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Light Microscopic Study of the Lichens *Xanthoria Parietina* and *Diploicia Canescens* Infecting Citrus Trees in Egypt

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ABSTRACT:

The lichens *Xanthoria parietina* and *Diploicia canescens* are the most abundant lichens infecting citrus trees at Sharkia Governorate. The freezing microtome was used to study the anatomy characters of both lichens as well as the physical contact between their thallus and the tissues of citrus tree's bark and twigs. The thallus structure of *Xanthoria parietina* exhibited the common organization of foliose lichens, consisting of an upper cortex, algal layer, medulla, lower cortex, and rhizines. No penetration was noticed except the loosely cork layer in the center point of contact.

Conclusion: The anatomy of the lichen *D. canescens* thallus showed a typical crustose lichen layers, i.e., upper cortex, algal layer, and medulla. The thallus was in direct contact with bark tissues due to the absence of lower cortex and rhizines. Superficial penetration, by the lichen hyphae through the cork tissues, was noticed, which caused disruption and separation of the cork layer.

Key words: Anatomy, Lichens, *Xanthoria parietina*, *Diploicia canescens*, Citrus.

INTRODUCTION:

The lichens are a symbiotic association, with unique structure, comprised of two unrelated organisms: a fungus and an alga. When fully integrated, they form a new biological entity with no resemblance to either of the components. Studying lichens did not have enough attention from plant pathologists as one of plant pathogens like fungi, bacteria, and viruses. The earliest idea about the harmful effect of lichens upon fruit trees was concentrated on the action of the lichen thallus in preventing gas exchange through the lenticels, competition for available sunlight and trapping moisture that increase the activity of other pathogenic fungi. From recent studies, the harmful effects of lichens upon higher plants, especially fruit trees, have been proved. This harmful effect could be due to physical or physiological actions.

At the physiological level, some lichens produce acids which strong photosynthetic inhibitors lead to defoliation, and others can produce enzymes that attack polysaccharides of plant cell walls and other pectin degrading enzymes (Legaz *et al.*2004). Bouaid and Vicente (1998) noted the degradation of chlorophyll in response to epiphytic lichen substances, on the other hand Adenikingu and Akinfenwa (1991) reported that suppressive effect of lichens on flowering of cocoa plants. Legaz *et al* (2004) illustrated that the inhibition of leaf growth produced by epiphytic lichens (*parmelia sulcata* and *Xanthoria perietina*) has been related to potential changes of auxin content of buds. Epiphytic lichens can also inhibit the appearance of leaves in *Quercus pyrenacia* (Legaz *et al*. 1988).

AT the physical level, the breakup of the surface layers of tree bark by the growth, development, and penetration of the epiphytic lichens has been documented and reported by many workers. Ascaso *et al*. (1980) reported that the hyphae of the lichen *Evernia prunastri* have been proved to extend to the medulla and penetrate the phloem and even individual xylem vessels of *Quercus pyrenaica*. The hyphal penetration of the same lichen growing on detached branches of *Fagus sylvatica* was carried out through lenticels and injuries as well as through the intact tissues (Estevez *et al*., 1980).

Legaz *et al*. (1988) mentioned that epiphytic lichens can have harmful effects on trees by the extensive penetration of the rhizines through the cork, cortex, and cambium, as far as living wood. These hyphae readily tear off areas of both phloem and xylem, and they disperse either individually or in small groups towards the vessels. In India, it was noticed that both crustaceous and foliaceous lichens infected the twigs and larger branches of sandalwood, cinnamomum, citrus, etc. The affected parts dry up gradually, resulting in dieback and death. Profuse fungal hyphae were observed in twigs infected for 1-2 years, while in freshly infected twigs, hyphal infection was only 2-10% (Nayer and Ramanujam, 1988). The lichen *Evernia prunastri*, growing epiphytically on *Betula pendula* was able to penetrate xylem vessels and its photophore (Monso *et al*.,1993).

In Egypt, many fruit orchards are infected with Lichens. At Sharkia, Governorate the lichens infect fruit trees, i. c. citrus, mango, peach, and apple were recently surveyed and identified (Koriem, 1990). The mycobiont and photobiont of the Lichen *Xanthoria parietina* infecting citrus trees were isolated and cultivated (Koriem, 1991).

The light and electron microscopic study of the lichen *X. Parietina* was carried out by Koriem (1999). He reported that the anatomy of the thallus showed typical foliose lichen layers and the rhizines were spreading over the cork tissues. No penetration was noticed except the loosely cork layer in the center point of contact. The ultrastructure indicated that the interaction between the symbionts ranged between wall to wall contact to intramembranous haustorium.

Abd El Wahab (2019) carried out pathological and histological studies on citrus lichen and their control. She found that anatomical characterization of *Xanthoria parietina* showed a typical foliose lichen layer, while *Diploicia canescens* showed typical crustose lichen structure.

Hegazy (2012) recorded that fruticose lichen *Usnea hirta* at Ain Quraychat in the Siwa Oasis, Western Desert, Egypt. The diversity of lichens in Egypt was later studied by Shawky and Abdi-Azeem (2018).

In Sharkia Governorate the lichen *Xanthoria perietina* is the most abundant lichen followed by *Diploicia canescens* on the trunk and twigs of fruit trees. Therefor the objective of this paper is to study the anatomy of the two lichens as well as the physical contact between its thallus and host tissue to create more attention for studying the lichen of Egypt than they have received.

MATERIALS AND METHODS

1- Collecting Lichen Samples:

Samples of infected young branches or pieces of citrus tree bark and branches were collected from many different orchards in Sharkia Governorate.

The samples were kept in refrigerator until conducting morphological and histological observations.

2- Lichens Identification:

Morphological and histological observations, as well as color test for lichen chemical constituents (Jahns, 1983), (Hale, 1979) were used for lichen identification. Also, the Keys of Hale (1979) and Duncan (1970) beside the color photographs by Jahns (1983) and koriem (1990) were used.

3-Histological observations:

Most results of the published studies are based on free hand cuts made with steel blodes. Since this technique does not allow obtaining slim cut with uniform thickness (Suzan Bissacot *et al*., 2009). Therefore, we used freezing microtome for a lichen specimen cut.

Pieces of thallus and fruiting bodies as well as pieces of citrus tree branches infected with lichens were carefully removed with razor blade and embedded in 20% plane agar then sectioned using freezing microtome. Sections 10-15µ were stained with 0.25% Lactophenol cotton blue, then examined using a light microscope.

The different layers of each lichen thallus as well as the presence of rhizines, sexual and asexual reproductive propagules (apothecia or soredia) were noticed. The contact between the lower surface of both lichen thallus and host tissues as well as the penetration by the thallus hyphae or rhizines through the host tissues were also examined.

RESULTS AND DISCUSSIONS:

1- Lichens Identification**:**

The morphological and histological observations and chemical color test as well as lichen keys indicated that the collected lichen samples from many locations in Sharkia Governorate belonged to two growth forms. Each one represents a separate lichen genus. The foliose growth form represented the genus *Xanthoria*, while the crustose growth form represented the genus *Diploicia*. One species belonging to the genus *Xanthoria* was identified; *Xanthoria parietina* (L.) Th-Fr., while one species belonging to the genus *Diploicia* was identified as *Diploicia Canescens* (Dicks.) Massal.

Ellis (2012) reported that strict substrate specificity is relatively rare in lichens, and that the number of epiphytic species is more or less confined to rather narrow range of substrates in terms of bark pH, waterholding capacity, and substrate hardness.

The observed morphological and histological characters of each identified species were as follows:

***Xanthoria parietina* (L.) Th. Fr.**

Thallus foliose, large, growing in rosettes or irregularly spreading, lobes 1-5 mm broad, horizontal, soft, appressed; tips rounded, crenulate, sometimes slightly ascending (Figs 1&2). Upper surface yellow to orange, lower side whitish, plicate with few scattered rhizines. 100-200 µ thick. Upper cortex paraplectenchymateous, colorless, covered with yellow crystals. Alga, *Trebouxia decolarans* and *T.irregularis* (Ahmadjian, 1993), in continuous layer. Medulla is composed of thick, more or less longitudinally arranged hyphae. Apothecia usually numerous, 1-5 mm across. Disc orange yellow, concave to plain. Thalline margin is somewhat paler than the thallus. Hymenium, 80-90 µ. Apothecium bright yellow granular. Hypothecium colorless ellipsoid, placodiomorph, 12-16 X 5-9 µ.

Reactions: Thallus and apothecia K+ purple; contained parietin.

Hosts: Twigs, young branches, and barks of citrus trees.

Fig 1: *Xanthoria parietina* on citrus branch with orange color.

Fig 2: Close-up of *X. parietina* showing abundant fruiting bodies (apothecia).

Diploicia canescens **(Disks.) Messal.**

Thallus crustose (no lower cortex), orbicular, rather loosely attached to the substrates, lobate effigurate at the circumference, lobes radiote - plicate, continguous, 1-4 mm long; center areolate, soraliate, pale ash - grey somewhat darker towards the periphery, 250-500 µ thick at the center 150-200 µ thick at the lobate margin Figs (3&4). Attached to the substrate by medullary hyphae or by hypothallus. Upper cortex distinctly paraplectenchymatous, colorless; apical cells at the lobate region greyish black. Hyphae of the prothallus dark in color. Apothecia absent, sterile lichen, the abundant scattered soredia (few algal cells surrounded by fungal hyphae) on the upper surface can be used for reproduction (Fig 4).

Reaction: Thallus K+ yellow contained atranorin.

Hosts: Pear, Peach, Citrus, and Mango trees.

Lichen collection, preservation, and identification methods for beginners were studied by Nayaka (2005).

Fig 3: *Diploicia canescens* on citrus bark. Note the scattered crustose thallus.

Fig 4: Close-up of *D. canescens* showing the lobate margin and scattered powdery clumps of soredia on the surface.

2- Anatomical observations:

Anatomical information of most lichen species is scarce and super facial. This is partially due to the technical difficulties found during the preparation of samples and sections suitable for optical microscopy analysis. There is a lack of pictures of anatomical sections as well as detailed by specialized plant anatomists in literature (Suzan *et al*., 2009).

A- Histology of the lichen *X. parietina* thallus:

The lichen *X. parietina* is the most abundant lichen infecting fruit trees in Egypt (koriem 1990). Numerous fruiting bodies, the sexual reproductive structures, were found on the upper surface of the thallus (Fig 2).

The anatomy of lichen *X. parietina* thallus showed a typical foliose lichen Layers i. e, upper cortex, algal layer, medulla, lower cortex and rhizines (Fig.5). The anatomy of *X. parietina* thallus was studied by koriem (1990) and Fatma M Abel El Wahab (2019).

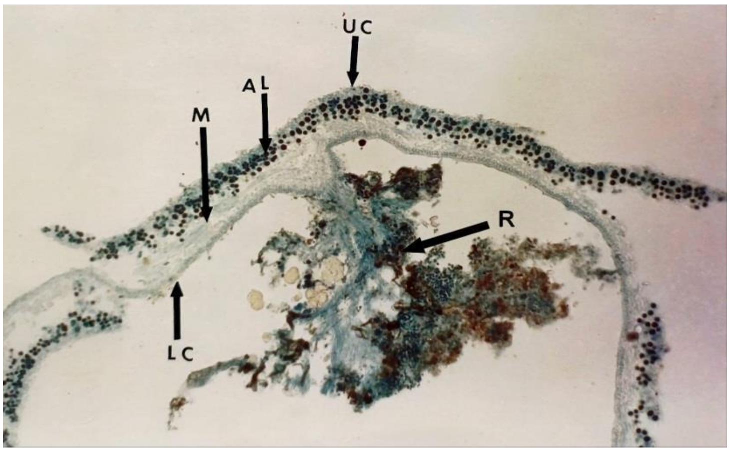

Fig 5: Cross section through the thallus *X.parietina* showing upper cortex (UC) algal layer (AL), medulla (M) lower cortex (LC), and rhizines (R).

Styburiski and Skubata (2023) reported that the increase thickness of the algal layer and entire *physcia adscendens* thallus together with the large photobiont cells may indicate a high level of air pollution with nitrogen.

Amina Daminova *et al*. (2024) mentioned that the highly pigmented thalli of *Xanthoria parietina* possess thicker cell walls and higher water-holding capacities than the pale thalli.

Honegger (1991) reported that the photobiont layer of heteroamorous thalli appears surprisingly uniform regarding photobiont cell numbers per thallus area. However, the photo-biont cell population is not homogenous about cell size and metabolic activity.

Fig (6) represents the anatomy of the fruiting body (apothecium)

showed the hymenium, which contains a thin layer of asci and paraphyses lining the inner surface of the disc (Koriem, 1990 and 1999).

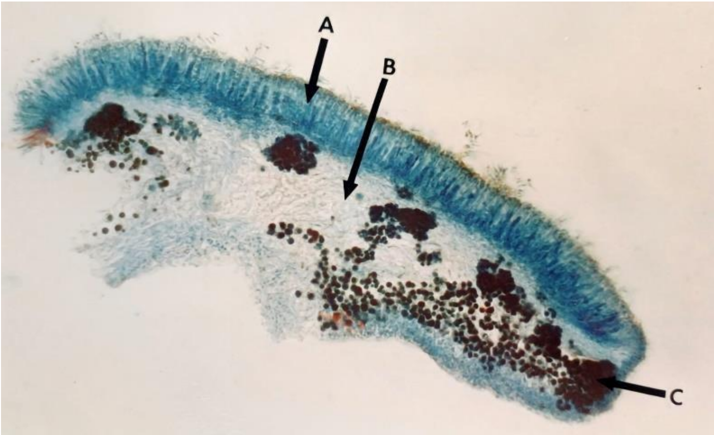

Fig 6: Cross section of apothecium of *X. paretina*. Note the hymenium layer (A) hypothecium (B) and thallus margin (C).

B. Histology of the lichen *D.Canescens* thallus:

The anatomy of the lichen *D. canescens* thallus showed a typical crustose lichen layer. The Lower cortex and rhizines were absent (Fig 7) therefore the medullary hyphae are in direct contact with host tissues.

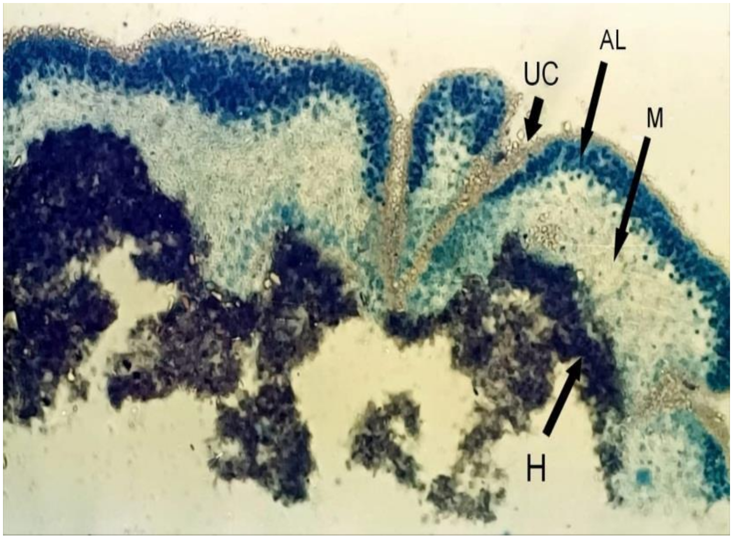

Fig 7: Cross section through the thallus of *D. canescens* showing the upper cortex. (UC), algal layer (AL), medulla (M) and host tissues (H). Note the absence of lower cortex and rhizines.

Diploicia. *Canescens* is a sterile lichen. No fruiting bodies were found, so soridia (some algal cells surrounded by fungal hyphae) in the upper surface can be used for reproduction (Fig 8).

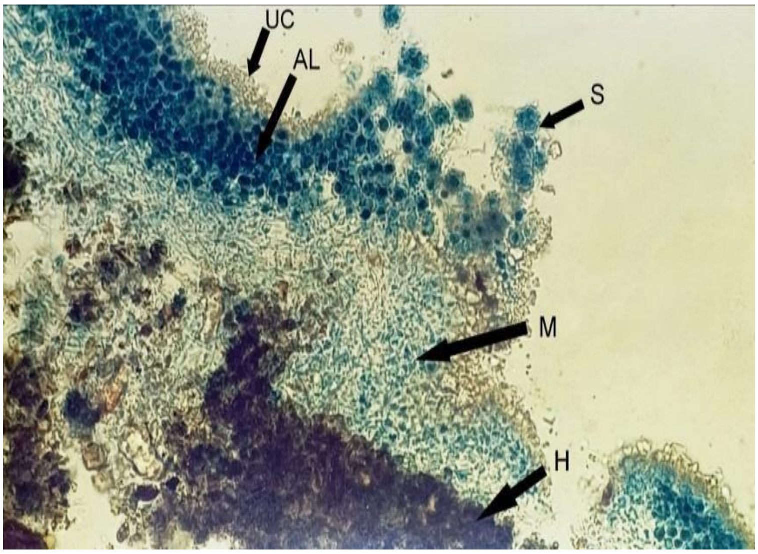

Fig 8: Cross section through the thallus *D. canescens* showing the upper cortex (UC), algal layer (AL), medulla (M), host tissues (H) and the dispersed soredia (S) Note the absence of lower cortex. **C. The contact between *X. parietina* and *D. canescens* thallus and their hosts:**

pieces of citrus tree, bark and twigs, infected with *X. parietina* and *D. canescens* were removed for sectioning to investigate the physical attachment of thallus and its hyphal or rhizinal penetration into bark tissues. Fig (9) shows a heavy bundle of *X. Parietina* rhizine hyphae, which helps the thallus to penetrate on and adhere itself to host tissues. Hawksworth (1988) reported that corticolous lichen-forming fungi can be exceptionally abundant on the twigs, branches, and trunks of trees and shrubs. The extent to which the host tissues are penetrated varies markedly, depending on the growth form of the lichen and the notice of bark tissues themselves.

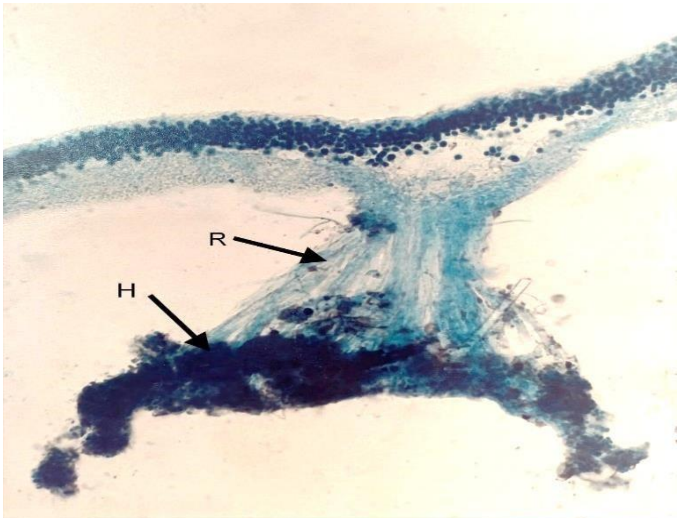

Fig 9: Cross-Section through *X. parietina* thallus, showing a heavy bundle of rhizines hyphae (R) towards the host tissues (H).

Fig (10) shows the attachment of *X.parietina* to the citrus tree bark, with rhizine hyphae forming foot-like structures and showing loosening and disruption of the cork layer. More extensive penetrations were reported by several authors (Legaz *et al*. 1988 and Monso *et al*. 1993).

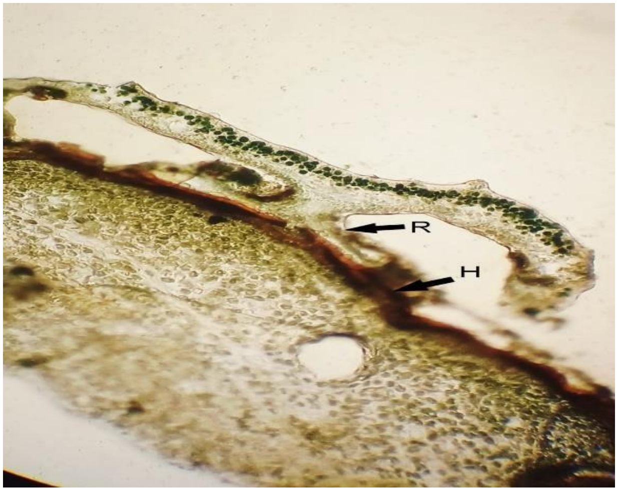

Fig (10) Attachment of *X. parietina* to citrus tree bark showing rhizine hyphae (R) forming foot-like structure. Note the loose and disruption cork layer in the center point of contact arrow. (⬆)

The contact between *D. Canescens* thallus and citrus bark is shown in Fig (11). Since the thallus with no lower cortex, the medulla is in direct contact with bark tissues. The noticed loosely cork layer could result from disruption caused by developing and expanding of lichen hyphae between the cork layers. This little degree of penetration agrees with Hawksworth (1988), who reported that penetration and disruption caused by crustose lichens can be more extensive than those with foliose lichens.

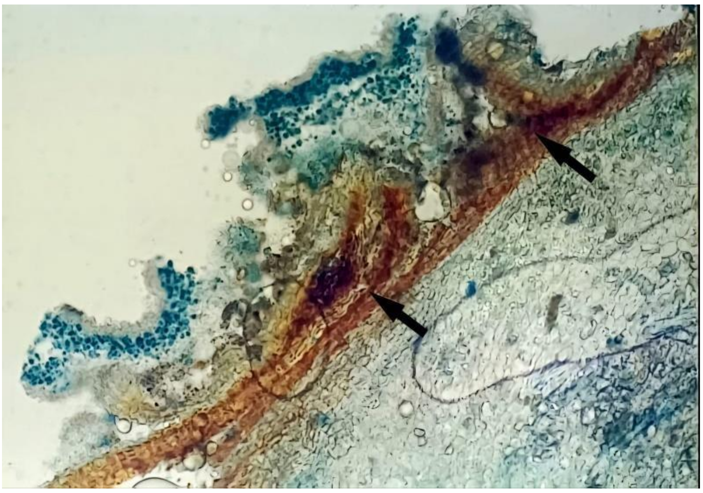

Fig 11: *Diploicia canescens* on citrus tree bark. Note the disruption and separation of cork layer (arrows) by the growth and penetration of medullary hyphae lichen thallus.

Conclusion:

In general, although no penetration was found with *X. parietina* lichen and only little degree of penetration was noticed with *D. canescens*, the damage and harmful effect of these two lichens in citrus trees could be explained. Alternate wetting and drying of the thallus could be arching and finally rupture of the bark tissues, but in addition to that mechanical action it has been postulated that cellulolytic enzymes may be involved which dissolve the membranes between the host cells facilitating their separation (Hawksworth, 1988 and Legaz 2004).

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