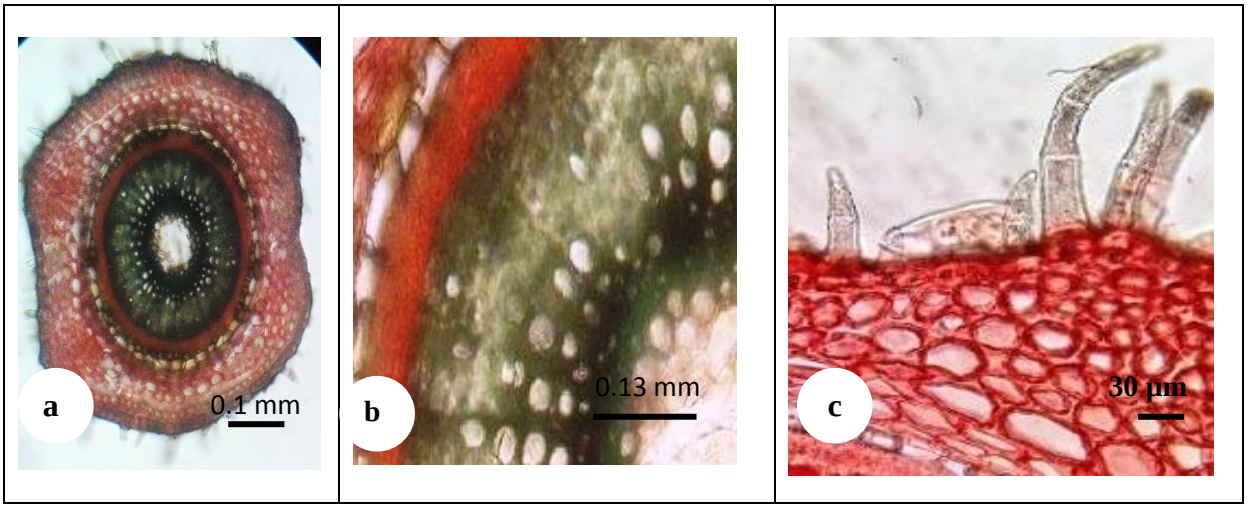

Figure 1: Cross section of the stem of *T. ciliatus* ssp *coloratus* colored with methyl green and Congo red – general view 10x (a, vegetative stage) and structural details view 40x (b, flowering - c, after flowering).

Leaf Anatomy

According to table 01, the values significantly ($p < 0.05$) vary from one period to another. The covering hair with the highest value was observed during the flowering period (42.7 ± 11.47 µm), while the weaker was obtained during the vegetative period (58 ± 10.2 µm). In addition, the endodermis also presented a variation with the lowest value was recorded in the flowering period (12.5 ± 2.63 µm) and the highest value was obtained in the vegetative period (19 ± 5.67 µm). However, the epidermis medullary parenchyma, xylem, phloem, and cortical parenchyma showed no significant difference from one period to another ($p > 0.05$).

The leaves of *Thymus ciliatus* are small, slightly expanded in opposite pairs, without stipules, these leaves are oblong and smooth, but typically ciliated at the base, slightly curled at the edges, and colored with a green hue. The observation of cross-sections of leaf *Thymus ciliatus* showed that the section was bifacial, dorco-ventral symmetry. The epidermis was composed of monolayer rectangular cells on both adaxial and abaxial surfaces. Epidermal cells were covered with a thin layer of cuticle. The latter has curvatures on the lower and upper sides, and within cavities are the glandular hairs (the piliferous crypt). The glandular trichome type peltate, was consisted of a basal cell, a stalk cell, and an 8-celled head. The essential oil is collected in a large subcuticular space, between the cell wall and the cuticle (pocket), released by the cuticle, following a mechanical action or a hygrometric variation tear and releases the essence which volatilizes (Serrato-Valenti et al., 1997). This organization consists of a long-term gland (do Socorro Serra Gama et al., 2013) (Figure 2).

The observation of the leaf surface displayed that glandular trichomes were spread on both leaf surfaces. Protector trichomes with the multicellular type were also observed under microscopy. Below the epidermis, the mesophyll was divided into a palisade and spongy parenchyma, with cellulosic walls. Between the two areas of the mesophyll, there were vascular bundles of the xylem and phloem of collateral type, arranged in a single line. The midvein vascular bundle was larger than the lateral vascular bundles. The latter is limited by an endodermis stratum, and it includes xylem and phloem bundles and a sheath of sclerenchyma colored in green due to their lignin thickenings. The stomata type were diacitic on the abaxial side. The stomata apparatus is surrounded by two cells. The two cells are more or less parallel to the stomata (Figure 3). They are of unequal sizes, and the largest tend to cover.

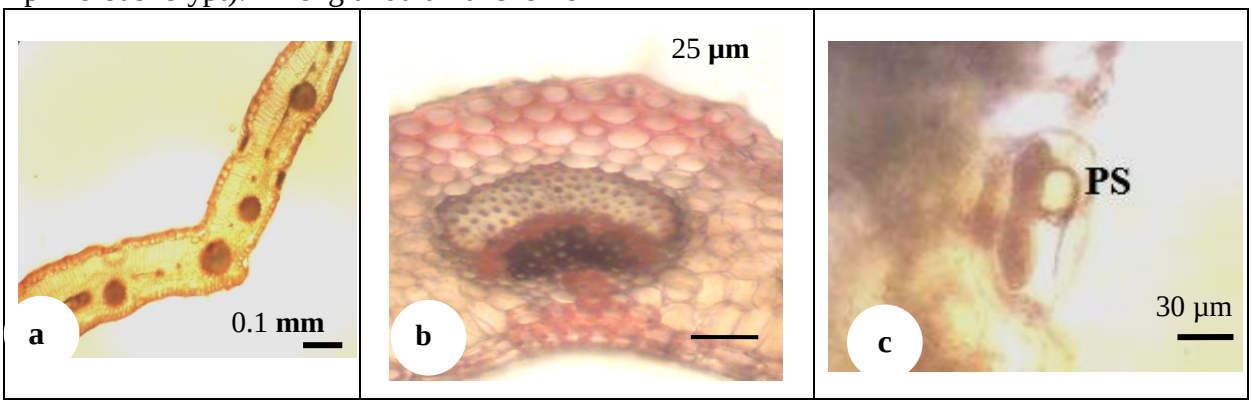

Figure 2: Cross section of the leaf of *T. ciliatus* ssp *coloratus* colored with methyl green and Congo red – general view 10x (a); structural detail of the central vascular bundle 40x (b); (c) Glandular trichome: PS.

Table 2: Anatomical measurements of *T. ciliatus* ssp *coloratus* leaves *(µm)*

Tissues	Before flowering	Flowering	After flowering
Peltate trichomes	26 ± 6.99^{b}	38.77 ± 8.18^{a}	37.77 ± 6.67^{a}
Tector hairs	12.5 ± 3.53^{a}	14 ± 6.99^{a}	15 ± 7.07^{a}
Epidermis	21 ± 3.94^{a}	24 ± 2.11^{a}	24 ± 3.94^{a}
Palisade parenchyma	72.5 ± 6.77^{a}	79 ± 7.38^{a}	71.5 ± 8.18^{a}
Spongy parenchyma	59.5 ± 4.37^{a}	55.5 ± 7.21^{a}	62 ± 9.14^{a}
Sclerenchyma	59.5 ± 5.50^{a}	65 ± 4.71^{a}	58.95 ± 6.32^{a}
Phloem	24.5 ± 3.68^{a}	25 ± 4.08^{a}	26 ± 4.59^{a}
Xylem	24 ± 3.16^{a}	26.5 ± 4.74^{a}	26.5 ± 3.37^{a}

Each value is expressed as mean ± standard error (SD). *Different letters within a line showing the significant difference using Tukey test ($p < 0.05$).*

The measurements of different histological structures according to Table 2, revealed at the flowering stage, the tissues reach their maximum sizes at the leaf level.

The analysis of variance revealed that the recorded values do not vary significantly from one period to another ($p > 0.05$), the variation in the epidermis is from 15 ± 7.07 to 21 ± 3.53 µm, in the palisade parenchyma is from 71.5 ± 8.18 to 79 ± 7.38 µm, spongy parenchyma is between 55.5 ± 7.21 and 62 ± 9.14 µm, pericyclic fiber 58.95 ± 6.32 to 59.5 ± 5.50 µm, the phloem from 24.5 ± 3.68 to 26 ± 4.5 µm and the same for the xylem from 24 ± 3.16 to 26.5 ± 4.74 µm. However, the sizes of the glandular hairs vary significantly ($p < 0.05$) (from 26 ± 6.99 to 37.77 ± 6.67 µm). This variation is related to the secretion of essential oils which increases during the flowering period.

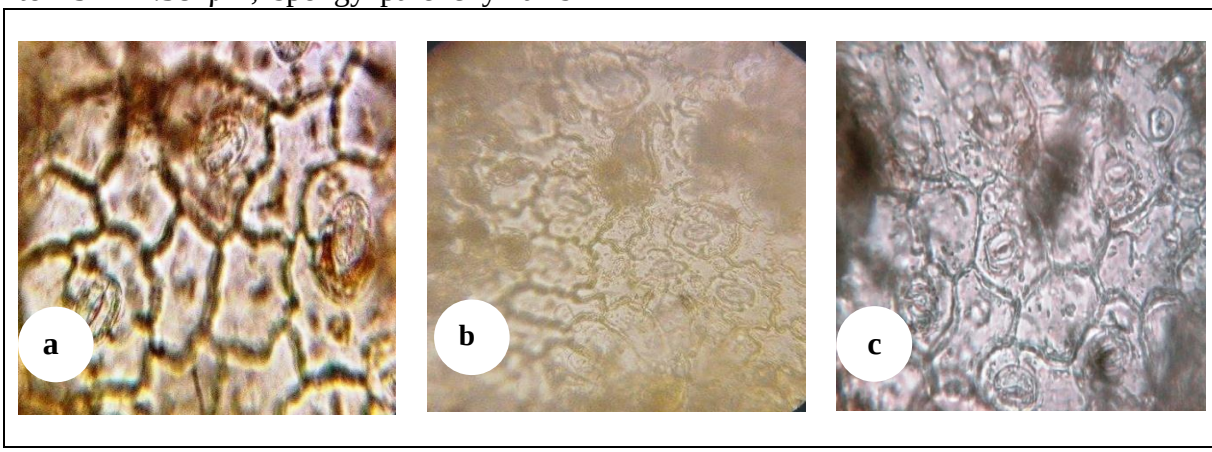

Figure 3: The morphology of stomata size in various stages of *T. ciliatus*.
Note: a Before flowering, b Flowering, c After flowering

DISCUSSION

Histological studies play a crucial role in the systematic of the genus Thymus as they are necessary for species discrimination. These studies undoubtedly enhance our understanding of the observed or cited chemical polymorphism.

Our results showed the general anatomical characteristics of the endemic *Thymus ciliatus* of the genus Thymus, and provided more data on this point in addition to those already reported (Lakusic

et al., 1999 ; Satil et al., 2005 ; Ecevit-Genç et al., 2018 ; Abd Elbar et al., 2019). The comparative anatomical analysis of the trichomes of *Thymus ciliatus* in this study indicated that glandular peltate-type trichomes are distributed on both leaves and stems. Nevertheless, capitate trichomes are distributed only on the stem's surface. The different types of trichomes observed in *Thymus ciliatus* were similar to those described by Benzine-challam et al., (2019) in the same genus as *Thymus guyonii* de Noé. The observed increases in the sizes of glandular trichomes in *Thymus ciliatus* leaves may be attributed to higher temperatures, which are directly reflected in the essential oil content of the plant. In addition, Several studies have reported that the highest productivity of essential oils is observed during the flowering period in the Thymus genus (Lakusic et al., 1999 ; Satil et al., 2005 ; Amarti et al., 2011 ; Giweli et al., 2013 ; Ecevit-Genç et al., 2018 ; Abd Elbar et al., 2019). The influence of environmental conditions on the yield and composition of essential oil in various plants has been reported (Zhang et al., 2015 ; Rahimmalek et al., 2017). The main components of the essential oil of *Thymus ciliatus* are thymol (Heni et al., 2015 ; Sadou et al., 2016) and carvacrol (Bousmaha-Marroki et al., 2007). Furthermore, the small leaves (macrophyll) serve as an acclimation strategy to reduce the intensity of surface transpiration (Feijó et al., 2014), allowing the plant to adapt to the Mediterranean climate.

In the present study, the protective hairs were divided into unicellular and multicellular hairs, with higher density, than the secretory trichomes on both leaves and stems. Indeed, such an observation has already been reported in certain species of Thymus such as *Thymus hirtus* (Guesmi et al., 2019). Eglandular trichomes are an expansion of the epidermal cells, playing a role in protecting the plant, particularly against desiccation, and may also reduce transpiration rates when stomata are closed (Cui et al., 2022). Moreover, our study indicates that stomata are diacitic type, these types are relatively present in thymus species (Satil et al., 2005 ; Zamfirache, 2021). In this study, we reported for the first time the presence of calcium oxalate crystals were observed in the leaf mesophyll, occurring shape Druses crystals. The presence of the crystals constitutes a form of storage and play a role removal of excess ions and electrolytes. Therefore, these structures played a significant role in species identification (Pietczak et al., 2018).

CONCLUSION

The development of secretory structures is related to essential oil yield as well as the biosynthesis and variability of terpene compounds. It was concluded that the most appropriate time for the harvest of this species is during the flowering stage. This study highlights the significance of the histo-anatomical variations in stem and leaf at different stages, as they are likely to affect their potential biological activities. The present study could be very useful for the verification and complete determination of the identity of this plant species, which has proved to be very promising for the therapeutic and food industries, in addition to improving the taxonomic differentiation of the Thymus species of the Lamiaceae family.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

AUTHOR'S CONTRIBUTION

Nina Sadou: onceptualization, methodology, formal analysis, investigation, writing—original draft preparation; Ratiba Seridi: soft-ware,

resources, data curation, visualization, project administration, funding acquisition; Hamdi Bendif: validation, review and editing, supervision; All authors have read and agreed to the published version of the manuscript.

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