

CHARACTERISTICS OF LEAVES TRICHOMES OF *FAGONIA* L. (ZYGOPHYLLACEAE) IN SAUDI ARABIA

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Abstract

The characteristics of the leaf epidermis play a distinctive role in support of taxonomic studies within plants. However, we did not find taxonomic studies in Saudi Arabia of leaf epidermis, especially the trichomes of species in the genus *Fagonia* L. that are considered one of the most important medicinal genera in the Zygophyllaceae family, recorded in the Flora of Saudi Arabia. In this paper, the trichomes of *Fagonia* species leaves are determined. The upper and lower surfaces of leaves in all studied species had many types of trichomes with varying densities, except *F. schwinfurthii* leaves were smooth with sparse trichomes on their petioles. All trichomes in the studied species were unbranched glandular. Each species had more than one type of glandular trichomes. Some of the trichomes were sessile and others had stalk. The studied species contained multiple forms of secretory cells. The most common shapes were the oblong and clavate secretory cells. Also, the secretory cells had other shapes, such as cylindrical, pyriform, spherical, capitate, pilate, digitiform, and hook-shaped. The secretory cell of some species contained a cupule, but *F. tristis* was distinguished by the radial shape of a cupule. In this study, the multiple differences in secretory cell shapes demonstrated the distinguish diversity of trichomes and recorded new shapes that were not previously observed in *Fagonia* L.

Keywords: *Fagonia* L., Zygophyllaceae, trichom, SEM, Saudi Arabia

INTRODUCTION

Zygophyllaceae is a widespread family, dicotyledonous and heterogeneous. It consists of trees, shrubs, herbs, and many halophytes, most of which are found in arid and semi-arid regions of the tropics and subtropics (Sheahan and Cutler, 1993; Ghazanfar, 1994). Zygophyllaceae is represented in the Kingdom of Saudi Arabia by six genera (Chaudhari, 2001). *Fagonia* L. is one of the most important medicinal genera of Zygophyllaceae and consists of annual and perennial plants (Beier, 2005; Chaudhari, 2001). *Fagonia* L. is recorded in the Flora of Saudi Arabia. Migahid (1996) mentioned that the genus *Fagonia* L. in Saudi Arabia includes nine species, while Collenette (1999) mentioned ten species, but only nine species are identified, and the tenth species was recorded as an unidentified species (*Fagonia* sp.), and Chaudhary (2001) recorded eleven species that belong to *Fagonia*.

Since 1753, when Linnaeus identified *Fagonia* in his book (Species Plantarum), more than 160 scientific names of the *Fagonia* species had been published. Therefore the classification and limitation of the species *Fagonia* difficult due to its complex diversity and elasticity, which change its morphology as response to surrounding environmental conditions (Danin, 1996; Zohary, 1972; Beier, 2005). This led some authors to identify many taxonomic units that are below the species' taxa (Beier, 2005). *Fagonia* species are known in Saudi Arabia by the common name Darma, Dreama, or Janbah, and the species *F. bruguieri* and *F. indica* are the most widespread in Saudi Arabia (Chaudhary and Al-jowaid, 2013).

Palmer and Tucker (1981) stated that the use of scanning electron microscopy (SEM) to study epidermis of leaves and presence or absence of some epidermal structures are important for taxonomic studies. Trichomes are one of the most important structures on the vegetative and reproductive parts of angiosperms (Werker, 2000; Wagner, et. al., 2004). The trichomes have great diversity in shape, cellular structure and function, they may be unicellular or multicellular, branched or unbranched, glandular or non-glandular (Werker, 2000; Yang and Ye, 2013). The trichomes play an important role in protecting the plant from herbivores and some disease-causing insects in addition to reflecting light and regulating temperature, which reduces water loss (Wagner et. al., 2004).

All species of *Fagonia* have some types of trichomes, although they may be scattered or confined only to certain parts of plants, such as small stems, petioles, fruits, or sepals (Beier, 2005). Their number and density vary according to the different species and the degree of maturity or age (Abdel Khalik and Hassan, 2012). The upper and lower surfaces of *Fagonia* L. leaves are containing glandular trichomes, which greatly contribute to the adaptation with the dry conditions (Fahn and Shimony, 1996).

The aim of this study is to investigate the diversity, density and morphological characteristics of glandular trichomes among the species of the genus *Fagonia* L. in Saudi Arabia by using light microscope (LM) and scanning electron microscope (SEM).

Materials and Methods

- Specimen collection

Samples of *Fagonia* species were collected from their natural sites in the kingdom of Saudi Arabia as shown in Table1).

-light microscopy examination

The plant samples were thoroughly washed with running water to get rid of the dust and impurities suspended in them. Thin strips were prepared from the leaves of plant species using the Beier (2003) method with some modifications. A thin piece is taken from the leaf surface by using a pointed needle and forceps, then transferred onto a clean glass slide, and finally covered with a cover glass. After that, the slide was examined by a Compound Light Microscope (Olympus), with an eyepiece plan of (40X) in the lab 116, a botany and microbiology department. Finally, the trichomes types were photographed by using iPhone 8 plus mobile camera.

-Scanning electron microscopy examination

SEM study of the leaf epidermis of *Fagonia* species was carried out to obtain a detailed study of the microscopic morphology of the trichomes. Samples of the leaf were cut into small portions, the samples portions were mounted on the double adhesive tape on stubs, sputter-coated with titanium. After that, they examined under SEM (JEOL-JSM-6060 LV) in the Electron Microscope Unit - Central Laboratory of the Departments of Science and Medical Studies at King Saud University.

The terms of (Simpson, 2010; Abdel khalik and Hassan, 2012) were used to describe and identify the patterns of trichomes with some modification.

Results

The upper and lower surfaces of the leaves were examined in all studied species of *Fagonia*. There are various types of trichomes that covered the leaves surfaces with varying degrees of density. Except for *F. schwinfurthii*, the leaves surfaces were smooth with sparse trichomes which covered the petioles. The leaves of *F. boveana* and *F. glutinosa*, were distinguished by thick Indumentums, which makes the leaves very sticky and led to adhesion of sand and impurities on their surface. The Indumentums in *F.boveana* were hispid indumentums compared to the rest of the other species.

All trichomes of the studied species were unbranched glandular type. Table (2), showed that each species contained more than one type of glandular trichomes. Some of them were sessile, like *F. schwinfurthii* and *F. ovalifolia*, Figures (11, 12,

17, 18), and some others contained stalks as *F. tristis*, *F. glutinosa*, and *F. boveana* Figures (9, 10, 13, 14, 15, 16). The rest of others species, *F. arabica*, *F. bruguieri*, *F. paulayana* and *F. indica* were sessile and possessed stalks Figures (1, 2, 3, 4, 5, 6, 7, 8). All of the observed stalks were multicellular or multiseriate, except *F. tristis*, which had unicellular stalk (Figure 15A). The stalks of all studied species were short, with the exception of *F. boveana* was distinguished by long stalk with multicellular and multiseriate (Figure 13, 14).

The secretory cells of the glandular trichomes were unicellular in all the studied species, except *F. schwinfurthii*, which was biseriate as Figure (11) and *F. boveana* contained glandular trichome with multicellular secretory cell, Figure (13F). The current study results displayed that the secretory cells were morphologically different from each other as shown in Table (2). The common form of secretory cell was oblong. It was oblong with the concavity of one side as in species *F. arabica* (Figures 1, 2 A, B), *F. paulayana* (Figures 5, 6 A), *F. indica* (Figures 7 F, 8 B) and *F. ovalifolia* (Figures. 17, 18 A, B), or oblong with the concavity of two sides as in species *F. arabica* (Figures 1, 2 C). Sometimes it was linear-oblong with a bent top as in the species *F. paulayana* (Figures 5, 6 D).

The other common form was the clavate shape as seen in *F. bruguieri* (Figures 3, 4B), *F. indica* (Figure 7B) and *F. boveana* (Figures 13 A, D, 14B). The clavate shape might be concavity of one side, as in the species *F. schwinfurthii* (Figures 11 B, C, D, 12 A), *F. indica* (Figure 7C), and *F. boveana* (Figures 13B, C, E, 14C, D), or concavity of two sides as in *F. schwinfurthii* (Figures 11A, 12B). It might be linear clavate with an apex curved in the species *F. bruguieri* as in Figures (3, 4A), or with a rugose top in species *F. indica* as in Figure (7D).

The others forms of secretory cells of the studied species trichomes were cylindrical shaped in both species *F. arabica* and *F. ovalifolia* as shown in Figures (1,2 D) and (17C, 18C, D) respectively. Pyriform shaped in both *F. bruguieri* and *F. boveana* as shown in Figures (3,4D, 13A). Capitata shaped which was observed in *F. arabica*, *F. indica* and *F. paulayana* species, Figures respectively (1 E, F, 2 E), (5, 6 B, C), (7E, 8A). Spherical shape in *F. glutinosa* and *F. tristis* (Figures 9, 10, 15), but the radial cupule gave the *F. tristis* secretory cells a special shape (Figures 15, 16). There are some distinct shapes were recorded in some species, such as hook-shaped, which was observed in *F. indica* (Figure 7A), pilate shape in *F. bruguieri* (Figures 3, 4C), and the digitiform in *F. boveana* (Figure 13F). There were some structures of secretory cells like, neck cells, cupules and apertures that might be present or absent of some species, as in Table (2).

It was observed dark color at the top of the secretory cells like cupule by examining some species such as *F. schwinfurthii*, *F. indica* and *F. arabica* by the light microscope, but scanning electron microscope showed them didn't had. There was accumulation of secretory substance at the top of the secretory cells, and sometimes it was densely secreted on the surface of the secretory cell, as shown in Figure (19). The cross-section of *F. ovalifolia* and *F. boveana* trichomes appeared as channels, Figure (20).

Table (1) List of the studied *Fagonia* species and their collection sites in Saudi Arabia

No.	Species	Collection area	Coordinates
1	<i>F. arabica</i>	Fayfa	N 17° 16' 30" E 43° 03' 59"
2	<i>F. bruguieri</i>	Al-Riyadh	N 24° 43' 25" E 46° 38' 09"
3	<i>F. paulayana</i>	Muhayil Asir	N 18° 29' 29" E 42° 4' 20"
4	<i>F. indica</i>	Khamis Mushayt	N 18° 20' 08" E 42° 46' 41"
5	<i>F. glutinosa</i>	Sekakah	N 29° 54' 49" E 40° 03' 08"
6	<i>F. schwinfurthii</i>	Al-Darb	N 17° 49' 60" E 42° 22' 31"
7	<i>F. boveana</i>	AL-Madinah	N 24° 28' 05" E 38° 57' 22"
8	<i>F. tristis</i>	Southeast of Tabuk	N 28° 13' 05" E 37° 15' 17"
9	<i>F. ovalifolia</i>	East of Tabuk	N 28° 27' 57" E 36° 33' 25"

Table (2) Types of glandular trichomes of the *Fagonia* species in Saudi Arabia

Species	Stalk	Head			
		Secretory cell	Neck cell	Cupule	Apertures
<i>F.arabica</i>	Sessile	Unicellular, oblong, concavity of one side	Absent	Absent	Absent
		Unicellular, oblong, concavity of two sides	Absent	Absent	Absent
	Multiseriate base	Unicellular, oblong, concavity of one side	Absent	Absent	Absent
		Unicellular, Cylindrical	Present	Absent	Absent
		Unicellular, Capitate	Present	Absent	Present
<i>F.brguieri</i>	Sessile	Unicellular, linear-clavate, apex curved	Absent	Absent	Present
		Unicellular, Clavate	Absent	Absent	Present
	Multiseriate base	Unicellular, Pilate	Present	Absent	Present
		Unicellular, Pyriform	Absent	Present	Present
<i>F.paulayana</i>	Sessile	Unicellular, oblong, concavity of one side	Absent	Absent	Absent
		Unicellular, Linear-oblong, apex curved	Absent	Absent	Absent
	Multiseriate base	Unicellular, Capitate	Present	Present	Present
<i>F.indica</i>	Sessile	Unicellular, oblong, concavity of one side	Absent	Absent	Absent
		Unicellular, Hook shape	Present	Absent	Absent
	Multiseriate base	Unicellular, Clavate	Absent	Absent	Absent
		Unicellular, Clavate, concavity of one side	Absent	Absent	Absent
		Unicellular, Capitate	Absent	Absent	Absent
		Unicellular, linear-clavate, a rugose top	Absent	Absent	Absent
<i>F.glutinosa</i>	Short, biseriata Stalk	Unicellular, Spherical shape	Absent	Present	Present
	Multiseriate base+ Short, biseriata Stalk	Unicellular, Spherical shape	Absent	Present	Present
<i>F.schwinfurthii</i>	Sessile	Biseriate, Clavate, concavity of one side	Absent	Absent	Present
		Biseriate, Clavate, concavity of two sides	Absent	Absent	Present
<i>F.boveana</i>	Multiseriate base	Unicellular, Clavate	Present	Absent or Present	Absent
		Unicellular, Pyriform	Present	Absent	Absent
		Unicellular, Clavate, concavity of one side	Present	Absent	Absent
		Multicellular, Digitiform	Absent	Absent	Absent
	Long, multicellular, multiseriate stalk	Unicellular, Clavate, concavity of one side	Present	Absent or Present	Absent
<i>F.tristis</i>	Short, oblong, unicellular or multiseriate stalk	Unicellular, Spherical with radial cupule	Absent	Present	Absent or Present
<i>F.ovalifolia</i>	Sessile	Unicellular, oblong, concavity of one side	Absent	Absent	Absent
		Unicellular, Cylindrical	Absent	Absent	Absent

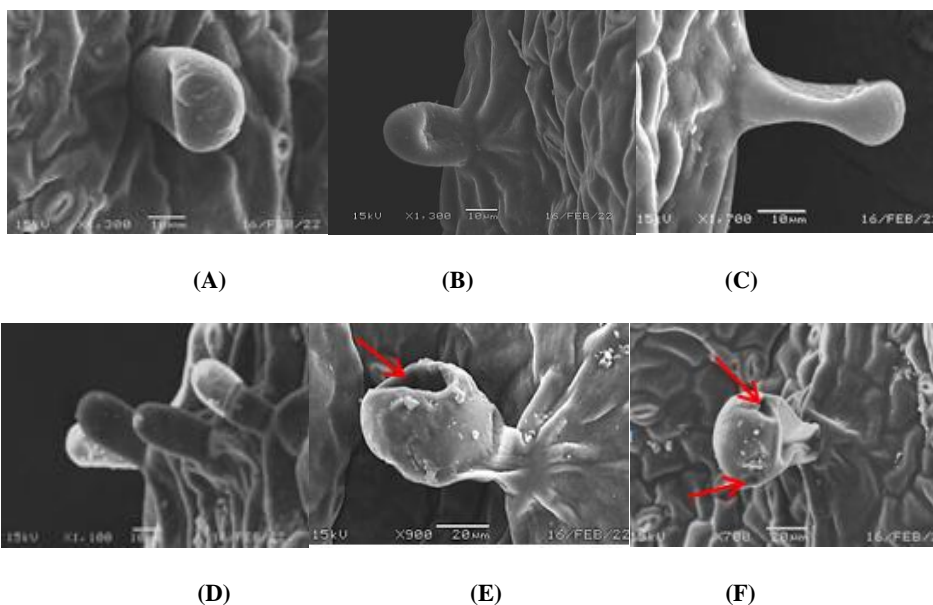


Figure (1): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F.arabica*. The red arrow indicates the apertures in the secretory cell.

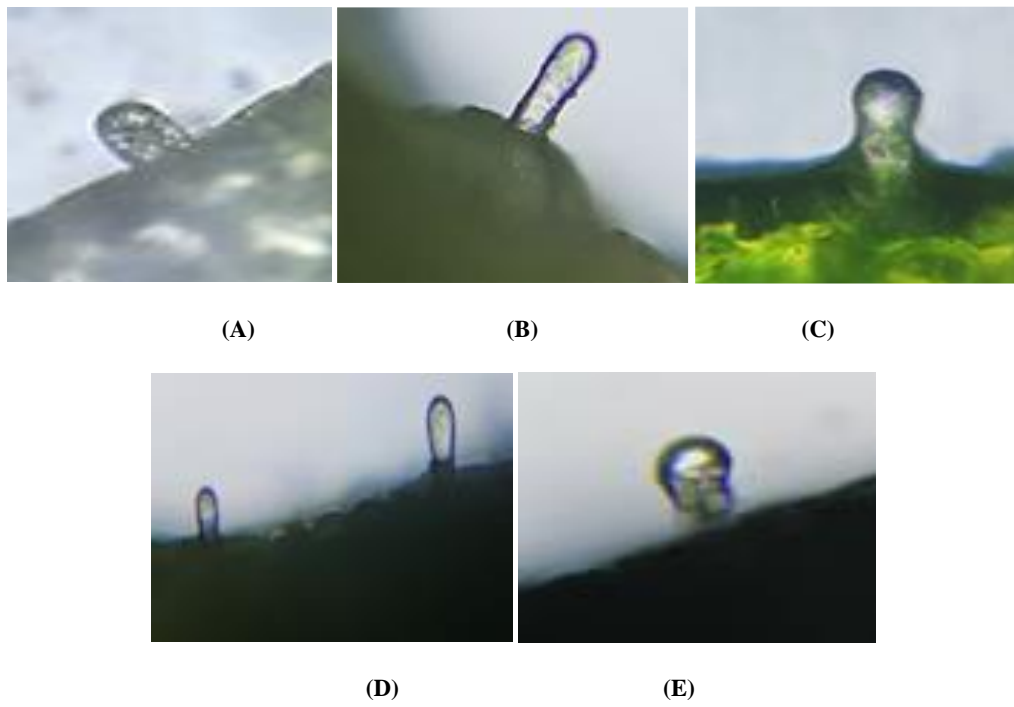


Figure (2): Light microscope (LM 40X) micrographs showing the glandular trichomes of *F.arabica* at (40x) magnification.

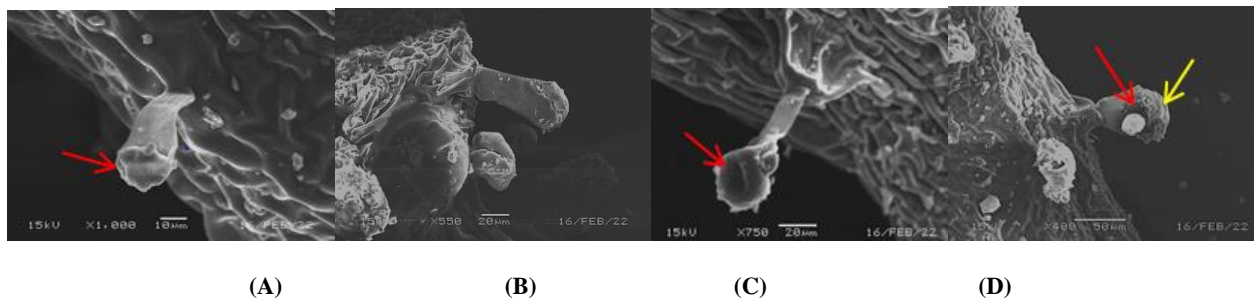


Figure (3): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F.bruguieri*. The red arrow indicates the apertures in the secretory cell, yellow arrow indicates the cupule.

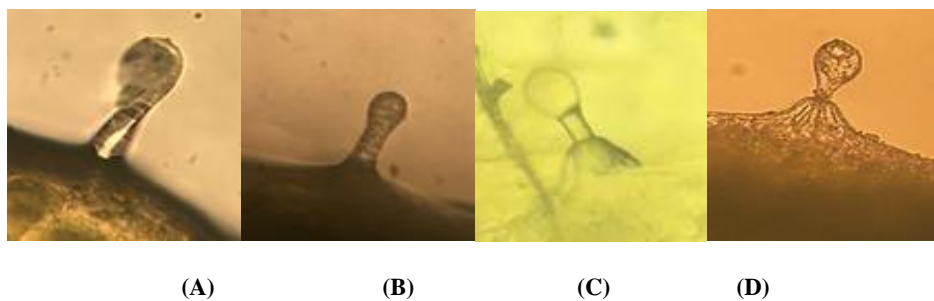


Figure (4): Light microscope (LM 40X) micrographs showing the glandular trichomes of *F.bruguieri* at (40x) magnification.

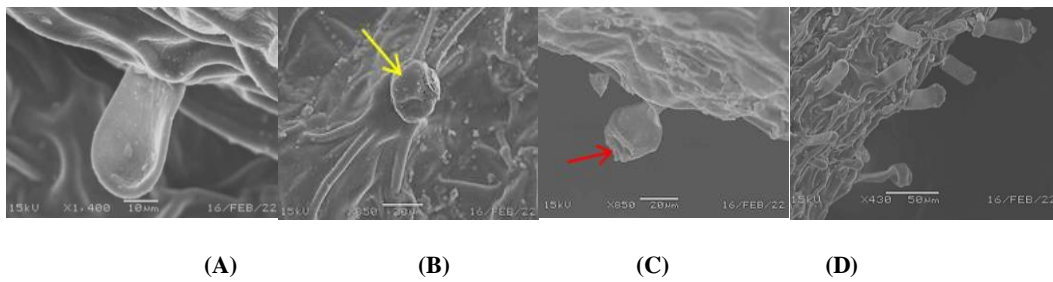


Figure (5): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F. paulayana*. The red arrow indicates the apertures in the secretory cell, yellow arrow indicates the cupule.

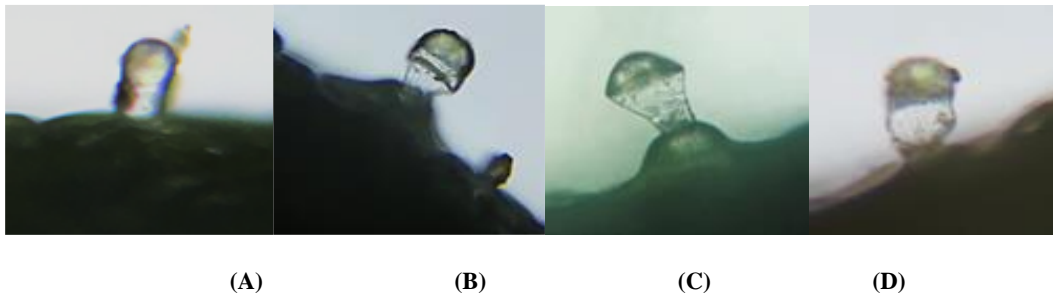


Figure (6): Light microscope (LM40X) micrographs showing the glandular trichomes of *F. paulayana* at (40x) magnification.

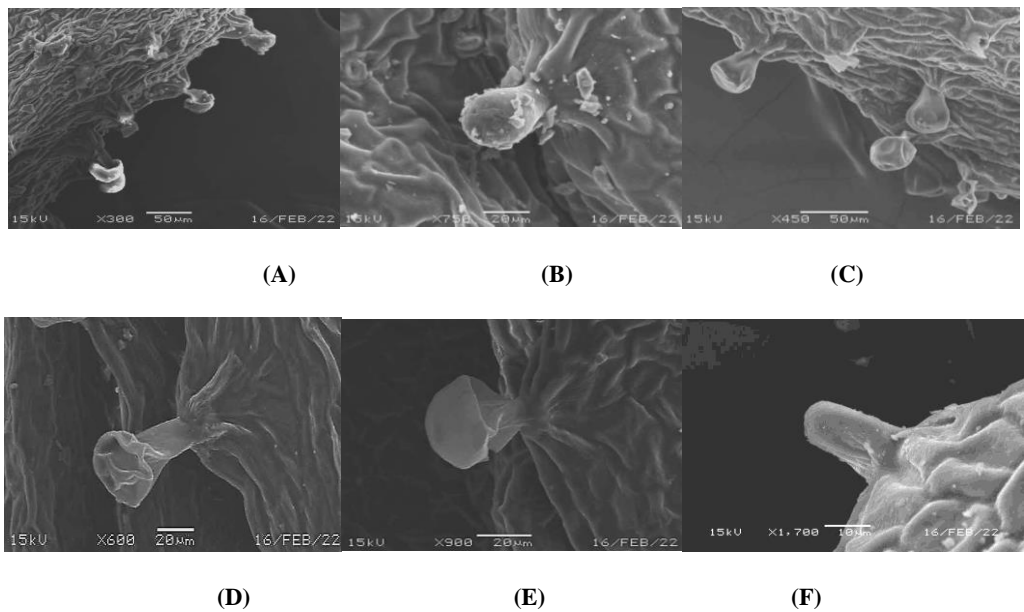


Figure (7): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F. indica*.

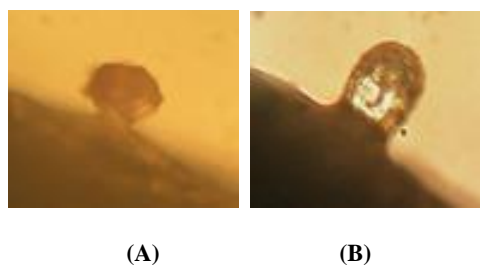


Figure (8): Light microscope (LM40X) micrographs showing the glandular trichomes of *F. indica* at (40x) magnification.

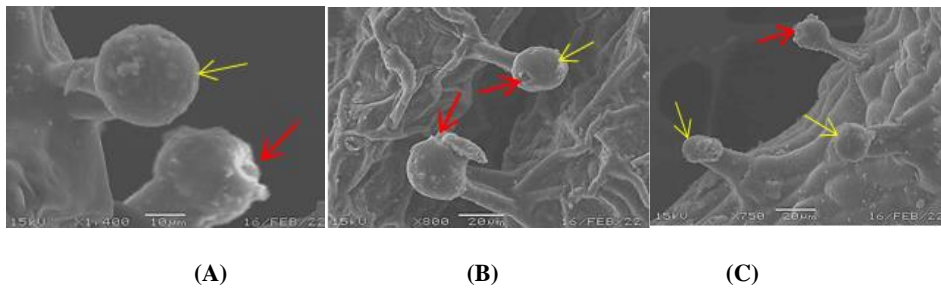


Figure (9): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F. glutinosa*. The red arrow indicates the apertures in the secretory cell, yellow

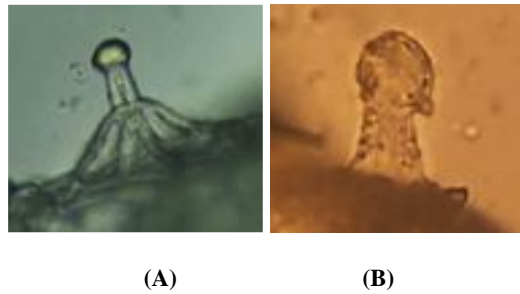


Figure (10): Light microscope (LM40X) micrographs showing the glandular trichomes of *F. glutinosa* at (40×) magnification.

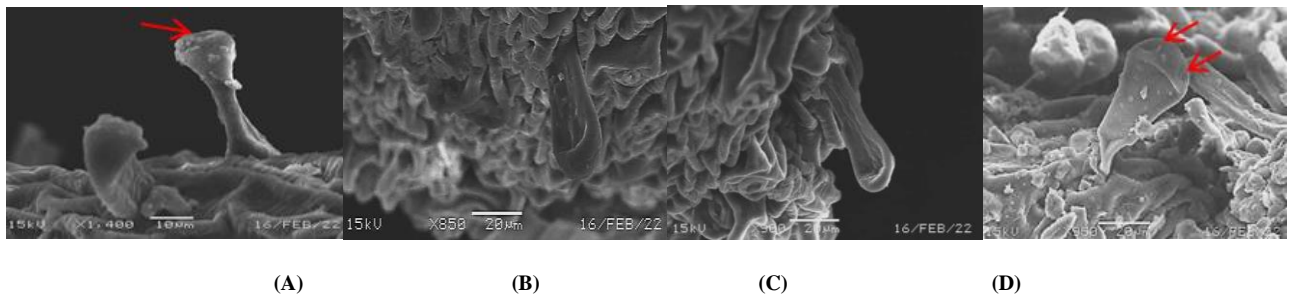


Figure (11): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F. schwinfurthii*. The red arrow indicates the apertures in the secretory cell.

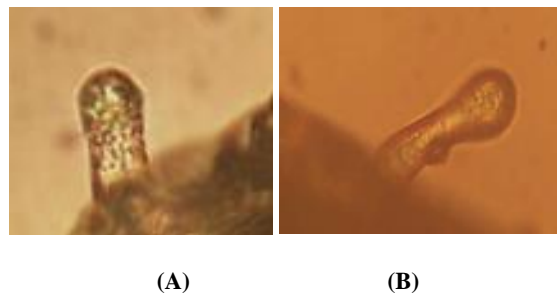


Figure (12): Light microscope (LM40X) micrographs showing the glandular trichomes of *F. schwinfurthii* at (40×) magnification.

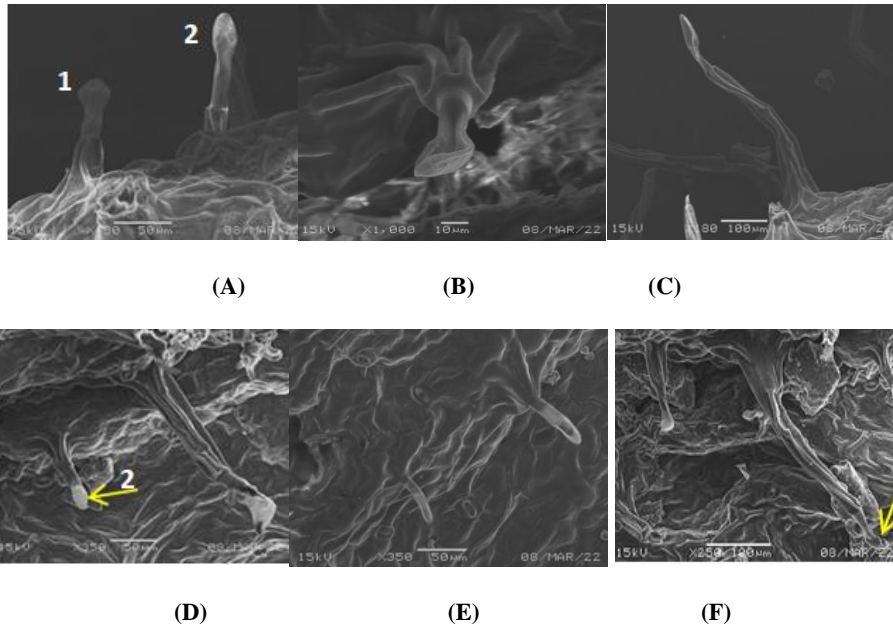


Figure (13): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F. boveana*. The yellow arrow indicates the cupule. (1) Pyriform secretory cell. (2) Clavate secretory cell.

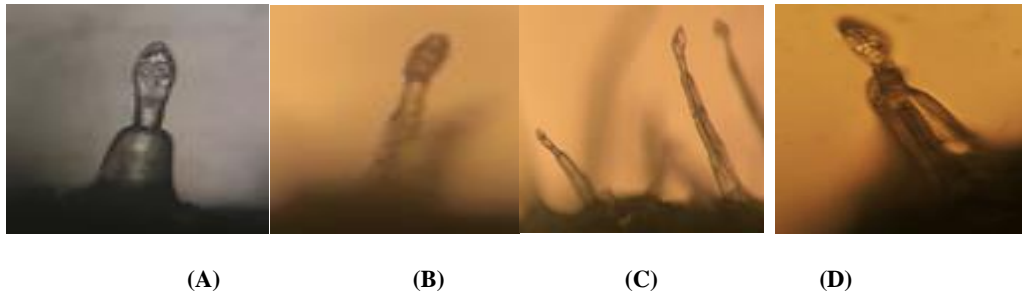


Figure (14): Light microscope (LM40X) micrographs showing the glandular trichomes of *F. boveana* at (40x) magnification.

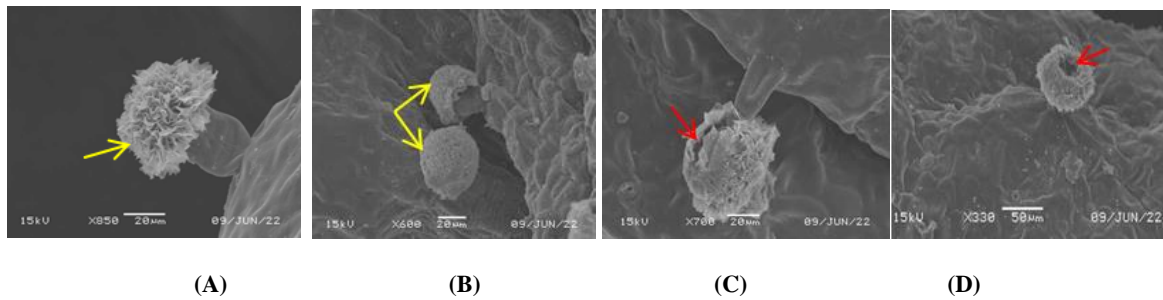


Figure (15): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F. tristis*. The red arrow indicates the apertures in the secretory cell, yellow arrow indicates the cupule.

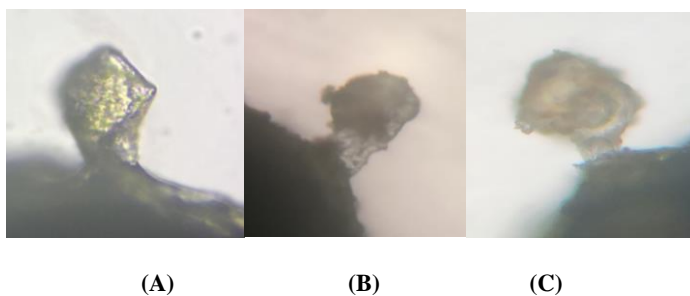


Figure (16): Light microscope (LM40X) micrographs showing the glandular trichomes of *F. tristis* at (40×) magnification.

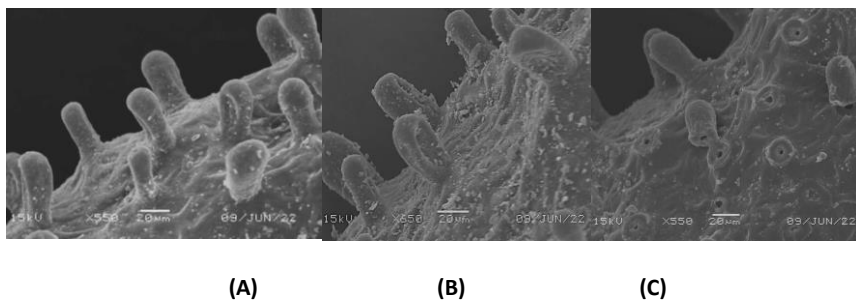


Figure (17): Scanning electron microscope (SEM) micrographs showing the glandular trichomes of *F. ovalifolia*.

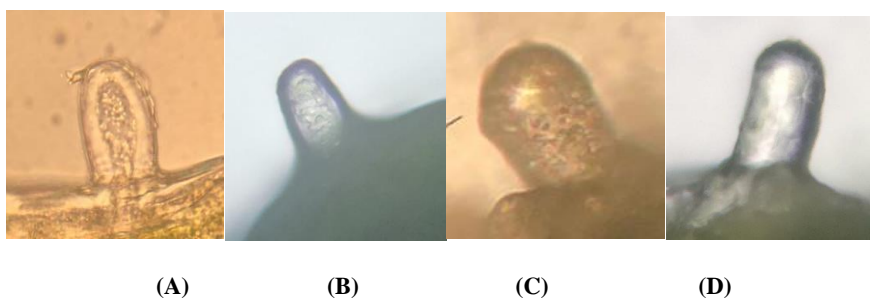
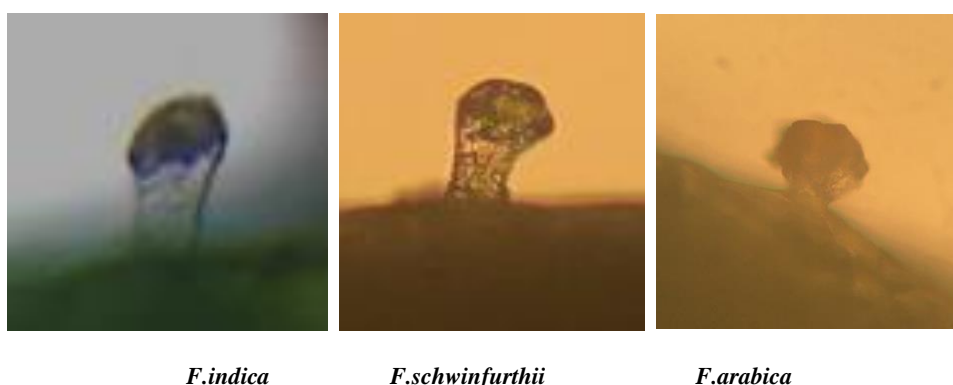


Figure (18): Light microscope (LM40X) micrographs showing the glandular trichomes of *F. ovalifolia* at (40×) magnification.



F.indica

F.schwinfurthii

F.arabica

Figure (19): Light microscope (LM40X) micrographs at (40×) magnification showing the concentration of substance secretory at the top of secretory cells.

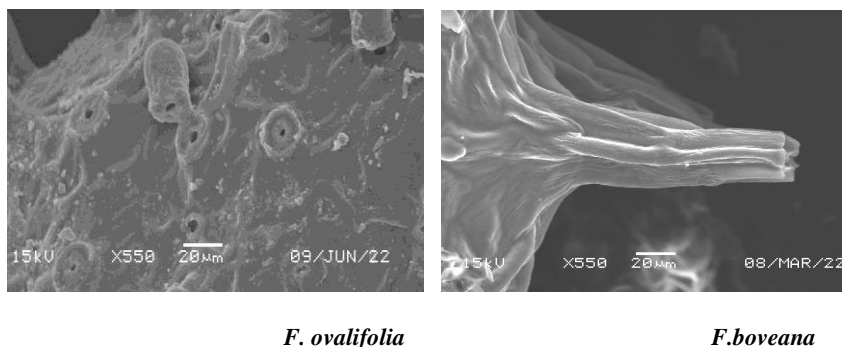


Figure (20): Scanning electron microscope (SEM) micrographs showing a transverse section of the glandular trichomes.

Discussion

The study of the epidermis of plant leaves is a great importance in enriching the phylogeny and taxonomy (Taia, 2005). The microscopic characteristics of foliar trichomes played an important role in plant taxonomic systems (Hardin, 1979). In our study, the Indumentums of the upper and lower epidermis of the studied species leaves were contained different types of trichomes that vary in density and texture, which agreed with description (Chaudhary, 2001).

All trichomes in the studied species were glandular, which agreed with some studies, such as (Fahn and Shimony, 1996; Abdel Khalik and Hassan, 2012; Moawed and Ibrahim, 2016), while differed with (Gabr, et. al., 2012; Beier, 2005) which mentioned that some species had glandular and non-glandular trichomes. This might be to the examination of trichomes for vegetative parts other than leaves such as stems or stipules spines. Also, the secretory cells in the glandular trichomes of some herbaceous samples are difficult to distinguish due to the method of drying and storage. The multiple differences between the trichomes attributed to environmental, genetic and hormones factors that affect the growth and development of the trichomes (Xiao, et.al, 2017).

The present study results indicated that some trichomes were sessile and others had stalks, which incongruent with some studies like (Abdel Khalik and Hassan, 2012; Gabr, et. al., 2012) who published that all trichoms had stalks, while agreed about *F. boveana* that had long stalk with multiseriate and multicellular. Fahn and Shimony (1996) explained that the difference in length was due to the continued division and elongation of the initial stalk cell. The differences in the numbers of stalk cells were explained by Beier (2005) that the mature glandular trichome contains a multicellular stalk, while the immature glandular trichome contains a unicellular stalk. The secretory cells were characterized by variety shapes with cupule at the top of some secretory cells, which agreed with a number of studies that were conducted on some similar species (Fahn and Shimony, 1996; Abdel Khalik and Hassan, 2012).

Some micrographs showed accumulation of secretory substance at the top of secretory cell resembling the cupule, and sometimes it was densely secreted on the out surface of the secretory cell by channels which secrete the secretory material outward through the apertures of the secretory cells, which corresponded with the explanation in study (Fahn and Shimony, 1996). This sticky substance causes adhesion the soil with plant and creates a thick layer that reduces the absorption of sunlight and protects the plant from herbivores and insects. With regard to the neck cell of the studied species, it was distinguished by a line separating between it and the secretory cell but some trichomes didn't had, which attributed to lack the apoplastic barrier between the cells in *Fagonia* trichomes, according of study (Fahn and Shimony, 1996).

Fahn and Shimony, (1996) reported that the glandular trichomes in *Fagonia* had special anatomical characteristics differed from other xerophytes. Besides the lack of apoplastic barrier, the formation way of the glandular trichomes was distinctive and unique, where a separation occurs between epidermal cells and the mesophyll cells in the leaves, which led to the formation of sub-epidermal chambers, thus providing a long path for transfer the water between cells and reduce the rate of transpiration.

Acknowledgements

The authors grateful to King Saud University, Riyadh, Saudi Arabia for providing research facilities.

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