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Anatomical study of the anther in *Peganum harmala* L. (Zygophyllaceae)

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ABSTRACT

The species *Peganum harmala* L. belong to the family Zygophyllaceae. This species is widely distributed in Libya as medicinal and poisonous plant. This study deals with anatomical structure of the anthers as well as the pollen development, through different stages of growth. The results of this study showed that the anther consist of 4-pollen sacs (tetrasporongiate), its wall development follows the dicotyledonous type. The anther wall consists of four types of different cell layers, these are, epidermis with one layer of cells, endothecium with radial fibrous thickening at maturity leading to the connection of each two sporangia, the anther splits longitudinally to shade out the pollen grains. The middle layer with 1-3 rows of cells, and tapetum with one layer of multinucleate irregular cells, the phenomena of cytomixis was observed. The sporogenous tissue under go meiosis resulting microspore tetrads, the microspore tetrads are of three types: tetrahedral, decussate, and isobilateral shapes. The pollen grains are tricolporate, ornamented, highly striped exine, and two-celled when shed out the anthers. The in situ germination of some pollen grains was observed.

Keywords: *Peganum harmala* L; tetrasporangiate; endothecium; pollen grains; in situ germination.

1. Introduction

Peganum harmala L. belonging to the family Zygophyllaceae, it is widely distributed in most of cities of Libya, especially the arid regions with sandy soil and barren lands [1]. It is not suitable for animal grazing because of its bitter taste, and repellent odors [2], *P.harmala* L. used since a long time ago as antibiotics for many bacterial, viral and fungal diseases [3], as will as to getting rid of intestinal parasites [4], in the cancer treatment [5], [6]. The high medicinal efficacy is attributed to a group of compounds (β-carboline alkaloids), which are found in all parts of the plant, and the seeds contain a high percentage of them, reaching 4-6% of their dry weight [7].

Therefore *P.harmala* L. is one of the most promising plants in the field of pharmaceutical manufacturing, and also considered as poisonous due to its toxicity if consumed in high quantities, especially when dryed, the components mainly effect the central nervous system, and causes many complications, including sudden rise in blood pressure, vomiting, headache and others [8].

P.harmala L. is shrub (30- 100 cm) hapit, contains simple, opposite leaves and pentamerous flower. The andrechium consists of a number of stamens (10-15) arranged in two whorls [1], where each stamen consists of a filament and anther. Embryological, anatomical studies showed that at the beginning of the flower bud development, the anther consists of a mass of homogenous meristematic cells that divide transversely, to give rise the anther wall. In addition to that, each anther of plant species belonging to the family Zygophyllaceae consists of four sporangia (tetrasporangiate). Moreover, the wall development of the anther follows the dicotyledon type of wall development [9].

The anther wall consist of four different types of cells: 1-Epidermis a single row of square to rectangular cells. Kapil & Ahluwalia [10], indicated that the epidermis in ***P.harmala*** **L.** is heavily cutinized to protect inner layers from external influences. 2- Endothecium is one layer of irregularly shaped cells, at maturity it develops fibrous thickenings that contribute to coalescence sporangiates, and anther split longitudinally to release pollen grains, this phenomenon has been seen in many Lilliacous plants [11]. 3- The middle layer in each of species of Zygophyllaceae is two or three layers [12], and it fade during development, the outer layer of it has fibrous thickenings. 4- Tapetum is of glandular cells, which is a microspores nutritious layer, and it is characterized by irregularity of cells. In *P.harmala* L. the cells become multinucleated and are in two irregular rows. Some times The nuclei fused together to form polyploid nucleous and It also has a role in the secretion of some organic substances, amino acids and enzymes necessary for the development of pollen grain wall [13].

The sporangenous tissue developos into a diploid microspore mother cells (M.M.C), of which during meiosis each M.M.C produces four haploid microspores (microspore tetrads). The microspore tetrads comes in different shapes including: tetrahydral, decussate, isobilateral, linear, L- shape, and T- shape [14]. Johari *et al* [12], reported that microspore tetrads in *P.harmala* L. are tetrahydral and decussate in shape.

Semerdjieva and Yankova-Tsvetkova [15], in the study of pollen grains and seeds of *P.harmala* L. and *Zygophyllum fabago* of some Bulgarian populations using the electron microscope, and light microscope the observations showed that pollen grains in *P.harmala* L. are oblate, spheroidal, elongated, oval in shape, colporate,exine and striato- rugulate.

Kamelina [16], has described the mature pollen grain of Zygophyllaceae as two-celled, and has reported three- celled pollen only in the genus *Fagonia* L., and *Tribulus terrestris.* Watson & Dallwitz [17], have also observed the three- celled mature pollen grains in *Tribulus terrestris*. Moreover, Masand [18], reported that the genera *Peganum*, *Seetzenia*, and *Zygophyllum* shed pollen grains at two-celled stage.

The pollen grain is encased by envelopes with a smooth, thin intine, and a thick, frilled exine, are on three- colporate in *P.harmala* [19]. Almosawi [20], pointed out that the pollen grains of *P.harmala* in Iraq is smooth, and tricolporate, through which pollen tubes comes out during germination process. In some plants, early pollen germination occurs in anther (in situ germination), this phenomenon has been seen in eleven plants from chasmogamy angiosperms as *Prunis amygdala* and apples [21].

This study aims to clarify the anatomical characteristics of the anther of *Peganum harmala* L. during different growth stages, and to follow the pollen grains microsporogenesis.

2. Materials & Methods

Field trips were made to the city of Gharyan, specifically the Qawasim area at the beginning of May - June 2021. The flower buds were collected in all different stages of development. Buds were fixed in Cornoy's fixative (1:3 alcohol/ acetic acid). The anthers were separated from other parts of the flower and stored in ethanol 70%. For dehydration, the anthers were run through different concentration of ethanol- xylose series with addition of paraffin wax crystals as described

by Johansen 1940. Anthers were embedded thoroughly in paraffin wax for at least overnight in the oven at a temperature ranges between 60-65°c. The anthers were arranged in pure paraffin blocks for sectioning. A rotary microtome was used for sectioning. The best sections obtained were cut at 10-20µm in thickness. Sections were stained with Safranin/ Fast green stain [22]. The slides were investigated by light microscope, and photos were taken with fixed camera microscope.

3. Results & Discussion

The young anther is tetrasporangia, and the sporangenous tissue is coated with four layers of cells (epidermis, endothecium, middle layer, and tapetum) Figure 1, which can be clearly distinguished from each other at the early microsporogenesis (during prophase I), Therefore, the anther development follows the dicotyledonous type, and that agrees with Yankova- Tsyetkova *et al* [9]; Johari *et al* [12]; Davis [14].

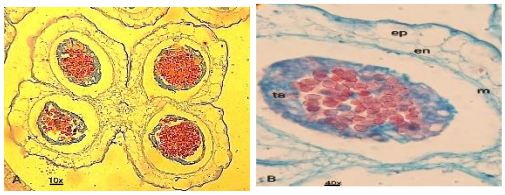

Fig.1. Crosse section of a young anther. A: showing tetrasporangia, B: anther wall composetion, ep: epidermis, en: endothecium, m: middle layer, ta: tapetum

The epidermis is a single row of square to almost rectangular mononucleate cells, which covered whit an extremely thick cuticle layer to protect the other layers of the anther wall. This was also mentioned by Kapil & Ahlwalia, [10], in *P.harmala* L. Endothecium is rather large consists of irregular- shaped cells, which when mature develops radial spiral thickening at maturity, which causes the fusion of each two sporangia, resulting an anther with two chambers Figure 2. The anther splits longitudinally to release the mature pollen grains, these results were agreed with the results obtained by Inamuddine & Faris [11], in their study of *Scilla automnalis* and *Urgenia maritima*.

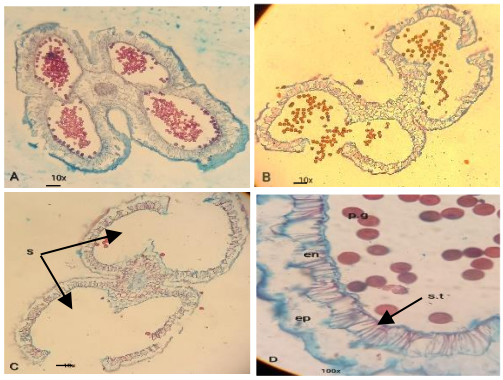

Fig.2. Crosse section of a mature anther. A: shows the beginning of the fusion of each two sporangi, B: anther with two chambers, C: the anther splits longitudinally to release the pollen grains, D: radial spiral thickening, and the anther wall contain only epidermis and endothecium, s: sporangia, ep: epidermis, en: endothecium, s.t: spiral thickening

The middle layer is one to three rows of elongated ephemeral cells Figure 3, which degrade during meiosis, and a few remnants of it present in mature anther. The mature anther contain only epidermis and endothecium Figure 2. The tapetum is the inner layer of glandular cells surrounding the cells of sporangenous tissue. The tapetal cells are irregular and rich in nutrients, and they supply the sporoangenous tissue and products of meiosis with nutrients needed during division stages [14]. It is distinguished from other cells of the layers Figure 4.

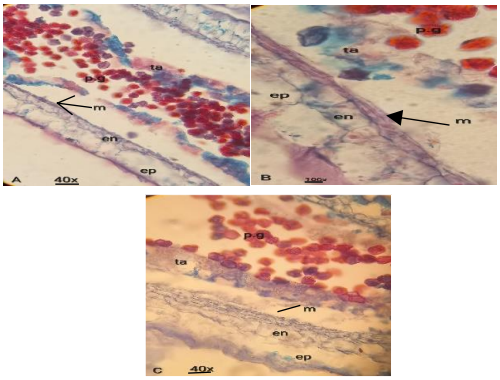

Fig.3. Longitudinal section of young anther, showing the rows of the middle layers. A one row of cells, B two rows, C three rows of cells, ep: epidermis, en: endothecium, m: middle layer , ta: tapetum, p.g: Pollen grains

The phenomenon of cytomixis is also observed, where some cells with two- more nuclei, and others without nuclei Figure 4, this

was agreed with observation of Faris & Inamuddine [23]. The tapetum also secretes pollen kitte and some organic substance, amino acids and enzymes which help in the exine formation and sporopollenin maturity [14]. During pollen development, the tapetal cells begin to separate from the middle layer and left a space, and gradually disappear as shown Figure 5.

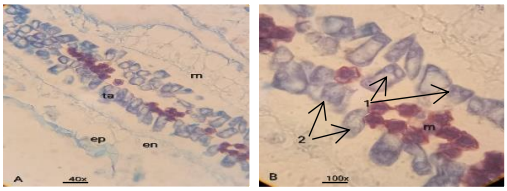

Fig. 4. Longitudinal section of young anther, shows the Tapetal cells with the phenomenon of cytomexsis, A: the tapetum layer with glandular cells, B: 1: cells with two nuclei , 2: cells without (lacking) nuclei , ep: epidermis, en: endothecium, m: middle layer, ta: tapetum, m: microspores

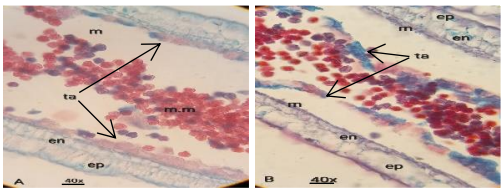

Fig. 5. Longitudinal section of young anther, A: the tapetal cells contact with middle layer, B:the tapetal cell begin to separate and left space, ep: epiderms, en: endothecium, m: middle layer, ta: tapetum, m.m: microspore monad

The sporangenous tissue is a mass of diploid cells (microspore mother cells), that undergoes meiosis and give raise to microspore tetrads. The results of this study showed that most of the pollen grains were normal, and some of them were small and abnormal Figure 6. The percentage of pollen grains viability showed that 55% of pollen grains were normal and viable, while 23% of pollen grains were abnormal (not viable). Such results leads to the fact that the percentage of viable pollen grains is high in anthers. The microspore tetrads formed are mostly tetrahedral, decussate, and isobilateral, thus this agree with Johari *et al* [12] Figure 7.

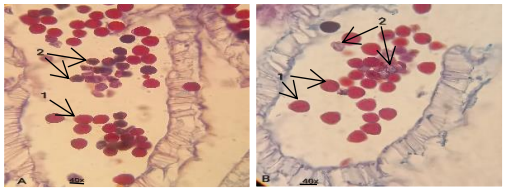

Fig. 6. Crosse section of a mature anther, A, B shows the pollen viability, 1: normal pollen grains, 2: abnormal pollen grains

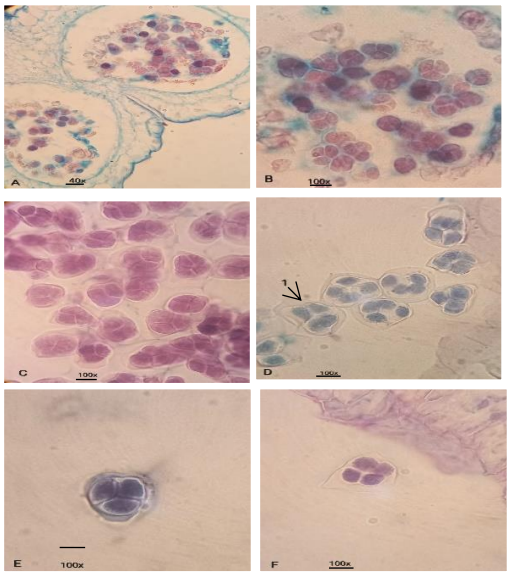

Fig. 7. Crosse section of a mature anther during microsporogenesis , A, B, C: shows the microspore tetrads, D: microspores tetrads separate from each other, 1: decussate, E: tetrahedral, F: isobilateral

The pollen grains are spherical or oval, ornamented with highly striped exine and smooth intine. The pollen grains consisting of 3 pores (tricolporate) through which pollen tube germination takes place Figure 8. It was also observed that early germination of some pollen grains inside the anther in a phenomenon known as in situ germination Figure 9. These results were agreed with Koul *et al* [21]; Faris & Inamuddine [23]

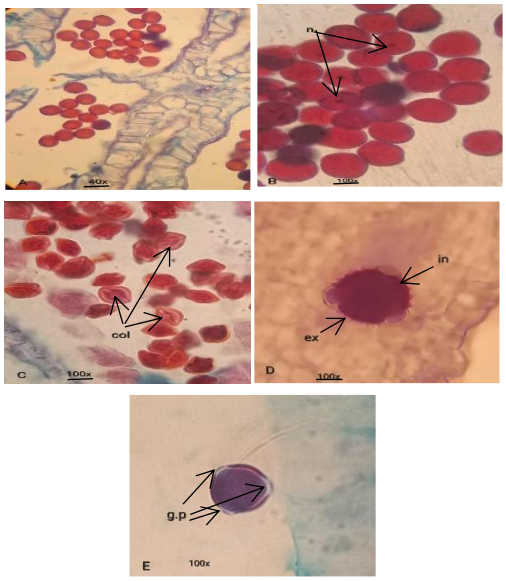

Fig.8. Crosse section of mature anther, A, B: shows the mature pollen grains, C: colporate, D: ornamented exine and smooth intine of p.g,E: three germ pores of pollen grain (tricolporate), n: nuclei, col: colporate, g.p: germ pores, ex: exine, in: intine

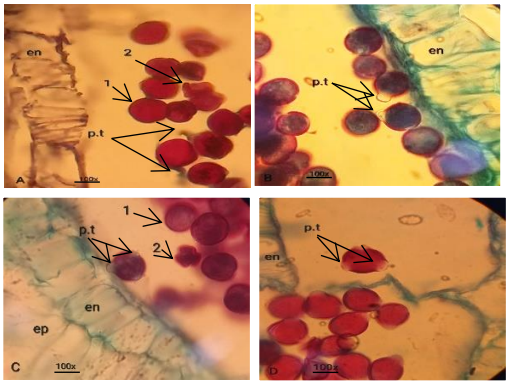

Fig.9. Crosse section of mature anther, A,B,C,D: the in situe germination in some pollen grains, 1: normal p.g, 2: abnormal p.g, p.t: pollen tubes, en: endothecium, ep: epidermis

Mature pollen grains mostly are two-celled when shed out of the anthers, with central large vegetative nucleus and preipheral spindle-shaped generative nucleus Figure 10. The three- celled Pollen grains were never observed in this study and agree with the observations of Kamelina [16], and Masand [18].

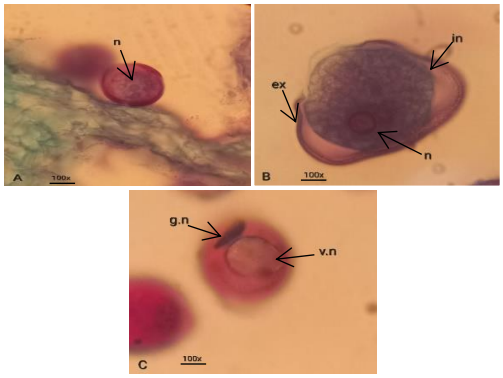

Fig.10. Crosse section of pollen grains, A,B: shows the one- celled of p.g (mononucleate), C, two-celled of p.g (Binucleate), n: nuclei, ex: exine, in: intine, g.n: generative nucleus, v.n: vegetative nucleus

4. Conclusion

The present study showed the anatomical characteristics of the anthers in *Peganum harmala* L. in Libya. The young anther is tetrasporangia, and the wall development of the anther follows the dicotyledons type. The phenomenon of cytomixis, as well as in situ germination of some pollen grains were observed in this study. The pollen grains are tricolporate, ornamented exine and two-celled when shed out the anthers.

5. Refernces

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