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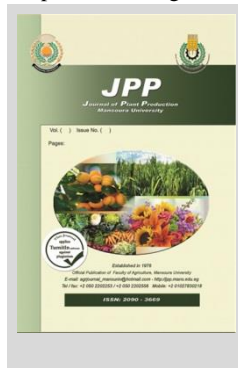
Anatomical and Ultra Structures of Floral Bud, Fruit and Nutlet and Biochemical Analysis of Seed Oil of Basil Plant (*Ocimum basilicum* L.)

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ABSTRACT

The present study focuses on the histological characteristics of Basil's reproductive organ (*Ocimum basilicum* L.). Various organs of reproductive growth were examined during the flowering and fruiting stages of plants development, that being floral bud, fruit and nutlet. Histological characteristics were analyzed microscopically and photomicrographically across transverse sections of different reproductive organs in Basil. Basil nutlet electron scanning microscope was being considered. Furthermore, at maturity phase the biochemical study of Basil fixed oil has been performed. Total fatty acids of Basil seed of which linolenic is the major constituent comprised 49.58% followed by linoleic which being the second major component comprised 23.46% and oleic consists the third major component comprised 10.13% of the total fatty acids of Basil seed. Worth noting that the variations found in the literature and in the current research in nutlet gross morphology and anatomy of the pericarp can be due, primarily, to varying genetic conditions in the studied taxa.

Keywords: *Ocimum basilicum* L., Basil, Anatomy, Ultra structure, Reproductive organs, Fixed oil

INTRODUCTION

The genus *Ocimum* Linn. belongs to family Lamiaceae and the order Lamiales. As a source of volatile oils, fixed oils, medicinal products and ornamentals, it is very important. About 150 species are common in the warm regions of the world in this genus. (Evans, *et al.*, 2008; Kumar, 2009; Sharopov, *et al.*, 2015.). They differ in habit of development, physiology and chemical and aromatic composition.

Ocimum basilicum L. (Basil) is native to Southern Asia and the Middle East but it has long been grown in Europe as an ornamental, culinary and medicinal plant. It is cultivated commercially in Southern and Eastern Europe, North Africa and USA. (Bunney, 1992; Kruger, 1992; Singh and Panda, 2005). Some researchers as (Domokos, *et al.*, 1993; Angers, *et al.*, 1996; Nour, *et al.*, 2009; Mostafavai, *et al.*, 2019; Abeer, *et al.*, 2020; Ghaleshahi, *et al.*, 2020) reported a great variation of fixed oil composition among basil cultivars. Basil as a new high-value essential oil crop is of great concern in many countries.

Basil seed oil has some medicinal properties. It is useful in catarrh, chronic diarrhea, dysentery, gonorrhea, nephritis, cystitis and internal piles (Angers, *et al.*, 1996; Nour, *et al.*, 2009; Mostafavai, *et al.*, 2019; Ghaleshahi, *et al.*, 2020). However, the available information on the anatomical structure of reproductive organs of Basil plant and seed fixed oil composition are extremely poor.

Therefore, it is amid in this study to provide further information on the anatomic properties of Basil plant reproductive organs. Also, phytochemical studies including percentages and composition of fixed oil of Basil seeds at maturity were carried out. Such knowledge may fulfill the lack in information concerning the anatomical and

phytochemical characteristics of the *Ocimum* genus with these huge economic species of the family Lamiaceae.

MATERIALS AND METHODS

This study was performed on Basil of the family Lamiaceae (Labiatae). Seeds were procured from the Experimental Station of Medicinal Plants, Fac. of Pharmacy, Cairo Univ., Egypt. The field work for the production of experimental plant material was performed at the Agricultural and Research Unit of the Fac. of Agric., Cairo Univ., Egypt. The experiment consisted of five replicates, each with a plot, the plot was 4 to 5 m and height 60 cm sides. The seeds have been seeded in 30 cm spacing hills; the plants have been diluted to two plants per hill. All work in the field was conducted in the vicinity of Basil as suggested.

Anatomical studies

A full microscopical study was done to investigate the histological structures of reproductive organs of Basil plant. Specimens represented different plant organs of reproductive growth were taken, including floral buds, fruits, nutlets and seeds.

Microtechnique practices were outlined at the Laboratory of Agric. Botany Dept., Fac. of Agric., Cairo Univ., Egypt. The procedures provided by microtechnique (Nassar and El-Sahhar, 1998) have been followed. At least 48 hours, materials were killed and fixed in (10 ml. formalin, 5 ml. glacial acetic acid, 85 ml. ethyl alcohol 70%). Upon fixing, samples were washed in 50% ethanol until they were put into paraffin wax M.P.

In butanol sequence. 56-58 For the majority of the period. The cross-sections cut to 20 μ m of a rotary microtome were stained with crystal violet / erythrosine

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before mounting in Canada balsam. Microscopically and photomicrographing the slides have been examined.

Nutlets with modern botanical techniques with electron scanning microscopy (SEM) were studied. The samples were mounted on the copper sample stub with dual-sided adhesive disks, and coated with a thin film of golden palladium, Edwards Sputter Coater S 150 A, UK.

Analysis in different positions was performed on the JXA840A electron samples Microanalyser – Jeol, Japan. Scan of electron microscopy was performed by Central Laboratory, National Research Center (NRC), Egypt.

Determination and analysis of Basil seed fixed oil

In order to obtain data on the fatty acids of basil crop, a chemical analysis was performed. For the extraction of lipid from plants, the process (A.O.A.C. 2000) was conducted with chloroform methanol to extract lipids (2: 1V/V). Three times with the aid of a CH₃OH: H₂O (1: 1 V/V) washing of the associated nonlipids. The chloroform lipids are dried with sodium sulfate and heated by heating up to 60 dc under the vacuum. the solvent is removed.

The lipid samples were saponified over-night with ethanoic KOH (20%) at room temperature. The fatty acids were freed from their potassium salts by acidification with hydrochloric acid (5N), followed by extraction with ether (or Pt. ether 40-60 °C), the ether extract was washed three times with distilled water then dried over anhydrous sodium sulfate, and filtered off (Vogel, A. J. 1975).

GC/MS technique was used to separate and detect the fatty acid constituents. Analysis was performed at Research Parks, Fac. of Agric., Cairo Univ.

Conditions used are as follows:

Instrument: HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector.

Column Description: TR-FAME (Thermo 260 M 142 P) (30 m, 0.25 mm Id, 0.25 µm Film) (70% Cyanopropyl-Polysilphenylene Siloxane) capillary column.

Conditions: Temperature of injector: 200 deg °C

Transfer line of temperature: 250 deg °C

Temperature Programming :

Initial	Rate (°C / min)	Temp (°C)	Hold time (minutes)
-	-	80	2
Ramp	3	230	5

The carrier gas (ml / min) He2: 1.5 ml / min

The amount of injected sample: (5 µl/1ml solvent)

The ionization energy: 70eV.

Compared the relative retention and mass species with those of authentic reference compounds (fatty acid methyl esters, purity of 98% of GC), the qualitative identification for the various constituents was performed.

The analysis tools for the chance merge and the NIST MS spectra system were used as well.

RESULTS AND DISCUSSION

Histological studies

Structure of the floral buds

In simple or many branching racemes, Basil plants bear lobiate white flowers. This flower is small, bisexual, hypogynous and bell-shaped both the calyx and the corolla. Infundibular to tubular, smooth, outward bent, firmly 2-lip, 5-lumped (1/4), lobes that are unchallenged, rounded afterwards, curve upwards, deltoid lateral teeth that sometimes have a prominent shoulder, deltoid lobes that sometimes close the often densely hairy calyx-throat.

Corolla strongly 2-lipped, 5-lobed (4/1), white, ascending backlip, similarly 4-lobed, flat or slightly concave anterior lip, a straight or curved corolla tube to the base, usually dorsal gibbous at the midpoint and dilating frequently to the throat. Two pairs of stamen are available. More pair attached close to the base of corolla, exerted, usually pubescent filaments at the root, also appendiculated, the former one attached to corolla-throat. Superior ovary, 4 chambered, single loculus ovule, axile, gynobase, bifid stigma.

The transverse sections of the floral buds of Basil plant as seen in Fig. (1) coincide to the above-mentioned characteristics of Basil flower. It is evident that the calyx consists of five united sepals. The posterior sepal which comprised the upper lip is the larger one, decurrent, with a prominent shoulder and curved. Calyx in transverse section consists of double epidermal layers with ground tissue in between and there are ten separated small vascular strands arranged in a ring through the ground tissue. It is clear that calyx epidermis is enclosed densely with trichomes of various kinds (Fig.3). The non-glandular hairs are uniseriate, pointed, straight or hook-like (sometimes curved), consisting of one to five thick-walled cells. The glandular hairs consist of capitate and peltate hairs.

Five single petals are in the corolla. The corolla tube has two epidermal layers of between two and four layers of parenchymatic cells that are loosely organized. The tubular portion of the corolla is covered by 10 different vascular strands inside the ring.

The androecium consisted of four stamens, each of which consisted of two four loculi, each of which were supported on the filament. The stamen is nearly rounded, constrained by the uniserial layer of almost square cells.

The filament is almost rounded. The tissue is made up of approximately four parenchyma cell layers. In the middle of the filament is a vascular strand. Pollen grains as seen in Fig. (2) are single (monad), isopolar, spheroidal, the equatorial outline is rounded or circular, usually hexacolate and have reticulate tectum.

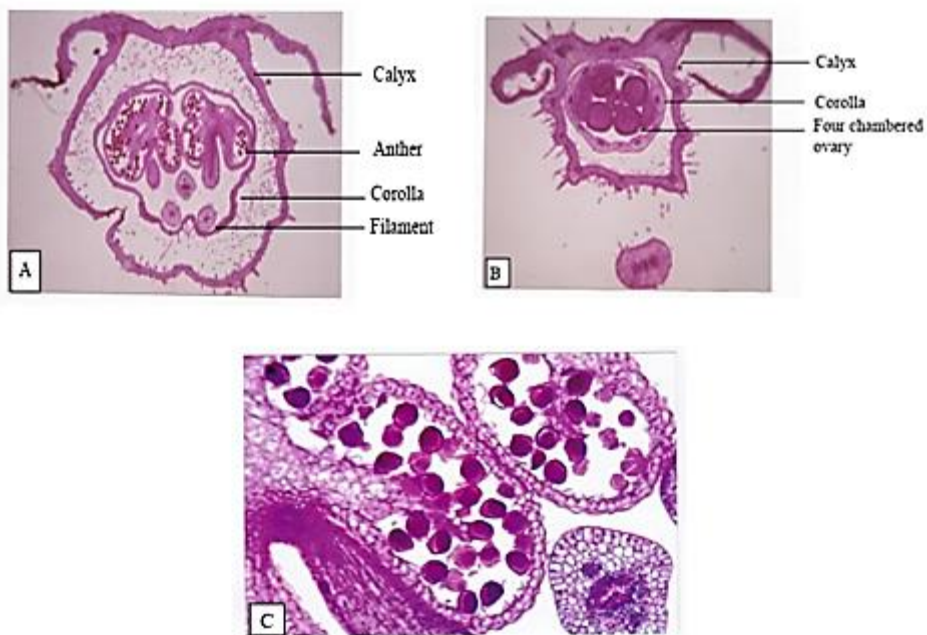


Fig. 1. Transverse sections of floral bud of *Ocimum basilicum* L.
 A- Through stamens. (x 35). B- Through ovary. (x 35)
 C- Magnified portion of A showing pollen grains. (x 180)

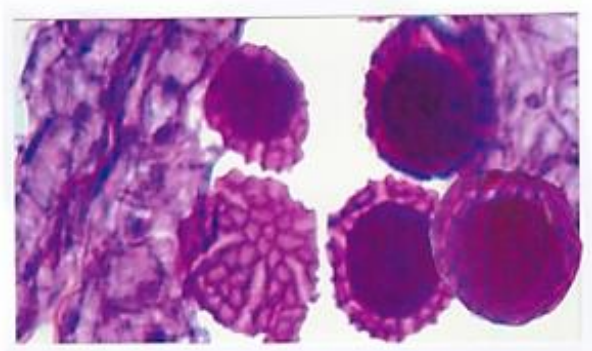


Fig. 2. Magnified pollen grains of *Ocimum basilicum* L.
 (x 720)

As shown in the Fig, (1-B), gynoecium consists of two united carpels, four gynobasic and chambered carpels. Each axil-placentated loculus consists of a single ovule. As far as the authors are aware, there was no thorough research on the floral buds of Basil anatomical structure.

Structure of the fruit, nutlet and seed

Transverse sections through mature fruit and nutlet of Basil plant are shown in Figures (4) and (5); respectively. Obviously, the fruit consists of 4 nutlets containing the mature calyx. In the transverse segment the nutlet is trigonous and contains one seed of dicotyledone (Fig. 5).

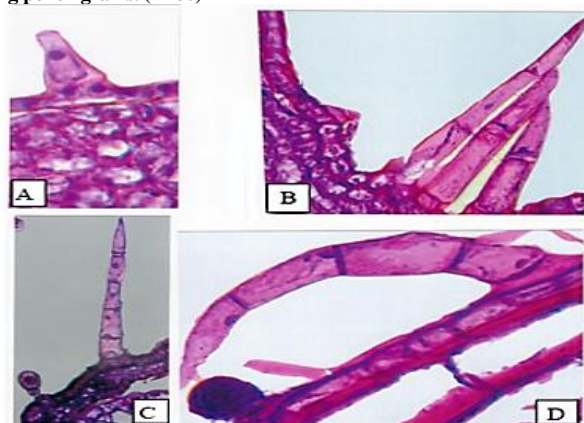


Fig. 3. Trichomes accompanied calyx in cross section of floral bud of Basil.

- A- Non-glandular uniseriate hair composed of one cell. (x 720)
- B- Non-glandular uniseriate, pointed, straight hairs composed of two to three thick-walled cells. (x 720)
- C- Glandular hair at left and non-glandular uniseriate straight hair at right composed of five thick-walled cells. (x 360)
- D- Glandular hair at left and non-glandular uniseriate, hook-like hair at right composed of 4 cells. (x 720)

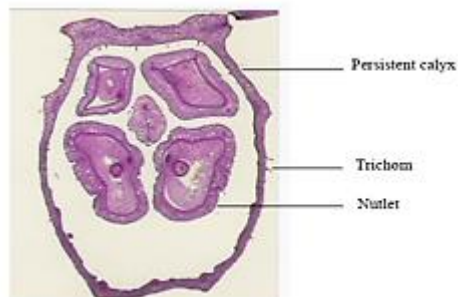


Fig. 4. Transverse section through mature fruit of *Ocimum basilicum* L. (x 52).

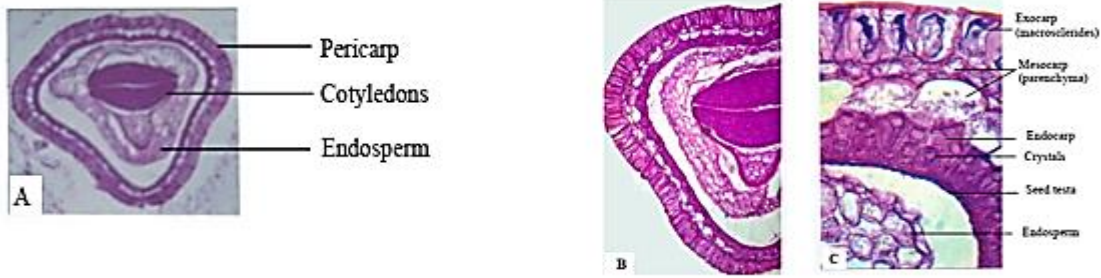


Fig. 5. Transverse section through outlet of Basil.

The pericarp of the nutlet consists of three main tissue regions which terms exocarp, mesocarp and endocarp followed by seed testa (a thin layer) which envelope slight volume of the endosperm and the embryo. Exocarp, the outer epidermis, consists of one layer which differentiated into columnar sclerenchymatic tissue (macrosclerides). Mesocarp consists of two layers composed of thin-walled parenchymatous cells. The endocarp, the inner epidermis,

consists mainly of a one layer of columnar to tubular or pillar-like cells. It contains abundant crystals and seems to act as storage cells.

Nutlet as seen by scanning electron microscope, SEM, (Fig. 6A, B and D) is ovate in outline, glabrous with reticulate sculpturing surface, 1.84 mm in long and about 1.15 mm in width. The hilum is almost rounded in shape and its diameter is about 140 μ m (Fig.6C).

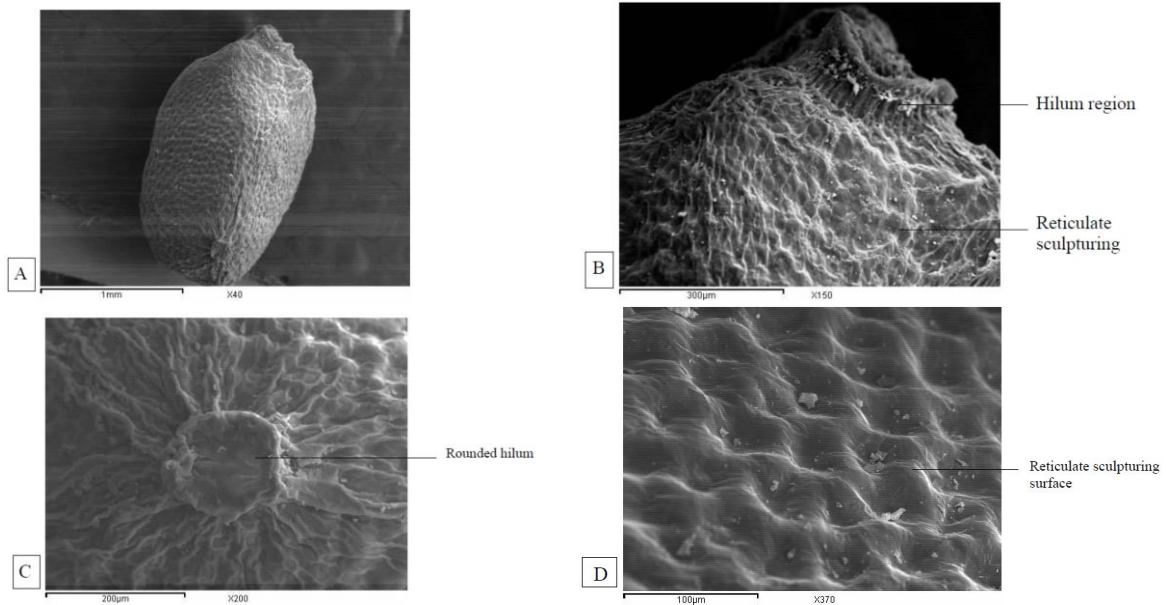


Fig. 6. SEM micrographs of *Ocimum basilicum* L. nutlet showing nutlet shape, hilum region and sculpture pattern. A- Gross morphology of the nutlet. (x 40). B- Hilum region. (x 150). C- Hilum shape. (x 200). D. Reticulate sculpturing pattern of nutlet surface. (x 370).

Analysis of the fixed oil

The chemical analysis proved that mature nutlets (seeds) of Basil contained 17.68% fixed oil. GC-MS technique was used to analyze the fatty acids of Basil seeds (Fig.7). The analysis detected 11 components of fatty acids. Seven of them were identified and the rest (four) were unknowns. Data presented in Table (1) indicate that the unsaturated linolenic (C18:3) was the major fatty acid and comprises 49.58% of seed total fatty acids, followed by unsaturated acids of linoleic (C18:2) and oleic (C18:1) and saturated palmitic (C16:0) which comprise 23.46, 10.13 and 8.28% of the total fatty acids of Basil seed; respectively.

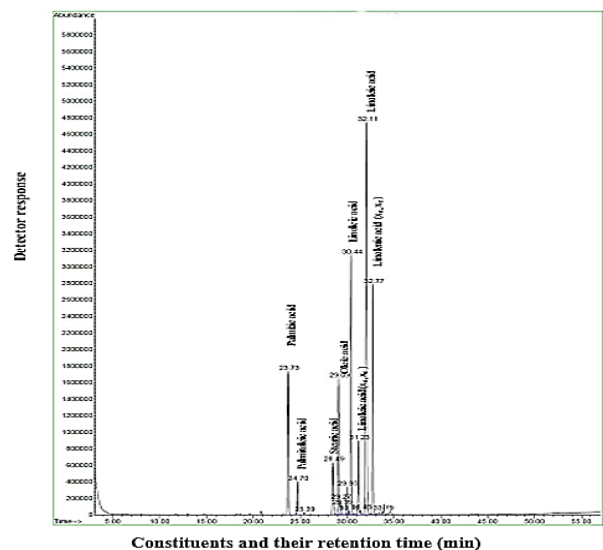


Fig. 7. GC-MS of fixed oil of Basil. Seeds

However, the remainder of fatty acids ranged between 0.14 % (saturated margaric, C17:0) and 3.85% (saturated stearic, C18:0).

Worthy to note that the fixed oil of Basil seed contains 12.27% saturated fatty acids of which palmitic consists 8.28% of the total fatty acids of Basil seed.

Whereas, unsaturated fatty acids consist 85.01% of the total fatty acids of Basil seed of which linolenic is the major constituent comprised 49.58% followed by linoleic which being the second major component comprised 23.46% and oleic consists the third major component comprised 10.13% of the total fatty acids of Basil seed.

Table 1. Fatty acids of Basil seeds, retention time, components and their percentages.

Retention time (min.)	Components		%
23.73	Palmitic acid	C 16:0	8.28
24.69	Palmitoleic acid	C 16:1	1.84
25.40	Margaric acid	C 17:0	0.14
28.49	Stearic acid	C 18:0	3.85
29.09	Oleic acid	C 18:1	9.23
29.27	Oleic acid (x _c , x _c)	C 18:1 (x _c , x _c)	0.90
29.39	Unknown	-	0.49
29.95	Unknown	-	1.67
30.15	Unknown	-	0.18
30.44	Linoleic acid	C 18:2	18.54
31.23	Linoleic acid (x _c , x _c)	C 18:2 (x _c , x _c)	4.92
32.11	Linolenic acid	C 18:3	35.49
32.77	Linolenic acid (x _c , x _c)	C 18:3 (x _c , x _c)	14.09
33.79	Unknown	-	0.08

Discussion

As much as the authors are aware no detailed investigation concerning anatomical structure of Basil fruit and nutlet has been executed. However, (Oran, 1997) studied the *Salvia* genus nutlet anatomy in Jordan and the obtained results revealed that nutlet pericarp consists of three separate regions: epicarp, mesocarp and endocarp. The epicarp is the epidermis and layers of hypodermis. The mesocarp is formed from undifferentiated tissue parenchyma. The endocarp primarily consists of a columnary (macrosclerides) or osteosclerides, a columnary tissue. The pericarp is centered on a fine layer that encircles the endosperm and the embryo. It is of one or sometimes two layers of thick-walled cells or clear brachy- scleroids.

Whereas, (Kahraman, *et al.*, 2009) stated that the nutlets of *Salvia indica* L. are rounded-trigonous in transverse section and ovate in their outline.

The mature nutlets have a length of 3.6 to 4.5 and a width of 3.0 to 3.5 mm. Hilum is round and white and is about 0.4-0.7 mm in diameter. The surface of the nutlets is glabrous, gradually tuberculated and black in mature color.

In this concern, (Coisin and Gostin, 2011) pointed out that nutlet surface of *Salvia glutinosa* L. is smooth, light brown, shiny, ovate-trigonous in outline with reticulate sculpturing surface and the nutlet is trigonous in transverse section. Mature nutlets are 2.88-3.24 mm long and 1.56-1.85 mm wide. The hilum is trigonous with 0.4-0.6 mm diameter. The pericarp of the nutlet could be differentiated into the four distinct regions namely exocarp (outer epidermis), mesocarp, sclerenchyma region and endocarp (inner epidermis). All, being partially in harmony with the present findings. All results above agree to some extend with the

results of (Hassan and Thobaiti, 2014). Worth noting that the variations found in the literature and in the current research in nutlet gross morphology and anatomy of the pericarp can be due, primarily, to varying genetic conditions in the studied taxa.

The previous report of (Minikeeva, *et al.*, 1971) found that Basil seed oil consists of linolenic (62.54%), linoleic (17.56%), oleic (9.40%), palmitic acid (6.75%) and stearic (2.14%). The seed oil of basil in Pakistan was therefore identified by (Malik, *et al.*, 1989) as having an elevated content of linolenic (48.5%) and linoleic (21.81%).

Similarly (Domokos, *et al.*, 1993) revealed that the seeds of Basil contain fixed oil consisting of linolenic (50–63%), linoleic (17–25%), oleic (9–15%), palmitic (6–9%) and stearic (2–3%). The fixed oil yield was 21.4%. All is nearly compatible with the test. In comparison, (Hiltunen, 1999, Nahak, *et al.*, 2011) the Basil fixed oil consists of linoleic (50.0%), linolenic (22.0%) and oleic (15%).

(Angers, *et al.*, 1996) noted that, basil seed fatty acids contain linolenic (57.4–62.5%), linoleic (18.3–21.7%), oleic (8.7–11.6%), palmitic (6.8–8.8%) and stearic (2–2.8%). (Nour, *et al.*, 2009) stated that, linolenic (49–62%), linoleic (12–32%), oleic (6–10%), palmitic (5–13%) and stearic (2–3%). (Ghaleshahi, *et al.*, 2020) recorded that, linolenic (63.8%), linoleic (20.2%), oleic (7.55%), palmitic (4.9%) and stearic (2.5%). (Mostafavai, *et al.*, 2019) found that, linolenic (42.45–61.85%), linoleic (16.73–24.93%), oleic (6.22–19.92%), palmitic (6.23–10.16%) and stearic (2.97–4.88%). (Abeer, *et al.*, 2020) showed that, linolenic (43.92%), linoleic (32.18%), palmitic (13.38%) and stearic (6.55%). Such difference in percentages of fatty acids might be due to the cultivar they used and the difference in the environmental and geographical conditions.

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التركيب التشريحي و الفوق ميكروسكوبي للبراعم الزهرية والثمار والتميريات والتحليل الكيموحيوي لزيوت بذور نبات الريحان

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تركزت الدراسة الحالية على فحص الخصائص التشريحية لأعضاء التكاثر في نبات الريحان (*Ocimum basilicum* L.) حيث تم دراسة تطور أعضاء التكاثر المختلفة خلال مرحلتي الأزهار والثمار وتمثل ذلك في البراعم الزهرية والثمار والتميريات. وفي هذا الصدد قد فحص التركيب التشريحي للقطاعات العرضية لهذه الاعضاء باستخدام الميكروسكوب الضوئي، كما تم فحص التمريرات باستخدام الميكروسكوب الإلكتروني. علاوة على ذلك (في مرحلة نضج الثمار)، تم إجراء دراسة كيموحيوية لزيوت بذور الريحان الثابت. يتضح من النتائج ان الحمض الدهني اللينولينيك هو المكون الرئيسي للأحماض الدهنية لبذور الريحان حيث يبلغ 49.58% يليه الحمض الدهني اللينوليك الذي يعتبر ثاني أكبر مكون يتكون من 23.46% وحمض الأوليك المكون الرئيسي الثالث يشكل 10.13% من إجمالي الأحماض الدهنية لبذور الريحان. تجدر الإشارة إلى أن التباين بين الأبحاث السابقة والدراسة الحالية في الشكل المورفولوجي والتركيب التشريحي للتميريات يمكن أن ترجع، في المقام الأول، إلى الظروف الوراثية المتغيرة في الأصناف تحت الدراسة.