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Incidence and ultramicroscopic characterization of *Cerotelium fici* (castagne) rust in *Ficus carica* L.

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Abstract

The aim of the research was to identify the emerging rust disease in fig cultivation. In commercial orchards in the Western Ghats of Tamil Nadu, India, trees infected with rust exhibited reddish-brown angular leaf spots on their upper surfaces while their lower leaf surfaces contained numerous orange to reddish, rust pustules. An intensive survey was conducted to determine the incidence and severity of rust in fig orchards of the different districts of the Western Ghats and found that the incidence was very severe (100 %) in Pannaikadu, Dindigul district. To confirm the pathogen and its infection module, scanning electron microscopy was used to analyze these rust pustules and their anatomy. Based on the microscopy study, the subjected pathogen was identified as *Cerotelium fici,* this is the first detailed report of *C. fici* infection on fig plants in India.

Key words: *Cerotelium fici,* morphology, disease occurrence, fig rust, survey

Introduction

Common fig (*Ficus carica* L.) belongs to the Moraceae family. It is one of the traditional edible fruits grown widely in tropical and sub-tropical regions of India (Patil and Patil, 2011). Other countries producing figs include Turkey, Egypt, Morocco, Spain, Greece, the United States (California), Italy, Brazil, and Japan. The edible fig is native to the Middle East and the Mediterranean. It grows best in subtropical climates with hot, dry summers and mild winters (Tous and Ferguson, 1996). However, this crop suffers great losses due to the incidence of a rust disease caused by *Cerotelium fici* (syn. *Physopella fici*) in the majority of the areas where the crop is cultivated. The widespread occurrence of this disease has been observed in the southeastern United States (Alabama, Florida, Louisiana, Mississippi, North Carolina, South Carolina, Texas and Georgia) (Eaker, 2015) and in Hawaii (Verga and Nelson, 2014). It is also found in Bermuda, the West Indies, Central America, and other tropical regions (Arthur, 1929) as well as in Yugoslavia (Mijuskovic, 1963), New Zealand (McKenzie, 1986), Australia (Simmonds, 1966), Turkey (Huseyin and Selcuk, 2004) and many other nations (HerbIMI, 2018).

In India, this disease was observed earlier by Butler (1914) followed by Cheema *et al.* (1954) and Desai (1998) in local varieties of *Ficus carica*. More recently *Ficus rumphi* was reported to be an additional host for *C. fici* (Kahn, 1994). *C. fici* was also been found to be parasitic on *Ficus glomerata* (Rai, 1980), *Ficus benjamina* (Kumar and Khurana, 2015), *Ficus religiosa*, *F. heterophylla*, *F. infectoria, F. palmata, F. racemosa, F. roxburghii* and *F. benghalensis* (Sinha and Singh, 1992; Pathak *et al.*, 2016; HerbIMI, 2018). It is also reported in Pakistan on *Ficus carica, F. religiosa* and *F. palmata* (Ahmad, 1956; Saba *et al.,* 2013), *F. asperifolia* in Ghana, *Ficus chlorocarpa* in China, *F. hochstetteri* in Kenya, *F. scobina* in Australia (herbIMI, 2018). Other host genera infected include *Brous, Sonnetia, Papyrifera, Maclura* and *Morus* (McKenzie, 2013). This pathogenic disease have serious outbreak in all the fig growing areas of India. In this context, we explored the identity of the pathogen and its causative nature in detail. This will helpful for the prevention, better management and development of resistant varieties in near future. The objectives of this study were: (i) to determine the occurrence of *C. fici* on *Ficus carica* in India; (ii) to identify the pathogen of the respective disease; (iii) to study the possible identity and morphology of the *C. fici*.

Materials and methods

Survey: An intensive survey was conducted in Tamil Nadu from October 2018 to April 2019, as weather during this period was highly conducive to the development of the rust disease in fig orchards. During the survey, rust spot symptoms caused by *C. fici* were observed on leaves (25 leaves/ plant) and fruits in two fig varieties (Poona and Dinkar) at Coimbatore (11.11005° N, 76.9208° E), Erode (11.504784° N, 77.2383862° E) and Dindigal (10.2787° N, 77.6286° E) districts in the Western Ghats forest range of Tamil Nadu state.

Sample collection: Three commercial orchard samples of *C. fici* infected leaves with typical symptoms were collected and microscopic examinations of the fungus were made by making transverse section of the fruiting structures from abaxial leaf surface and then placing these in wax rings containing glycerine.

Morphological confirmation: A morphological analysis was done with a light microscope (Mediline OX44 7XZ) at 400X magnification. Specimens from Dindigul district were also examined by SEM (FEI-Quanta 250). For this purpose, the leaf specimens with rust pustules were cut with a razor blade into small pieces of approximately 5 × 5 mm. The specimens were initially fixed in 2.5 % glutaraldehyde in 0.02 M sodium phosphate buffer (pH 7.2 at 4°C) for 24 h. This was followed by

30 min washes in 0.02 M sodium phosphate buffer at 4°C. Then the specimen was post fixed in 1 % osmium tetroxide prepared in the aforementioned buffer at room temperature for 2 h. The specimens were then dehydrated in a series of ethanol baths at 10, 20, 30, 40, 50, 60, 70, 85, 95 and 100 % v/v, two times for 30 min each. For the SEM analysis, the samples were kept in a desiccator and air-dried at room temperature overnight. The dried samples were then mounted onto carbon stubs with double sided sticky tape. SEM analysis was carried out using an acceleration voltage of 10 kV and at 6000X magnification (Parthasarathy *et al.*, 2017).

Results and discussion

The results of the survey carried out at Tamil Nadu state in India are presented in Table 1. Percent Disease Incidence (PDI) was higher in the Dindugal district (100 %) followed by the Coimbatore district (100 %). Results on PDI suggest that the disease is in a more-severe and epidemic form in the studied locations. Typically, the pale reddish brown, to reddish-brown angular leaf spots were observed on adaxial surfaces while on the same leaves the corresponding abaxial surface contained numerous orange rust pustules. At an advanced stage angular spots coalesced and formed necrotic patches while nearby leaf edges turned upwards. Almost all infected leaves abscised prematurely, leaving bare branches and unripe fruits. Raised, blister-like pustules also appeared on the surfaces of the fruits (Fig. 1). Fruits on a completely defoliated tree were not able to ripe fully. The fungus on the infected leaves was similar in all the examined orchards and confirmed as *C. fici* based on uredial characters (10.52-2.74 × 18.80-20.45 µm) as per the description and illustrations (McKenzie, 1986; Verga and Nelson, 2014). Urediniospores occur usually as hypophyllous on leaves and fruits, mainly scattered or sometimes tending to be grouped around edge of a leaf or in dew drop runs on leaf surfaces. Urediniospores are circular, ellipsoid to polygonal or round shaped. Spore granules are yellowish to faintly orange, framed with a thick, light brown, denticulate wall membrane (Fig. 2 and 3). Uredioniospores are

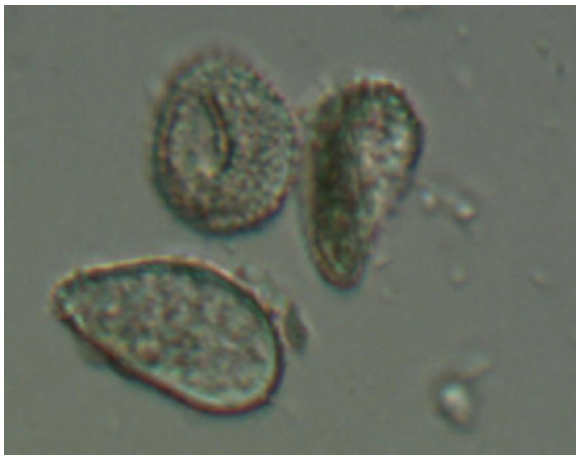
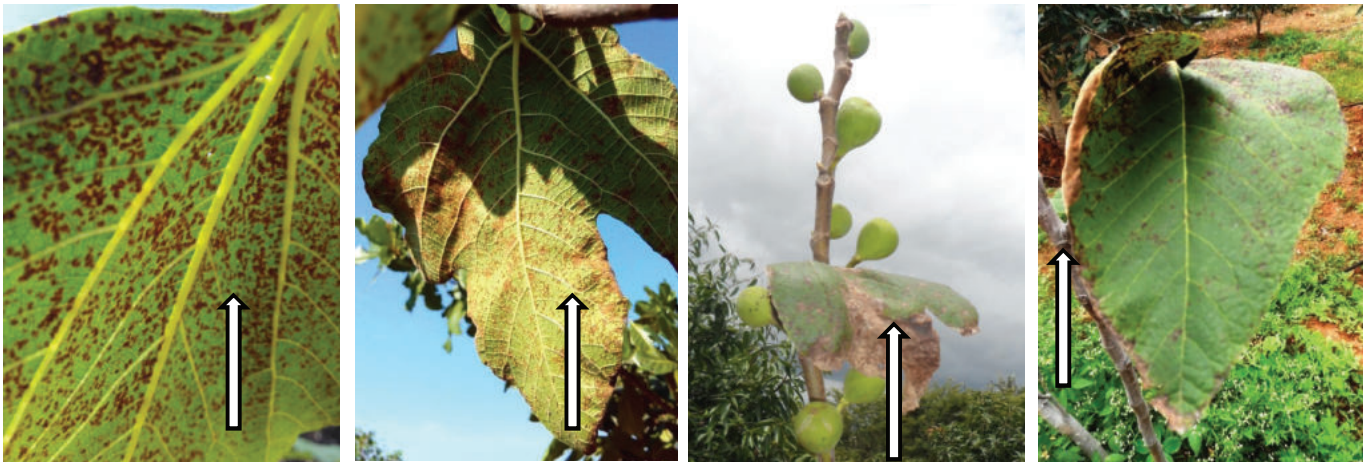


Fig. 2. *Cerotelium fici* Urediniospores

globose, obovoid, and vary in size from 19.6 – 31.9 × 14.7 – 24.5 µm (average 24.5 × 20.0 µm), spore walls are 0.5 -1 µm thick, pale and sparsely echinulate (Laundon and Rainbow, 1971). This description corresponds with Mijuskovic (1963) and McKenzie (1986). According to Huseyin and Selcuk (2004), spermogonia and aecia are unrevealed. Besides uredinia and urediniospores, telia appear as well on lower leaf surfaces. Telia are very small, white and scattered with numerous teliospores in chains of 2-7 spores, angular-spherical or broadly-ellipsoid, 19-22 × 10-17 µm; spore wall is 1-1.5 µm thick, smooth and hyaline. These results agree with the previous reports except seasonal occurrence of disease incidence in India. The other cyclic stages like aecium and telium were not found to be present in this study. From our survey, based on morphological and microscopical observations, we concluded that fig trees cultivated in the foot districts of Western Ghats forest ranges in Tamil Nadu were severely infected all around the year with the rust fungi *C. fici.* Hence, it is advised to adopt preventive measures at beginning stage of the infection especially during cold weather conditions (October to April) in southern hills.



Numerous orange rust pustules on abaxial surfaces Necrotic patches nearby leaf edges

Fig. 1. Common fig leaf rust symptom

Table 1. Incidence and severity of rust of *Ficus carica* recorded from different orchards of Tamil Nadu, India

District	Location	Number of trees observed	Number of trees affected	Disease incidence (%)
Coimbatore	Kallar	175	171	98
Erode	Thalavadi	400	380	95
Dindugal	Pannaikadu	150	150	100
Mean		241.66	233.66	97.66

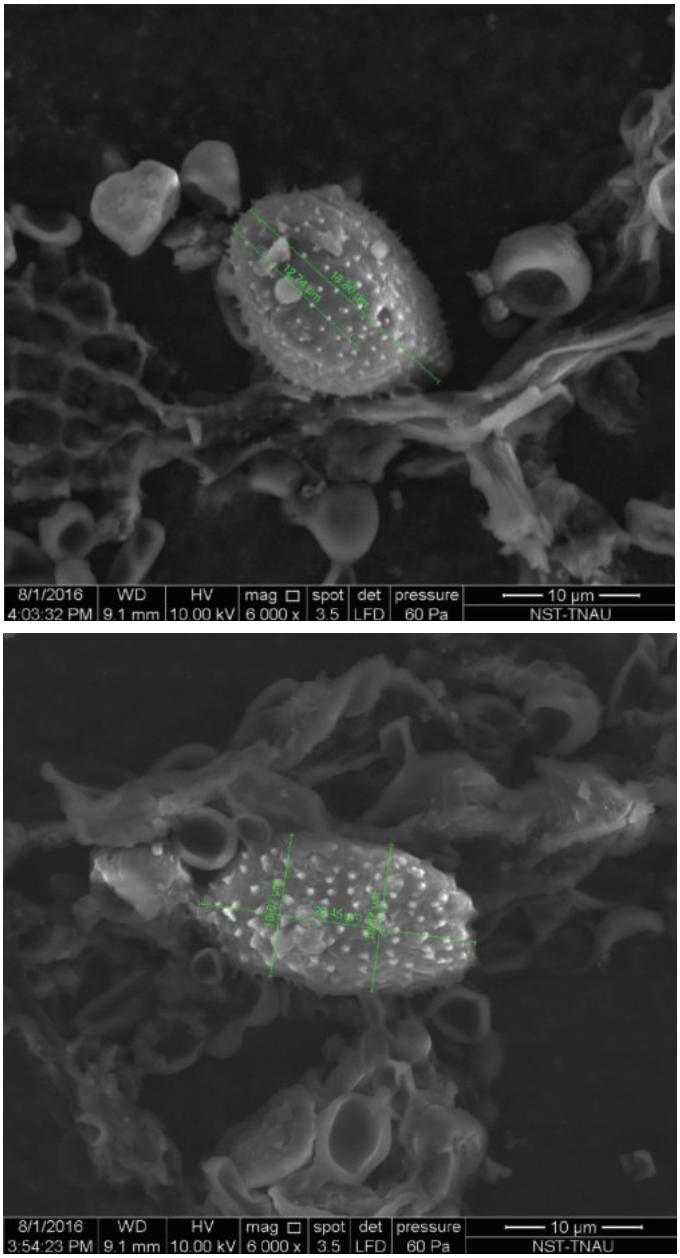


Fig. 3. SEM image of *Cerotelium fici*

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