

ANATOMICAL ASPECTS OF STEM GALLS INDUCED BY RHOPALOMYIA SPP. ON THEIR HOST PLANTS

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ABSTRACT

Seriphidium herba-album and *Tanacetum sinaicum* are important medicinal plants belonging to family Asteraceae; covering large areas within St. Katherine protectorate wadies and mountains. These plants are suffering from number of biotic stresses including plant galls (tumors) induced by the gall midge *Rhopalomyia* spp.. Permanent slides of galled and non-galled tissues were made in order to assess the anatomical induced effects in the host plants. Induction of a nutritive tissue surrounded by a storage tissue was recorded in both plants. A newly developed vascular tissue connecting gall tissues with the stem was formed in both plants. Formation of neo-vascularization within the gall tissue was a key feature within *Tanacetum sinaicum* galls. Induction of hairy non-glandular protective tissue was characteristics to *Seriphidium herba-album* galls. Despite sharing common anatomical features, galls induced in both plants were morphologically different. It was suggested that galls of both plants were induced by different *Rhopalomyia* species. More studies are needed to accurately identify the gall inducer species.

Key words: Plant galls; St. Katherine Protectorate; *Tanacetum sinaicum*; *Seriphidium herba-album*; *Rhopalomyia* spp.; Plant histology

INTRODUCTION

St. Katherine protectorate is one of the richest spots of floral diversity in the Middle East up to date. Nearly 1261 plant species have been recorded in Sinai; 472 species of which were recorded only in St. Katherine protectorate (Boulos, 1999; Fayed and Shaltout, 2004). Abd El-Wahab (2004) recorded

about 323 plant species in St. Katherine Protectorate, 128 species of which were medicinal. These 128 medicinal species belong to 40 taxonomic families and included 12 endemic species. Labiatea was the most represented family, followed by Asteraceae and Leguminosae.

Flora of St. Katherine Protectorate is suffering from number of biotic stresses mainly animal grazing (Semida, 2006) and gall-inducing insects (El-Akkad and Zalat, 2000; Zalat *et al.*, 2000). Gall-inducing insects can suppress plant defenses (Tooker and Hanks, 2004; Allison and Schultz, 2005), consume plant resources, change patterns of plant biomass accumulation, alter photosynthetic rates (Arriola and Isaias, 2015) and affect height and number of shoots produced per plant (Hinz and Müller-Schärer, 2000)

According to Elzen (1983), galls can be defined as “structures formed as a result of abnormal growth activates of plants in response to a gall inducer organism”. Plant galls can also be defined as cells, tissues or organs of abnormal growth formed due to an increase in plant cell volume (hypertrophy) and/or cell number (hyperplasia) in response to feeding and/or other stimuli by a foreign organism (Raman *et al.*, 2005). Galls are akin to tumors in animals (Luz *et al.*, 2014). Most galls are caused by nematodes; insects and mites, while a very small percentage can be caused by bacteria, fungi and viruses (Mani, 2013).

Galling insects are a type of herbivores (endophytic herbivory) that, in order to complete their life cycle, obligatorily induce galls on the host plant in order to gain food and protection (Stone and Schönrogge, 2003; Carneiro *et al.*, 2009). Galling insects reflect a parasitic relationship, in which the gall

inducer alters the host plant resources to be more easily accessed or consumed (Stone and Schönrogge, 2003). Insects that manipulate host plant development showed strategies that remodel plant cells content and structure (Giron *et al.*, 2016). Gall induction involved hijacking of plant cellular machinery and development resulting in creating completely new plant structures with features and functions of a novel organ (Shorthouse *et al.*, 2005).

Rhopalomyia is one of the largest genera of Cecidomyiidae (gall midges), with over 250 species worldwide (Gagné and Jaschhof, 2004). With very few exceptions, species of this genus were restricted to plants of the family Asteraceae, on which they induce mostly complex galls in roots, stems, buds, leaves or flower heads (Dorchin *et al.*, 2009). The gall midges *Rhopalomyia* spp. (Diptera: Cecidomyiidae) were recorded by (Kamel, 2017) as the most abundant gall-inducing species in St. Katherine protectorate. The authors reported that the gall-inducing insect *Rhopalomyia* spp. was associated with two important medicinal plants belonging to family Asteraceae namely, *Seriphidium herba-album* and *Tanacetum sinaicum*. Accordingly, the present study aimed at assessing the anatomical induced effects of the gall midges *Rhopalomyia* spp. on the host plants *T. sinaicum* and *S. herba-album*.

MATERIALS AND METHODS

Study area: Southern Sinai is a semi-arid desert ecosystem, characterized by an ecological uniqueness due to its diversity in geologic structures, landforms and climate that promoted diverse vegetation (Zahran and Willis,

2009), and a variation in soil properties (Abd El Wahab *et al.*, 2006). In 1996, the Egyptian Environmental Affairs Agency (EEAA), declared up to 40% of Southern Sinai as protected areas centered upon the town of St. Katherine (1,600 m above sea level). From the mountain of St. Katherine (2,641 meters above sea level m. a. s. l.; the highest point in Egypt and marking the watershed of the peninsula) wadi systems drain eastwards towards the Gulf of Aqaba, and westwards towards the Gulf of Suez. St. Katherine protected area is situated in the southern part of Sinai between 33°57' to 34°00' South and 28°26' to 28°34' East (Ayyad *et al.*, 2000).

Study localities: The chosen localities were found within the high mountain region that was declared as “The world heritage site” in 2002 (Kamel *et al.*, 2012). Samples of galled and non-galled stems of *S. herba-album* and *T. sinaicum* were collected in May 2017 from Wadi- El Arbaain, Farsh- El Hemar, and Farsh- Shoab, St. Katherine protectorate, Southern Sinai Table (1).

Table (1): Studied localities within St. Katherine Protectorate

NO.	Studied sites	Longitude (North)	Latitude (East)	Altitude (m. a. s. l)
1	Wadi- El Arbaain	28.55269	33.94931	1619
2	Farsh- Shoaby	28.5527	33.9668	2013
3	Farsh- El Hemar	28.54647	33.96775	2030

Plant materials:

Seriphidium herba-album (Family: Asteraceae): *Seriphidium herba-album* (Asso) Sojak (white wormwood, armoise herbe blanche) (syn. *Artemisia herba-alba*) is a medicinal shrub with strong aromatic that grows from 20 to 50 cm tall, covered with fine glandular hairs which give it a grayish aspect; its

flowers are naked, hermaphrodite, yellow, and grow in stalkless, nodding flower heads, 2 to 5 flowers per head (Boulos, 2002). *Rhopalomyia* sp. gall midges induce large, densely white pubescent galls on stems of *S. herba-album*. Galls were with several chambers, situated on stem sides and one larva developed in each gall (Kamel *et al.*, 2012; Skuhravá *et al.*, 2014).

Tanacetum sinaicum (Family: Asteraceae): *Tanacetum sinaicum* (Fresen.) Delile ex Bremer & Humphries [Syn. *Chrysanthemum sinaicum* (Delile ex DC.) Nab elek, *Pyrethrum santolinoides* DC., *Santolina sinaica* Fresen. and *Tanacetum santolinoides* (DC.) Feinbrun & Fertig] is an aromatic tomentose-canescens medicinal shrub of 20-50 cm tall; stems are many from the woody base, erect, leafy, slender and becoming stiff, leaves are oblong–elliptic in outline (Boulos, 2002). The gall midge *Rhopalomyia* sp. induce open cup shape galls on leaves and stems of *T. sinaicum*, each gall was composed of a single chamber in which a single larva developed (Kamel *et al.*, 2012).

Anatomical analysis: Collected non-galled stem and whole galls containing larvae were immediately fixed in an FAA solution (5% [v/v] formaldehyde, 50% [v/v] ethanol, and 5% [v/v] acetic acid. Permanent slides were dehydrated by passage through a graded series of tert-butyl alcohol, embedded in paraffin, sectioned, deparaffinized and stained by safranin (O'Brien and McCully 1981). Anatomical sections of non-galled stems and galls (at region of connection with the stems) were performed. Slides were examined under a light microscope and photographed.

RESULTS

Observations of the galls tissues: Galls induced by *Rhopalomyia* spp. midges in the host plant *S. herba-album* stems sides were large, densely white pubescent (Fig.1). Galls were formed of more than one chamber in which one larva was suited (Fig.1). Galls induced by *Rhopalomyia* sp. midges in the host plant *T. sinaicum* stems were open cup-shaped galls composed of a single chamber (Fig.2). Galls collected from both plants contained larvae, but no pupa were recorded in any of the galls collected.



Fig. (1): Galls induced by the gall midge *Rhopalomyia* sp. on stems of *S. herba-album*. A. The outward appearance of the gall. B. An opened gall under microscope with larval chambers (white arrows)



Fig. (2): Galls induced by the gall midge *Rhopalomyia* sp. on stems of *T. sinaicum*.

Anatomical induced effects of *Rhopalomyia* sp. in *S. herba-album* and *T. sinaicum* stems: Non-galled stem sections had collerated vascular bundles surrounded by sclerenchymatic sheath (Fig. 3a). Cross sections of non-galled *S. herba-album* stems presented a typical primary structure of the shoot. Cortex tissue was formed of 1-3 continuous layers of collenchyma cells developed under epidermis. The gall tissues were analyzed by microscopic observations of the gall sections. The stem vascular bundles were connected to the gall tissue by newly developed xylem vessels (Fig. 3b). The cells of the gall chamber where the larva occurred were compact as compared to the empty chamber (Fig. 3c and d).

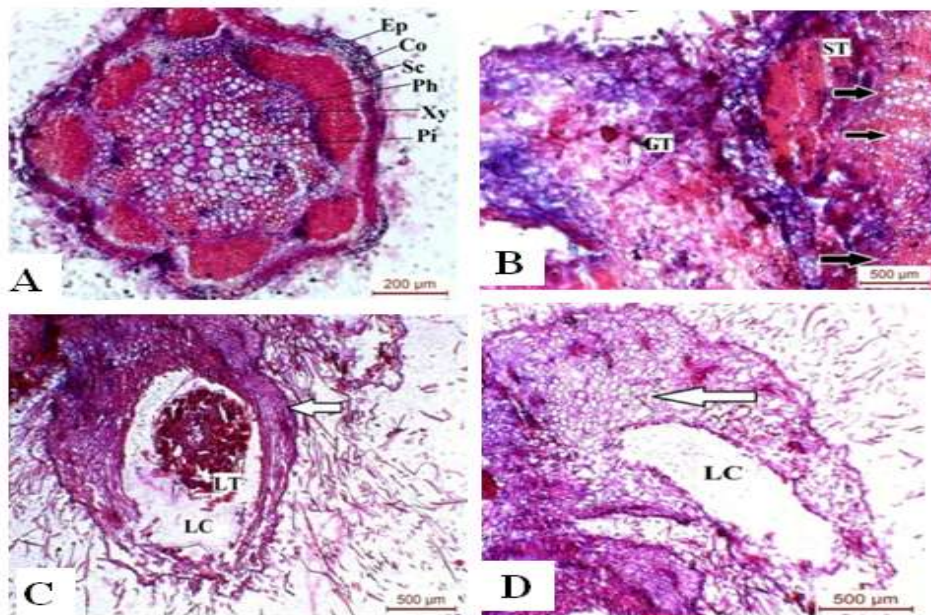


Fig. (3): Cross sections of *S. herba-album* non-galled stems (A) and Cross sections of *S. herba-album* galled stems (B, C and D). B. Vascularization connected to gall tissue (black arrows). C. larval chamber contained larva with compacted cells nutritive tissue (white arrows). D. larval chamber contained no larva with storage nutritive tissue (white arrows). Ep, Epidermis; Co, cortex; Sc, Sclerenchyma; Ph, Phloem; Xy, Xylem; Pi, Pith; ST, Stem tissue; GT, gall tissue; LT, larval tissue; LC, larval chamber.

Non-galled stem sections of *T. sinaicum* present a typical primary structure of the shoot. Vascular bundles of the stems were collerated and surrounded by sclerenchymatic sheath. The cortex tissue was formed of collenchyma tissue below the epidermis. Xylem secondary growth was observed within the stem sections (Fig. 4a).

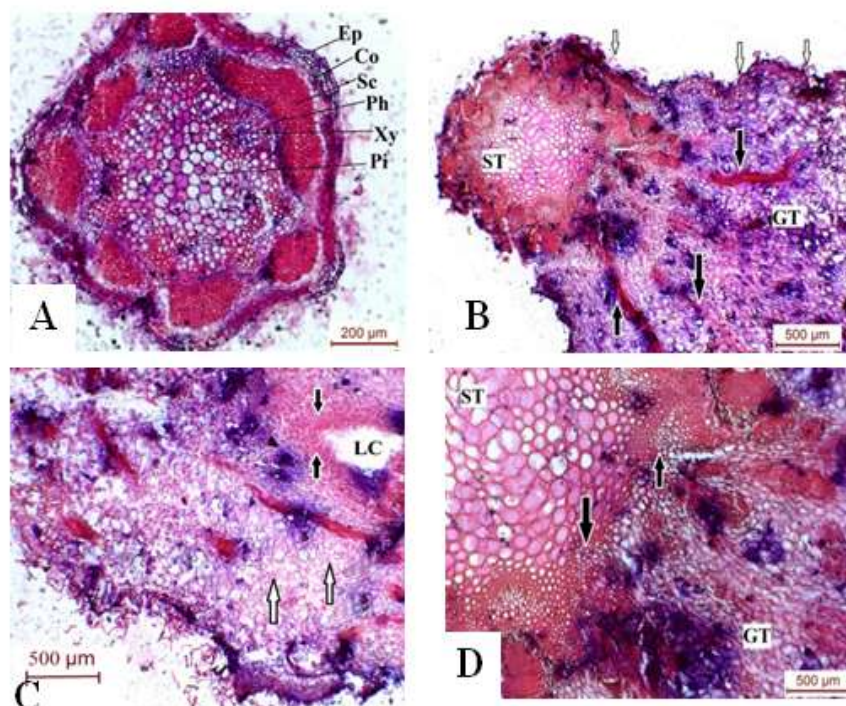


Fig. (4): Cross sections of non-galled *T. sinaicum* stems (A) and Cross sections of galled *T. sinaicum* stems (B, C and D). B. Neo-formations of vascular tissues within gall tissue (Black arrows) and non-glandular trichomes (white arrows) covering plant surface. C. larval chamber with both compact nutritive tissue (black arrow) and storage nutritive tissue (white arrows). D. Vascularization connected to gall tissue (Black arrow). Ep, Epidermis; Co, Cortex; Sc, Sclerenchyma; Ph, Phloem; Mxy, Meta-xylem; Pxy, Proto-xylem; Tr, Trichomes; Pi, Pith; ST, Stem tissue; GT, gall tissue; LT, larval tissue; LC, larval chamber.

DISCUSSION

Rhopalomyia spp. are gall midges that induce galls within two important medicinal plants belonging to family Asteraceae namely, *S. herba-album* and *T. sinaicum*. Both plants cover large areas in St. Katherine Protectorate; each of which had a characteristic shape of galls.

Galls induced in *S. herba-album* stems were large, densely white pubescent galls with several chambers, situated on stem sides and one larva develops in each gall. Whereas, galls induced in *T. sinaicum* were open cup shape galls. Each gall was composed of a single chamber in which a single larva developed. These results were consistent with previous findings of (Kamel *et al.*, 2012; Kamel, 2017).

Cross sections of non-galled stems in both plants were similar. Both stems showed typical primary structure and the presence of collerated vascular bundles surrounded by sclerenchymatic sheath. Cortex tissue was formed of 1-3 continuous layers of collenchyma cells developed under epidermis.

Cross sections of galls of both plants showed the presence of nutritive tissue. This feeding tissue showed unique characteristic shape of cells as compared to other parts of the gall. The tissue was composed of small and compacted cells indicating continuous consumption by larvae. Induction of nutritive tissues was a key feature of many gall-inducing insects (Stone and Schönrogge, 2003; Harris *et al.*, 2006 and Tanaka *et al.*, 2013). Anther storage parenchymal tissue with large and expanded cells was recorded surrounding the nutritive tissues in both plants. Nutritive cells characteristics

vary depending on the family and species of gall-inducer, and can show differentiation between peripheral ‘storage nutritive tissue’ and the inner ‘typical nutritive tissue’ on which the inducer feeds (Bronner, 1992).

Cross sections of galls in both plants showed the presence of extensive newly developed vascular tissues connecting the stem vascularization with the galls tissues. This feature was also recorded in several insect-plant galling systems (Yamaguchi *et al.*, 2012; Tanaka *et al.*, 2013). The formation of new vascular tissue within gall tissue to nourish the galls cells were recorded in *T. sinaicum* stems galls. Neo-formations of vascular tissues within galls were also recorded by (Wool, 2005; Bedetti *et al.*, 2013).

In addition to nutritive and storage tissues, *S. herba-album* galls showed the induction of hairy non-glandular protective tissue covering the whole galls. Additional protective tissues may contain diverse features such as secretory trichomes or hairs or spines or glands in order to protect the inducer from abiotic stress and/or natural enemies, though, not all galls have them and they are found nowhere else on the plant (Harris *et al.*, 2006; Pincebourde and Casas, 2016; Stone and Schönrogge, 2003).

Although morphologically different, galls induced by *Rhopalomyia* spp. in both plants shared number of common features. These included induction of nutritive tissues, induction of storage tissues and formation of newly developed vascular tissue connecting gall tissue to stem tissues. As identified by Kamel, (2017) both plants were infested by *Rhopalomyia tanaceticola*. However, Skuhrová *et al.*, (2014) reported that *S. herba-album* was infested by *Rhopalomyia navasi* while *Tanacetum* sp. was infested by *Rhopalomyia tanaceticola*. Russo, (2007) reported that adult gall midges were

taxonomically indistinguishable. However gall midges could be taxonomically identified based on pupal identification (Jones *et al.*, 1983). In many cases, the appearance and structure of galls were specific to gall-midge species, and each gall was induced on particular plant taxa. Therefore, the galls were regarded as an extended phenotype of respective gall-midge species (Ganaha *et al.*, 2004). Although the majority of gall-inducing insects display a strong level of fidelity to one species of host plant (Ramam *et al.*, 2005), some species were not so restricted and can induce galls on plant species other than their preferred hosts (Wool, 2005). According to Carneiro *et al.*, (2009) gall midges were highly host specific and host plant species gall morphology was reliable indicator of distinct gall midges species. Accurate identification of gall midges species at the DNA level was important even when they induce the same sort of gall on the same host organ and species (Ganaha *et al.*, 2004).

Accordingly, more studies are needed to solve this controversy. Morphological and molecular identification of pupae and adults of *Rhopalomyia* spp. obtained from *Tanacetum sinaicum* and *Seriphidium herba-album* are extremely important.

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الملاحم التشريحية للأورام النباتية المستحثة بأنواع ذبابة العنصر (روبالومايا) على سوق النباتات العائلة لها

[٢]

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المستخلص

يتبع نبات الشيح (سريفيديم هربا البام) ونبات المر (تنكاسيتم سيناكم) العائلة النجمية. وهما نباتان طبيان يغطيان مساحات كبيرة بالوديان والجبال بمحمية سانت كاترين. وتعاني هذه النباتات من عدة ضغوطات إحيائية مثل الأورام النباتية المستحثة بأنواع من الحشرات من جنس روبالومايا. تم عمل قطاعات دائمة لدراسة الملاحم التشريحية لهذه الأورام. وقد أظهرت قطاعات كلا النباتين وجود نسيج مستحث مغذي يحيط به نسيج آخر خازن. كما ظهرت أنسجة وعائية حديثة تربط بين أنسجة الأورام وسوق كلا النباتين. بينما تكونت أنسجة وعائية حديثة داخل أورام نبات المر. وقد أوضحت الأورام المستحثة على نبات الشيح تكون أنسجة وقائية مكونة من شعيرات غير غدبية. وتتشابه الأورام المستحثة على كلا النباتين في عدة ملاحم تشريحية إلا أنهما يختلفان في ملامحهما الظاهرية. وتشير هذه النتائج أن أورام كلا النباتين مستحثة بأنواع مختلفة من حشرة روبالومايا. ويوصي الباحثون بإجراء مزيد من الدراسة لتحديد نوع الحشرة المستحثة لتلك الأورام.