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Structural and ultrastructural aspects of rust infection *Cerotelium fici* (Castagne) on *Ficus carica* L. from Purandar, Maharashtra, India

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Abstract

Maharashtra, with Purandar being the highest fig-cultivating region, has gained importance for its high-quality fig fruits. However, cultivation is hindered by a fungal disease, fig rust, caused by *Cerotelium fici*, which has been spreading aggressively over several years. To the best of our knowledge, this is the first morphoanatomical report of *C. fici* on *Ficus carica* from the Purandar region with identification based on uredospore morphology, SEM characteristics and biotrophic infection evidence. The anatomical study reveals the infection pattern of *C. fici* at various stages in the leaves and stems of *F. carica*. The uredospores germinate and enter through stomata, primarily progressing through parenchymatous cells and then entering the vessels to reach the pith. In addition to the scanning electron micrograph of uredospores, to the best of our knowledge, the hyphae of *C. fici* are reported here for the first time with respect to the leaves and stems of the plant, based on a thorough review of available literature. There is a clear distinction between the damage that occurs to parenchymatous cells in the leaves and ray cells in the stem. The localized histochemical detection of phenolic compounds, increased occurrence of rhomboidal crystals, and the development of tyloses in proximity to infection sites are consistent with stress-related structural and biochemical responses to pathogen invasion. The study focuses on the host–pathogen interaction, infection strategies and structural aspects of host *F. carica* and pathogen *C. fici* through microanatomy and Scanning electron microscopy.

Keywords *Ficus carica*, Rust, *Cerotelium fici*, *Phakopsora nishidana*, Uredospores, Defensive strategies

1 Introduction

Rusts are the most complex fungi, with > 7000 species and a 5-spore-stage (Pycniospore, Aeciospore, Urediospore, Teliospore, and Basidiospore) life cycle that alternates between haploid and dikaryotic stages with 2 taxonomically unrelated hosts [1, 2]. Rust fungi are the largest group of obligatory biotrophs, growing, feeding, and reproducing only on the host plant, which makes it difficult to grow in culture. Due to this, rust fungi are among the most devastating plant pathogens, affecting almost every agricultural crop in the



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world [3]. Wheat stem rust (*Puccinia graminis* f. sp. *tritici*) alone results in yearly global losses approximated at \$2.9 billion, whilst soybean rust (*Phakopsora pachyrhizi*) threatens crops over four continents, resulting in yield reductions above 80% during severe infections [4, 5]. Rust infections in fig production can result in output losses of up to 50% in commercial orchards during severe outbreaks, leading to considerable economic repercussions for regions reliant on this GI-certified crop [6]. The threat also impacts the fig tree (*Ficus carica* L.), which is a plant of significant commercial value within the Moraceae family. Figs are the predominant orchard crop in the Purandar and Bhore tehsils of Pune district, with Purandar, Saswad, and Khedshivapur being the main locations [7]. However, fungal diseases are becoming more prevalent, with 94% of farmers reporting widespread infections that lead to crop loss. Roughly eight to nine fungal diseases affect fig trees all over India [8]. Out of those, fig rust caused by *Cerotelium fici* is the most prevalent disease affecting *F. carica* [9]. Our survey in the Purandar region indicates that fig rust caused by *C. fici* is among the most widespread diseases impacting a significant portion of fig cultivations.

Furthermore, the determination of the exact identity of the rust pathogen is indispensable for any region-specific disease management. Molecular and microscopic analyses can help in resolving the prevailing taxonomic confusion between *Cerotelium fici* and *Phakopsora nishidana* and also help elucidate various pathogenic mechanisms [6–14]. Recent reports from South Africa (Boshoff et al., 2022) identified the pathogen as *P. nishidana* through molecular data, whereas a 2024 study from Korea (Ha et al., 2024) confirmed *C. fici* using morphological criteria, underscoring the persistent necessity for integrative taxonomic approaches that incorporate morphological, molecular, and anatomical evidence [14, 15]. Limited research exists on the anatomical studies of infection pathways of the rust fungus in figs, indicating a substantial knowledge gap concerning the host–pathogen relationship. This study sought to elucidate fungal pathogenicity in the host and determine the mechanism of action through histology techniques. An anatomical method was employed to ascertain the mechanism of fungal motility and infection and the degree of cell wall degradation to different wood components. Future studies correlating meteorological factors with rust severity could facilitate predictive modelling, enabling timely fungicidal applications and supporting sustainable cultivation practices for fig growers in affected regions.

2 Materials and methods

2.1 Sample collection and host identification

Leaf and stem samples of *F. carica* displaying rust symptoms were systematically collected from orchards in several villages within Purandar Taluka, including Kalewadi, Jadhavwadi, Udachi Wadi, Guroli, Pargaon Chowfule, Pargaon Memane, and Malhargad, during the summer (March–April) and winter (November–December) periods from 2023 to 2025. The samples were systematically collected from 6–7 commercial fig orchards located in 6–7 different villages within Purandar Taluka throughout six harvest seasons across three years (2023–2025). During each season, 60–70 infected leaf samples were collected, comprising 8–10 leaves per sampling event, from 4–5 highly affected trees in each orchard. Composite samples comprised 4 to 5 leaves and 2 to 3 stems per tree. This specific strategy was intentionally selected to enhance pathogen prevalence for comprehensive microscopic and histochemical examinations. This sampling strategy

focused on optimizing pathogen prevalence for microscopic and histochemical analysis and does not represent a systematic epidemiological survey; consequently, no conclusions regarding disease incidence, prevalence, or spatial distribution within the region should be inferred from these data.

The host was identified as *Ficus carica* L., using local floras and checked against certified specimens and GI certificates; Dr. Vidya Patankar, Department of Botany, PDEA's Waghire College, Saswad, confirmed the identity. A voucher specimen, which includes infected leaves material for fungi identification, has been deposited in Ajrekar Mycological Herbarium of the National Fungal Culture Collection of India (NFCCI) at Agharkar Research Institute in Pune, under the accession number AMH-10805. Additionally, it has been submitted to the Herbarium of the Department of Botany at PDEA's Waghire College in Saswad, Pune, with the accession number PMoFC-1.

2.2 Pathogen identification approach and methodological constraints

Cerotelium fici was identified using standard techniques for obligate biotrophic rust fungi, which are unable to flourish or re-isolated on artificial medium owing to their metabolic dependency on living host tissue [3]. The classical Koch's postulates are inapplicable to this group of pathogens. Therefore, identification was based on (1) uredospore morphology (dimensions, form, surface ornamentation) analyzed through light and scanning electron microscopy; (2) disease symptoms aligning with established descriptions; (3) anatomical evidence of biotrophic infection mechanisms; (4) consistent pathogen-disease correlation across all surveyed locations; and (5) voucher specimen deposition (AMH-10805, PMoFC-1) for expert validation.

2.3 Microanatomy, histochemical localization and scanning electron microscopy (SEM)

Samples were fixed in FAA solution, including 90% ethanol, 5% formalin (37–40% formaldehyde), and 5% glacial acetic acid (90:5:5 v/v), in accordance with Berlyn and Miksche [16]. The prolonged fixation duration of two months served three purposes—complete disintegration of latex that would otherwise impede sectioning, adequate softening of lignified stem tissue, and thorough fixative infiltration into dense vascular tissue. The elevated ethanol content (90%) reduces the extraction of cellular components while preserving structural integrity, in accordance with protocols for latex-bearing and woody plant tissues. Each sample was examined using 10–15 sections per slide to ensure representative observation of pathogens. Leaf sections were prepared at a thickness of 25 µm, whereas stem sections were prepared at a thickness of 10–15 µm. Non-infected, asymptomatic tissues from rust-free trees within the same orchards were processed simultaneously alongside infected samples as negative controls, following identical fixation, dehydration, embedding, sectioning and staining protocols to ensure direct comparability of results. Dehydration was subsequently performed using a graded series of tertiary butyl alcohol, as stated by Johansen [16, 17]. Samples were embedded in paraffin wax, and transverse sections were prepared with a Leica RMz125 6TS rotary microtome. Histological staining utilized safranin-fast green for differential tissue visualization and cotton blue-lactophenol for the delineation of fungal structures.

Histochemical analyses were performed on fresh transverse sections to identify specific biomolecules: alkaloids were visualized using Wagner's Reagent, lipids with Sudan B, phenolic compounds with ferric chloride, and lignin through phloroglucinol-HCl

staining, following the protocols established by Eklavya, O'Brien and McCully, Jensen and Harborne [18–21]. These qualitative staining techniques reveal the existence and location of these chemicals in the host tissue; however, they do not yield quantitative concentration values. Microphotographs of these histological and biochemical data were frequently captured in order to better understand the chemical aspects of host–pathogen interactions. Microphotography was performed using a Magnus CH20I LED microscope paired with a Magcam DC 5 camera, facilitating high-resolution image acquisition. Morphometric measurements were conducted on 25 structures per category (including 25 uredospores and 25 uredinia), sourced from 10 unique samples (roughly 2–3 measurements per sample), to guarantee representative sampling across independent specimens. All data is presented as the mean $\pm$ standard deviation (SD) with range values. Measurements were conducted using MagVision image analysis software, which was calibrated with a stage micrometre (10 μ m divisions) before the measurements were taken. Fungal spores obtained from leaf surfaces were stained with safranin before microscopic analysis. The dimensions of uredinia were measured from 25 representative pustules (13 from the lower epidermis and 12 from the upper epidermis) utilizing Magvision software, with statistical analysis.

Dried leaf samples from a minimum of three independent infected specimens were subjected to sputter-coating with gold for ultrastructural analyses and examined using a JEOL JSM-6360A Scanning Electron Microscope (SEM) at magnifications from 100 $\times$ to 10,000 $\times$, facilitating detailed studies of surface morphology. The images shown in results (Fig. 4) are representative micrographs chosen from various fields of view among independently generated samples, facilitating detailed explorations of surface morphology.

3 Results

3.1 Field disease assessment

Throughout each collection period, physical disease surveys were implemented in all sampled orchards. According to visual field surveys, the proportion of trees in the surveyed orchards that were visibly affected varied between 72 and 94%. Infection levels were more pronounced during the winter months (November–December), which coincided with periods of high humidity and low temperature. Disease severity was visually assessed, with around 25–50% leaf area affected during initial seasonal infections (considered as moderate) and surpassing 75% at the infection's peak (considered as severe); these are approximate field observations and were not established using formal quantitative disease assessment scales. The majority of infected trees, approximately 38–45%, were located in plantations with a history of mechanical pruning wounds. Stem infections were also observed in these trees. Interviews with farmers in heavily impacted orchards during peak infection periods suggested perceived yield losses of 30–50%; these are informal estimates and lack validation from controlled agronomic evaluations [6]. These field observations establish a contextual framework for the morphological and histochemical characterisation discussed in subsequent sections.

3.2 Morphology of rust

The fungal pathogen *C. fici* induced characteristic morphological alterations in infected *F. carica* plants, causing leaf deformation, necrotic lesions, and pronounced curling of

the leaves that could reflect disruption of normal cell development and growth processes, which causes low-grade fruit formation (Fig. 1a, b, d). The abaxial leaf surfaces were ornamented with distinct powdery reddish-orange pustules containing abundant urediniospores. The dense urediniospore masses within pustules that serve as the primary inoculum source are observed (Fig. 1c). These spores are known to disperse through wind and precipitation, facilitating rapid dispersion across orchards. In severely infected orchards, pustular formations were additionally observed on the adaxial leaf surfaces (Fig. 2e), indicating advanced disease progression in leaves. The stem infestation was documented in *F. carica* (Fig. 5 and 6) with disease establishment facilitated by mechanical wounding from pruning activities, followed by favourable environmental conditions formed by unseasonal precipitation events. The pathogen exploited susceptible host entry points during periods of high humidity and moisture availability, leading to spore germination and subsequent colonisation. Severe infections significantly compromise photosynthesis capacity and nutrient translocation, leading to stunted growth and reduced plant vigour. Consequently, infected plants exhibited diminished branch and leaf development, ultimately resulting in decreased fruit production and substantial yield losses.

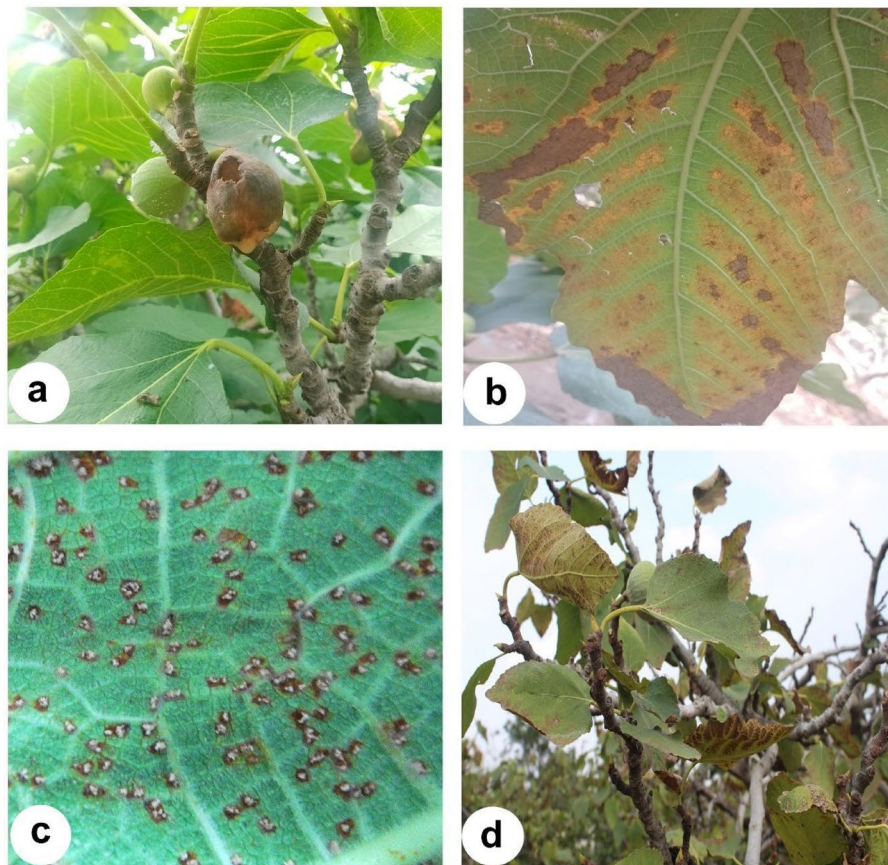


Fig. 1 Photographs of the fig orchards infected leaves and fruits with rust **a** Decaying of Fig fruits due to unseasonal rains **b** and **d** Rust infection on leaves **c** Microscopic view of rust pustules observed on the abaxial surface of leaves

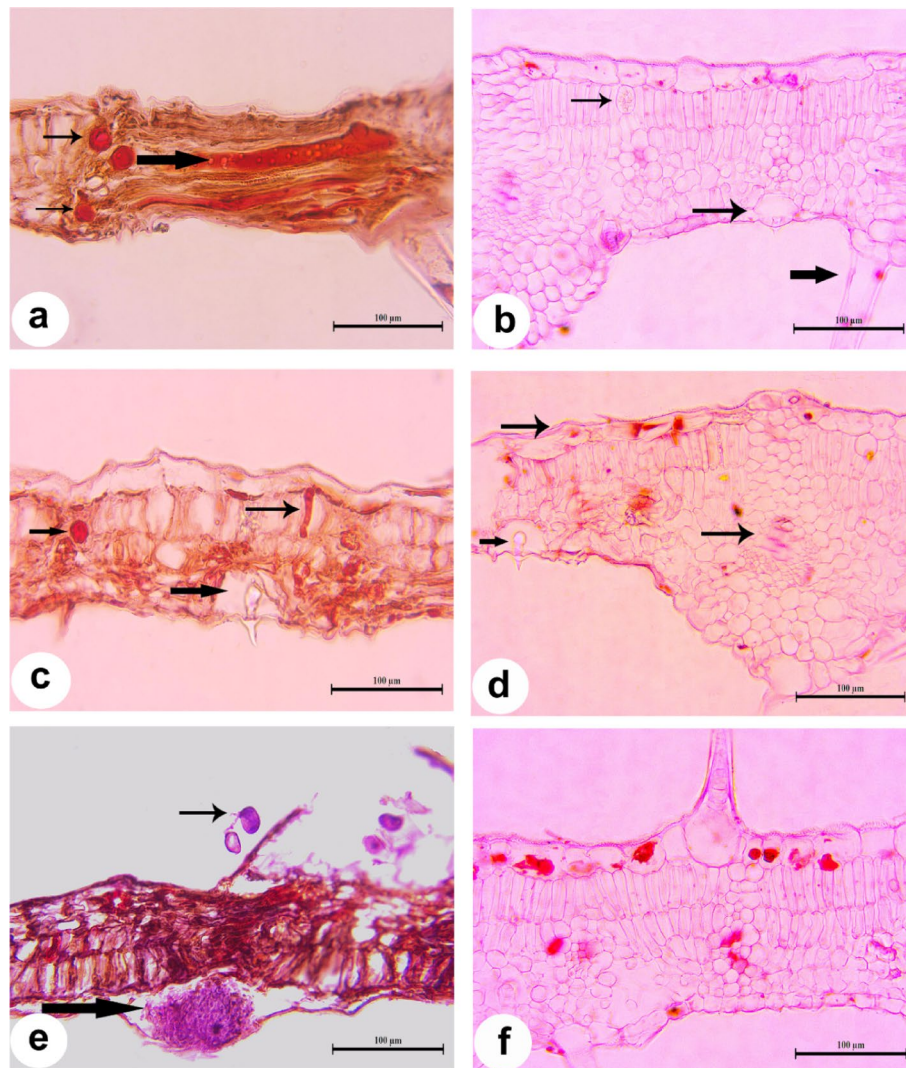


Fig. 2 **a, c, e** T.S. of leaf of *Ficus carica* showing rust infection inside the cells. **a** and **c** Haustoria-like structure formed in the mesophyll (pointed arrows) with mycelia progressing (broad arrow) **e** Uredosorus on the lower and upper sides of the leaf filled with uredospores **b, d, f** T.S. of a normal (healthy) leaf of *F. carica* with intact cellular structures like stone cells, cystoliths, cystolith hair, idioblasts and trichomes

3.3 Leaf anatomy: healthy baseline and infection-induced changes

3.3.1 Normal healthy leaf structure

The healthy leaves of *F. carica* in transverse section displayed the typical dorsiventrally organized structure (Fig. 2b, d, f). The upper epidermis consisted of a single layer characterized by the presence of unicellular, non-glandular trichomes (Fig. 2b), followed by 1–2 hypodermal layers. The palisade tissue of the mesophyll region normally comprised two layers of elongated parenchymatous cells containing calcium carbonate deposits (Fig. 2f). The spongy mesophyll typically consisted of 2–3 layers of loosely arranged parenchyma cells. The single-layered lower epidermis was characterized by the presence of cystoliths (Fig. 2c, f). Healthy tissue exhibited intact cellular structures including stone cells, cystoliths, cystolith hairs, idioblasts, and trichomes (Fig. 2b, d, f), with no evidence of uredinia, hyphal structures, or tyloses formation.

3.3.2 Fungal invasion-induced anatomical alterations

Infection of *C. fici* inside the leaf was characterized by the presence of intracellular fungal hyphae and specialized feeding structures (haustoria) within the leaf tissues (Fig. 2a). The formation of uredinia on the upper epidermis (adaxial side) (Fig. 2e) highlighted the severity of rust infection. The palisade tissue, which normally comprised two layers of elongated parenchymatous cells containing calcium carbonate deposits in healthy tissue, exhibited complete structural disruption in infected specimens due to hyphal proliferation and haustorium formation (Fig. 2a). The spongy mesophyll displayed extensive cellular lysis interspersed with phenolic depositions. The single-layered lower epidermis, characterized by the presence of cystoliths in healthy tissue (Fig. 2c, f), was severely compromised in infected leaves.

Heavily infested leaves exhibited numerous rust-coloured uredinia protruding through both the lower epidermis (Fig. 2e, 3a–d) and the upper epidermis (Fig. 2e), with ruptured

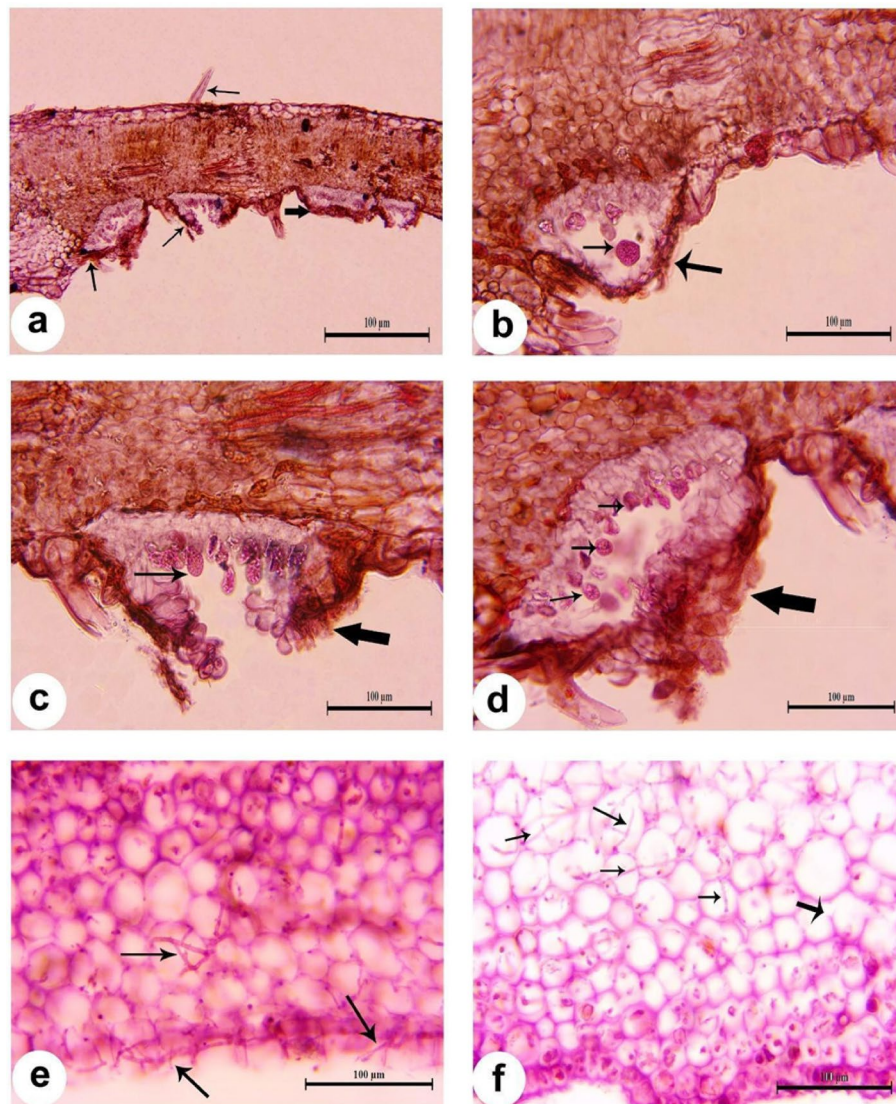


Fig. 3 a–d T.S. of *Ficus carica* leaf with uredinia on the lower side of leaf Uredospores observed inside the uredosorus (pointed arrow) c Mature uredosorus ruptured, marking the release of uredospores (thick arrow) e and f T.S. of midrib of *F. carica* with thin mycelia of *Cerotelium fici* progressing from epidermis to the cortex (pointed arrows)

uredinia causing extensive damage to surrounding epidermal cells (Fig. 3a and c). Cells adjacent to infection sites accumulated tannins, appearing reddish-brown in colour. The morphometric study of uredinia ($n=25$) indicated substantial size variations. Lower epidermis uredinia measured 183–185 μm (mean $184.3 \pm 1.2 \mu\text{m}$, $n=13$), while upper epidermis uredinia measured 30–32 μm (mean $31.4 \pm 0.8 \mu\text{m}$, $n=12$). Lower surface uredinia were substantially larger, indicating increased disease severity on the abaxial surface (Fig. 3b–d).

Some leaf transverse sections demonstrated completely ruptured uredinia with crushed lower epidermal cells (Fig. 3a and c). The fungal mycelia were observed proliferating inside the parenchymatous cells along the lower side of the leaf (Fig. 3e and f). The mycelia formed a thin, reticulated network lining the lower epidermis. Hyphal structures were located within vascular bundles, particularly in vessels (Fig. 3e), indicating the progression of the pathogen through the vascular tissue. Tannin depositions were mainly observed in mesophyll cells. Uredospores scraped from the leaf surface were circular to elliptical measuring a mean length of $24.5 \pm 0.8 \mu\text{m}$ (range: 23.74–25.34 μm , $n=25$) and a mean width of $18.3 \pm 0.6 \mu\text{m}$ ($n=25$). These anatomical alterations are systematically compared with healthy tissue in Table 1.

Histochemical staining revealed the presence of alkaloids, phenolic compounds (Fig. 7a, b), lipids (Fig. 7c, d), and lignin (Fig. 7e, f). Histochemical staining signals for phenols and alkaloids were more intense in infected tissue compared to healthy controls, indicating greater staining intensity rather than quantitatively determined concentration differences. The infection path is typical of rust fungi, shown by entry into the leaf through stomata, formation of an appressorium, and proliferation of hyphae to form haustoria for establishing contact with plant cells for nutrition. The host's production of phenolic compounds, which block the cells, highlighted the infection pattern.

3.4 Scanning electron microscopy

SEM study of an infected leaf of *F. carica* revealed the characteristic circular to ellipsoid echinulate uredospores of *C. fici* with spines (Fig. 4a and b). The lower side of the leaf shows stomatal openings and numerous hairs densely covering the leaf surface (Fig. 4c). The infected portion of the leaf displayed a ruptured uredosorus structure that was filled with numerous urediniospores (Fig. 4d).

3.5 Stem anatomy: healthy baseline and infection-induced changes

3.5.1 Normal healthy stem structure

The transverse section of a young *F. carica* stem is composed of a single-layered epidermis covered by a waxy cuticle, followed by 2–3 hypodermal layers. For protection, young stems have hairs, while in older stems, the epidermis is converted into periderm. The cortex consists of intact, organized parenchymatous cells. The endodermis and pericycle remain indistinct. The pericycle follows vascular bundles that are organized in a ring, including both collateral and open types. Xylem vessels in healthy stems exhibit uniform wall thickness, clear unobstructed lumina, and no tyloses formation. Uniseriate ray cells are organized and not colonized. The pith consists of loosely arranged parenchyma cells with intact cellular structure. Rhomboidal crystals are present at baseline levels.

Table 1 Comparative anatomical features of healthy and infected *Ficus carica* leaf and stem tissues. Features absent in healthy tissue are marked as "Absent"; those present in infected tissue but absent in healthy tissue are highlighted. Figure references indicate where changes are visible

Sr. No	Anatomical feature	Healthy tissue	Infected tissue
<i>Leaf</i>			
1	Epidermis	Single-layered, intact with trichomes (Fig. 2b)	Ruptured by uredinia (183–185 µm lower; 30–32 µm upper) (Fig. 2e, 3a-d)
2	Palisade mesophyll	Two organized layers, elongated cells with calcium carbonate deposits (Fig. 2f)	Complete structural disruption, hyphal proliferation, haustorium formation (Fig. 2a)
3	Spongy mesophyll	2–3 layers, loosely arranged parenchyma (Fig. 2d)	Extensive cellular lysis with phenolic depositions (Fig. 2a, c)
4	Cellular structures	Intact stone cells, cystoliths, idioblasts, trichomes (Fig. 2b, d, f)	Damaged, tannin accumulation in cells adjacent to infection (Fig. 2a, c)
5	Hyphal structures	Absent	Present as thin reticulated networks along lower epidermis (Fig. 3e, f)
6	Hauatoria	Absent	Present in mesophyll, intracellular (Fig. 2a, c)
7	Vascular bundles	Clear vessel lumina (Fig. 2)	Mycelia progressing through vessels (Fig. 3e)
8	Phenolic compounds	Baseline levels (Fig. 2)	Mesophyll cells adjacent to infection sites reveal stronger staining, indicating phenolic accumulation (Fig. 2a, c)
<i>Stem</i>			
1	Epidermis/cortex	Intact epidermis with cuticle, 2–3 hypodermal layers	Deformed, degraded cells with wall degradation (Fig. 5a)
2	Cortical parenchyma	Intact, organized	Extensive cellular disruption, phenolic depositions (Fig. 5a, c)
3	Vessel walls	Uniform thickness, intact	Thinning observed near infection sites (Fig. 5c, e)
4	Vessel lumina	Clear, unobstructed	Hyphal colonization, mycelia present (Fig. 5b, d, f)
5	Tyloses	Absent	Present in vessels, partial to complete occlusion (Fig. 6a-f)
6	Ray cells	Uniseriate, organized	Colonized by hyphae, serve as infection pathway (Fig. 6e)
7	Pith	Loosely arranged parenchyma, intact	Invaded by fungal mycelia (Fig. 5a, c)
8	Lateral vessels	Normal arrangement	Formation of lateral vessels (compartmentalization) (Fig. 6c, d)

3.5.2 Fungal infection-induced anatomical alterations

The fungal invasion of *C. fici* had progressed in the stem, resulting in the deformation and degradation of epidermal and cortical cells, primarily targeting parenchymatous cells by degrading their cell walls (Fig. 5a). The transverse section (T.S.) of the stem revealed extensive cellular disruption within the cortex accompanied by phenolic compound depositions. The fungi travelled through ray cells to the parenchymatous pith (Fig. 5a and c).

In heavily infected stems, vessel wall thinning was observed in proximity of infection sites, along with the formation of lateral vessels as a potential compartmentalization response to fungal invasion (Fig. 5c, e and 6c, d). Fungal mycelia penetrated wood cells via vessel elements, while uniseriate ray cells (Fig. 6e) promoted access to other xylem cell types and parenchymatous pith. The thin, continuous running mycelia were observed in the lumen of vessels and parenchymatous cells just beside the vessels (Fig. 5d). The progressing mycelia from the vessels of leaves to the stem were observed prominently (Fig. 5b, d, f). Pith consisting of loosely arranged parenchyma cells was also invaded by the fungal mycelia.

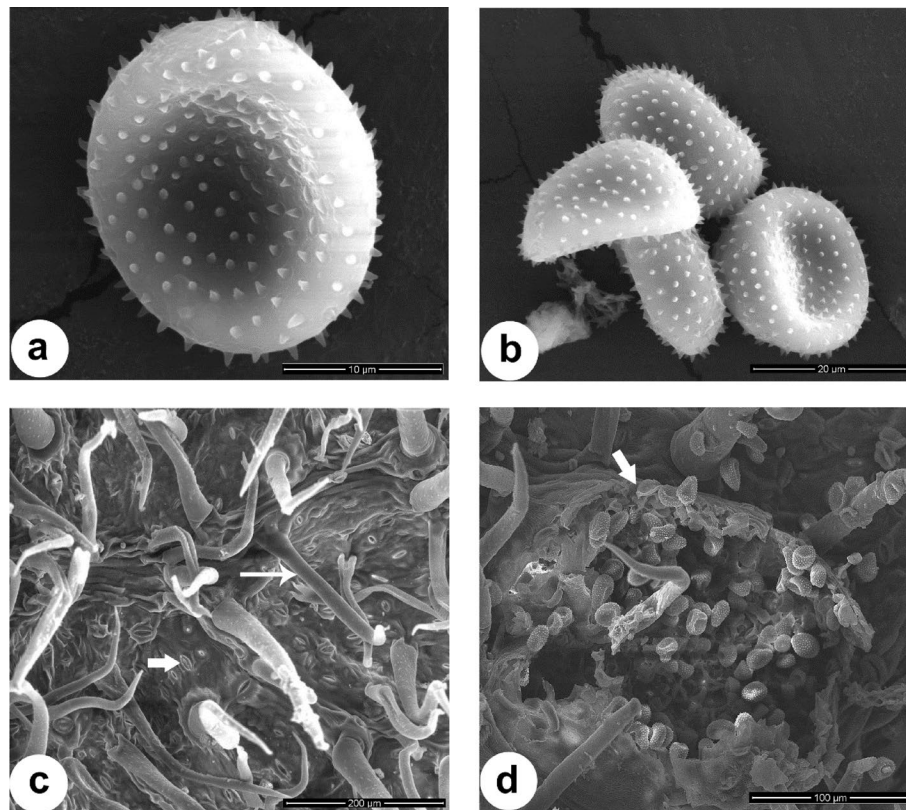


Fig. 4 **a** and **b** Scanning electron microscopy photos of uredospores of *Cerotelium fici* on the leaf surface of *Ficus carica* with the echinulate surface **c** Scanning electron microscopy photo of the lower side of *F. carica* leaf showing trichomes and stomata. **d** Ruptured pustule with numerous urediniospores

Tyloses were observed in xylem vessels (Fig. 6a, b, f) in proximity to fungal hyphae (Fig. 5f), which is consistent with a role as physical barriers potentially limiting the spread of hyphae from ray cells to xylem elements. The formation of an increased number of rhomboidal crystals is distinguishable in infected plants. The radial longitudinal sections showed *C. fici* hyphae in the vessel lumina and formation of tyloses by parenchyma cells protruding in the vessel lumina to prevent further spread of infection. Ray cells revealed a stronger histochemical signal for phenolic chemicals in regions adjacent to infection sites, an observation consistent with stress-induced metabolic alterations. Stem infection-induced changes compared with the healthy baseline are summarized in Table 1.

4 Discussion

4.1 Pathogen identification and comparison with literature

The current study supports the identification of the pathogen as *C. fici* based on morphoanatomical criteria, consistent with the reports of its widespread occurrence in New Zealand, Montenegro, Mexico, India (Tamil Nadu), and Korea [10–14]. According to the study of Ali et al. in 2016 [22], unlike many other rust species, *C. fici* has no known pycniospores, aeciospores, teliospores, or basidiospores, which is consistent with our morphological observations [23].

The obligatory biotrophic nature of *C. fici* restricts the application of standard Koch's postulates, as rust fungi lack vital metabolic pathways for autonomous development and

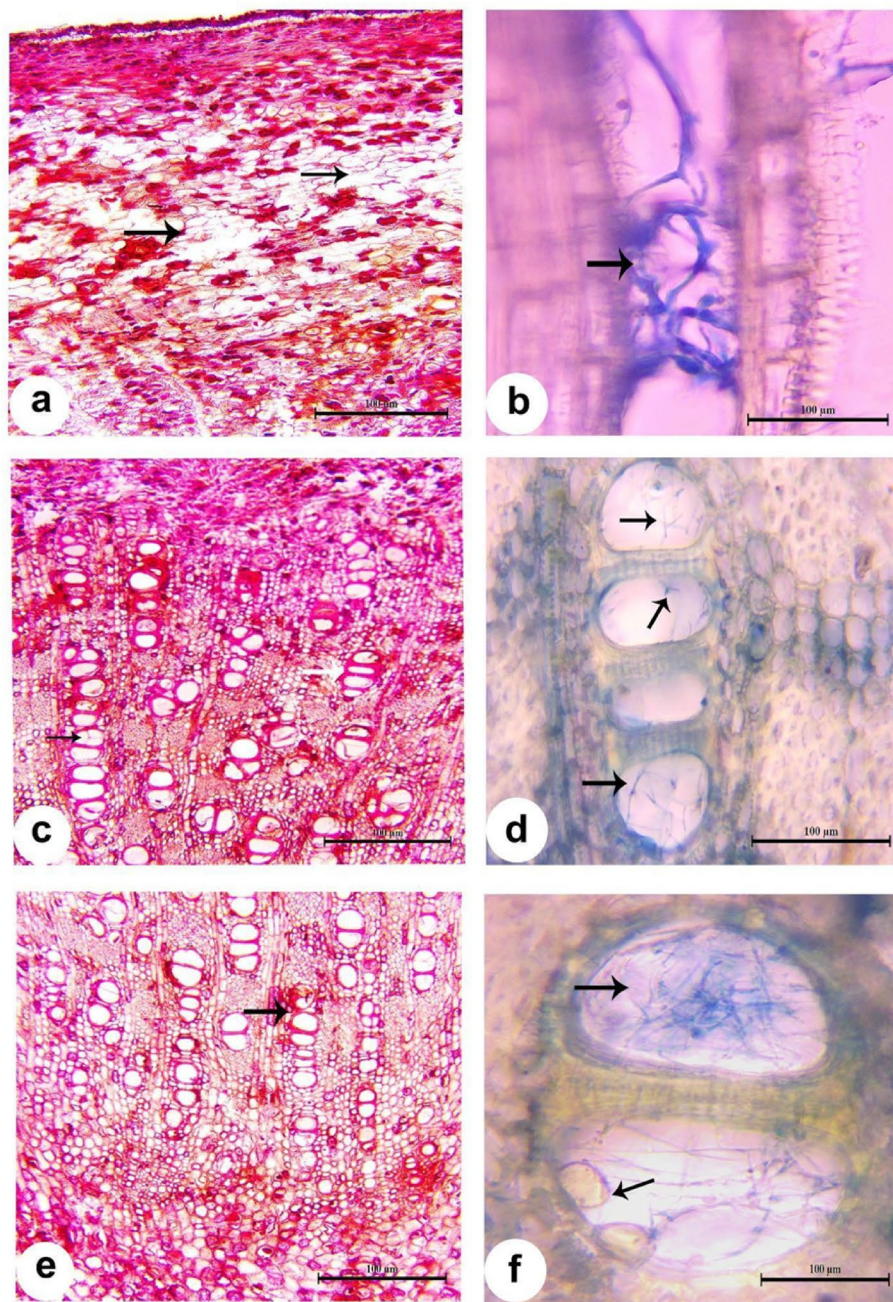


Fig. 5 **a, c, and e** T.S. of *Ficus carica* stem showing cortical cell damage and vessel deformations due to infection of *Cerotelium fici* **b** T.S. of *F. carica* stem with mycelia (blue color) of *C. fici* traveling through the vessels **d** and **f** thin mycelia of *C. fici* (blue colored) observed crowding the vessels in T.S. of *F. carica* stem **f** tyloses in the lower vessel defending against the pathogen attack

cannot be grown axenically [24]. This study utilized revised criteria for obligate pathogens: morphological characterization aligning with published descriptions of *C. fici* [10–14], SEM validation of diagnostic echinulate uredospores (Fig. 4), anatomical evidence of a biotrophic infection strategy (haustoria, stomatal entry, vascular colonization), and consistent field association. This approach is consistent with all currently published *C. fici* reports worldwide and is commonly used in the study of obligate biotrophic rusts for pathogen identification in the absence of axenic culture. Molecular phylogenetic

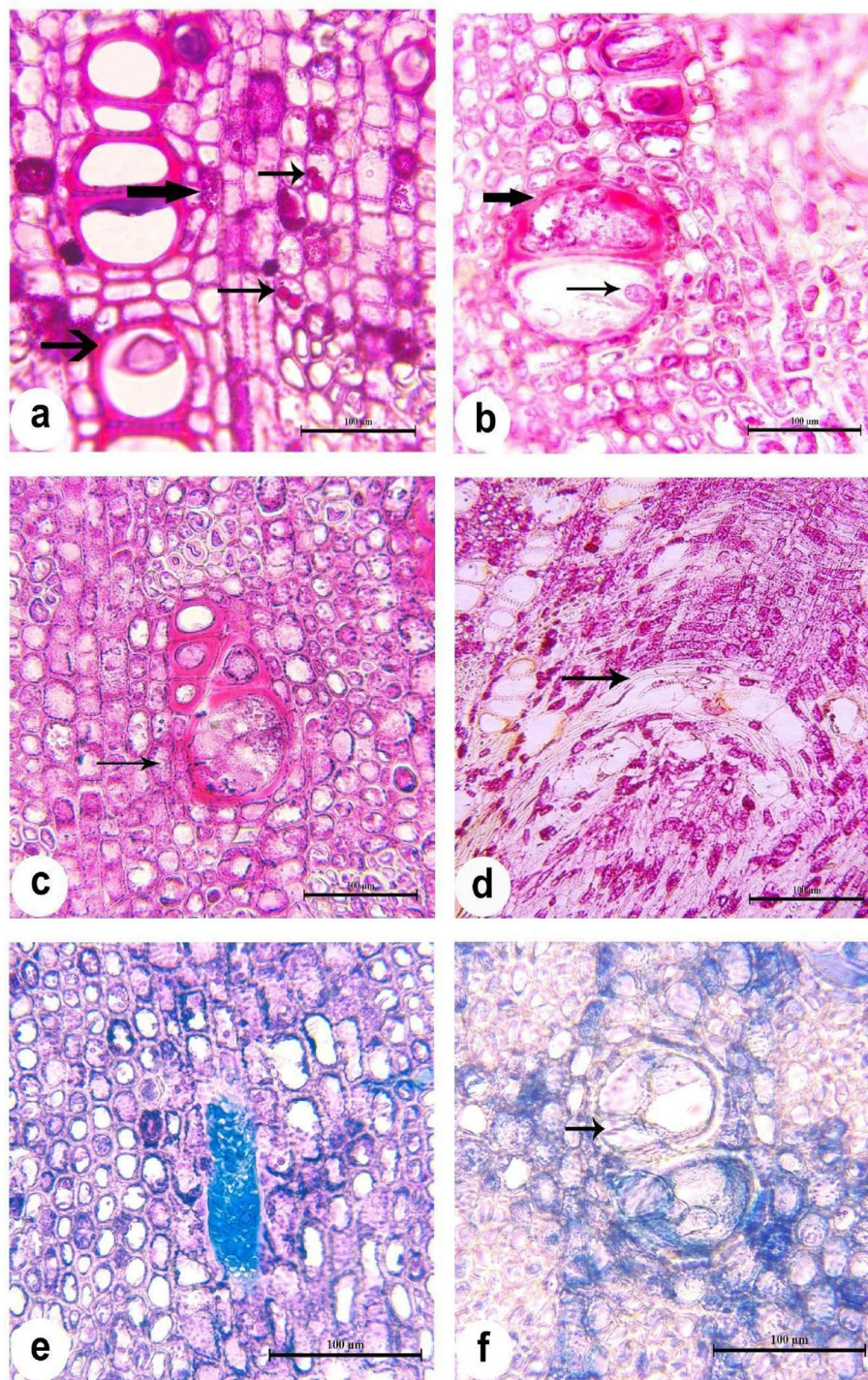


Fig. 6 a–c T.S. of *Ficus carica* stem with just forming tyloses in vessels (pointed arrow), various phenolic depositions observed in the surrounding vessel cells c completely occluded vessel due to infection d compartmentalisation of vessels and an activated stress response against pathogen attack were observed e ray cell completely colonised by *Cerotelium fici* (blue color) f tyloses occluding the vessels completely

confirmation through ITS and LSU sequencing is required to definitively resolve the taxonomic boundaries within the *C. fici*-*P. nishidana* species complex, as morphological identification alone is insufficient to distinguish these taxa with certainty, especially given molecular evidence from South Africa reclassifying the same pathogen as *P. nishidana* [15].

The size of uredospores (mean: $24.5 \pm 0.8 \mu\text{m}$) closely aligns with the latest published findings: 20–26 μm in Korea (Ha et al., 2024) and 22–28 μm in South Africa [14, 15]. In contrast to previous literature that mentioned circular, ellipsoid, or polygonal round-shaped spores, our SEM observations and recent reports consistently document echinulate surface ornamentation as a diagnostic feature [13, 14]. Our morphological observations—uredospore, echinulate ornamentation, and uredinia structure—align with the updated CABI diagnostic description (2022) and the Korean study, offering substantial morphological evidence for the identification of *C. fici* [6, 11, 14, 23].

The taxonomic differentiation between *C. fici* and *P. nishidana* is still challenging. Boshoff et al. (2022) identified South African fig rust as *P. nishidana* using ITS and LSU sequencing, observing morphological resemblances to *C. fici*, whereas Ha et al. confirmed *C. fici* in Korea through uredospore morphology and life cycle characteristics [14]. The molecular data from South Africa underscore the imperative of DNA sequencing in future study to definitively define species boundaries and uncover potential hidden diversity within the *C. fici*-*P. nishidana* complex.

4.2 Foliar infection patterns and uredinia development

The reddish-brown pustules that are the characteristic of chlorophyll degradation and premature leaf abscission were observed on both adaxial and abaxial leaf surfaces, correlating with previous phytopathological descriptions [6]. Anatomical studies revealed that uredospores penetrate leaf tissues through stomata, corroborating the ultrastructural findings of Lorrain et al. [3]. The rapid germination of uredospores within 14 h under optimal conditions corresponds with the stomatal entry strategy of rust fungi, wherein germ tubes are guided toward stomata and develop appressoria to facilitate entry, consistent with minimal tissue damage—essential for sustaining biotrophic infection.

The intracellular hyphal proliferation resulted in the disruption of epidermal and mesophyll cells (Fig. 2a, c, e), aligning with the data presented by Farr and Rossman [25]. The transverse section of fig leaves revealed pustules on both the adaxial and abaxial epidermis (Fig. 3a–d), with larger pustules measuring approximately 185.66 μm on the lower epidermis and smaller pustules measuring around 32.81 μm on the upper epidermis (Fig. 2e). The significantly larger uredinia on the lower epidermis ($184.3 \pm 1.2 \mu\text{m}$ vs $31.4 \pm 0.8 \mu\text{m}$ on the upper epidermis) suggest greater spore production capacity on abaxial surfaces, which could reflect more favourable conditions for spore development and dispersal from that surface. To the best of our knowledge, the hyphae of *C. fici* are being reported for the first time in the midrib region of the lower side of the leaf covering and integrating with the lower epidermis (Fig. 3e, f), based on a review of accessible published literature.

The haustoria-like structures found in palisade cells (Fig. 2a, c) correspond to the biotrophic interface described for rust fungi, where haustoria invaginate the host plasma membrane and function as specialised sites for nutrient uptake and delivery of effector proteins that modulate host defences [3]. This biotrophic infection strategy,

characterized by haustoria formation and minimal tissue necrosis, aligns with recent genomic and ultrastructural studies (2022–2023) demonstrating that rust fungi deploy specialized effector proteins to suppress host immune responses while maintaining host cell viability, a hallmark distinguishing biotrophs from necrotrophic pathogens [3, 13]. Quantitative morphometric differences (Table 1) and anatomical alterations documented in this study reveal the comprehensive host–pathogen interaction patterns.

4.3 Stem infection and vascular tissue colonization

The colonization of fig stems by *C. fici* uredospores occurs through natural openings, including lenticels and wounds, and via vascular connections from leaves, revealing the fungal potential for systemic infection. The thinning of vessel walls (Fig. 5c, e), consistent with Mengiste, suggests activity of fungal cell wall–degrading enzymes such as cellulases and pectinases that locally weaken secondary walls to facilitate hyphal movement between xylem elements while still preserving host viability [26]. Such enzymes could target pectin and cellulose components of the cell wall, which may contribute to tissue invasion, though direct enzymatic evidence was not obtained in this study. The observation of hyphae in both transverse and longitudinal vessel sections reveals the severity of infection in the stem (Fig. 5b, d, f).

Tyloses-induced vessel obstruction (Fig. 6a, b, f), completely absent in healthy stems, fits within the well-known compartmentalisation model of xylem defence, where parenchyma cells intrude into vessel lumina to restrict pathogen movement and isolate infected segments of the vascular system [27, 28]. The gradual degradation of cells from the epidermis to the cortex, observed in areas of damaged cells (Fig. 5a, c, e), corresponds with the anatomical pathway of infection that progresses through ray cells to the pith (Fig. 5e and 6e).

4.4 Host defence responses and anatomical modifications

Histochemical staining revealed more intense signals of phenolic compounds (Fig. 7a, b), alkaloids, lipids (Fig. 7c, d) and lignin (Fig. 7e, f) in tissues adjacent to infection sites, indicating localized distribution patterns in infected tissues relative to healthy ones. Although these chemicals are frequently associated with plant defence in the literature [3, 26], our qualitative histochemistry identifies presence and spatial distribution rather than quantitative levels. Confirming their defensive roles requires quantitative methods such as HPLC, spectrophotometry, GC–MS, along with appropriate bioactivity assays. The identified localization patterns align with stress-responsive secondary metabolism and possible compartmentalization techniques to restrict pathogen dissemination, as recorded in other rust–host systems [27]. The development of tyloses is a recognized physical defence mechanism validated by anatomical observation, irrespective of chemical quantification.

The structural modifications collectively align with the mechanistic framework, indicating that *C. fici* may employ haustoria and cell wall degradation to maintain biotrophy, while the host response evidenced by tyloses formation, phenolic deposition, and xylem compartmentalization may signify coordinated structural defence mechanisms, as documented in other rust–host systems.

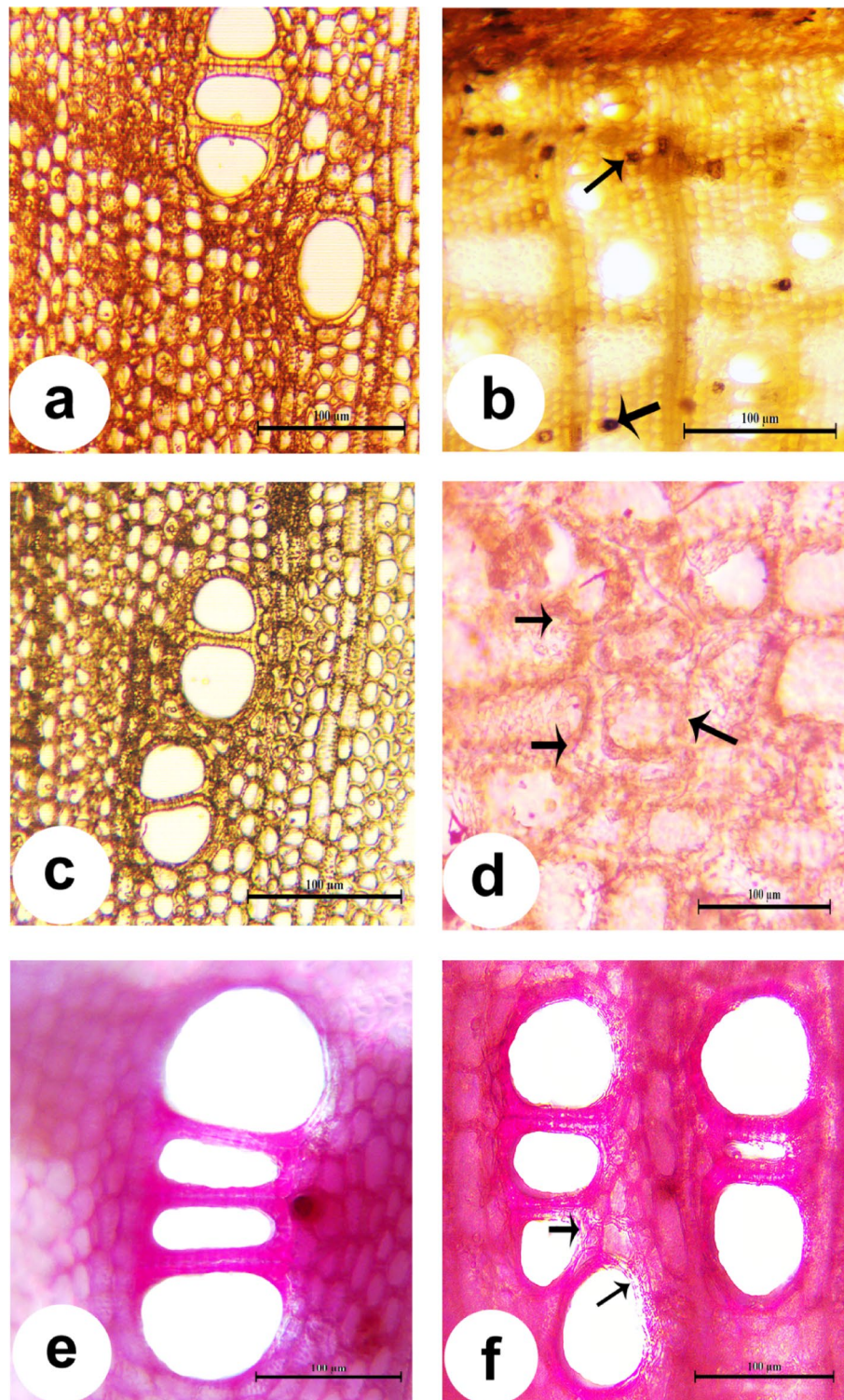


Fig. 7 Transverse sections (T.S.) of *Ficus carica* stem illustrating histochemical localization of phenols, lignin, and lipids in non-infected (a, c & e) and infected tissue (b, d & f). a Phenol distribution in non-infected stem; b phenol accumulation in infected stem-arrows indicate dark brown deposits at sites of fungal colonization; c lipid content in non-infected stem; d lipid distribution in infected stem-arrows indicate orange-red globules consistent with lipid droplet accumulation phenol accumulation in infected stem-arrows indicate dark brown deposits at sites of fungal colonization; e lignin deposition in non-infected stem; f lignin modification in infected stem-arrow indicates vessel wall thinning associated with vascular disruption.

4.5 Implications of disease management

Although field-level epidemiological quantification (lesion density per cm², disease severity indices, % leaf area affected) would enhance these anatomical findings, our study establishes the fundamental understanding of infection mechanisms that facilitate future quantitative evaluations.

5 Conclusion

This systematic morphoanatomical study has provided crucial insights into understanding the host–pathogen interaction between *F. carica* and *C. fici*. The significant anatomical alterations, like destruction of parenchyma cells, mycelial occlusions in xylem vessels, formation of tyloses and ray cells acting as the prime connecting path for rapid infection spread from cortex to pith, compartmentalization in vessel formation. The uredinia and uredospores were observed in the infected stem and leaf, reveal the pathogenic mechanisms of biotrophic fungi. This study contributes to bridging the gaps in host–pathogen interactions of fig and rust, leading to the formation of new strategies in handling the disease. The morphoanatomical observations contribute to the structural understanding of plant-pathogen interactions, especially in the biotrophic systems, and highlight the forthcoming agricultural challenges due to sudden changes in climatic conditions and the need to control them by developing resistant cultivars. Although our morphoanatomical technique offers extensive insights into host–pathogen interactions, the obligate biotrophic characteristic of *C. fici* precluded conventional pathogenicity testing via controlled inoculation experiments. Future research should combine molecular identification techniques (ITS, LSU, and potentially whole-genome sequencing) with field-based epidemiological methods to conclusively determine causation and clarify taxonomic ambiguities between *C. fici* and *P. nishidana* observed in various geographic areas. Furthermore, quantitative chemical analysis (HPLC, GCMS, metabolomics) would unequivocally delineate compound identities, concentrations, and antimicrobial activity to validate the defensive functions of the stress-responsive chemicals identified using histochemistry. Transcriptomic and effector analyses of spontaneously infected tissues may clarify the molecular mechanisms governing the biotrophic interaction strategies identified in this anatomical study.

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Author contributions

V.P. designed the experiment and supervised the research, while also fulfilling the responsibilities of the corresponding author. P.K. conducted the experimental work, encompassing both microscopy and anatomical analysis. V.P. and P.K. collaboratively examined the data and prepared the manuscript text. All authors contributed to the editing and approved the final version of the manuscript.

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Data availability

The data supporting the findings of this study are accessible from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The plant material comprised leaves sourced from cultivated fig trees (*Ficus carica* L.) located in private orchards within Purandar taluka, Pune, Maharashtra, India. No wild or protected species were included in the sampling process. *Ficus carica* is categorised as Least Concern according to the IUCN Red List and is not included in the CITES appendices. All procedures adhered to institutional policies and national guidelines regarding non-protected, cultivated plants. No experiments involved species that are endangered or regulated, and there was no international transport of regulated materials. Prior permission was obtained from orchard owners and landholders before collecting the leaves. No further governmental permits or licenses were necessary, as *Ficus carica* L. is a commonly cultivated, non-protected fruit tree, and the sampling was restricted to leaves. Collection activities were not carried out in protected forest areas, national parks, or wildlife sanctuaries.

Consent for publication

All authors consent to the publication of this manuscript and agree with its contents.

Competing interests

The authors declare no competing interests.

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