

Histopathological Features of Infections Caused by *Fusarium Equiseti* (Mart) Sacc. In Onion Plants from Kebbi State, Northern Nigeria

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Abstract

Histopathological features of onion tissues infected with *Fusarium equiseti* showed infection progress from the roots where the pathogen move through the vascular system to colonize the whole plant. At first, in the intercellular spaces of the root cortex but soon invaded the cells, followed by colonization of the cells by its hyphae and microconidia. At later stages, the cortex tissue became completely disorganized and decomposed as the pathogen advanced to the shoot system via the vessel elements.

Keywords: Onion; Histopathology; Infection; *Fusarium equiseti*; Tissue; Cells;

Introduction

Onion (*Allium cepa*) is an important vegetable among the cultivated Allium. It ranks second in value after tomatoes on the list of cultivated vegetable crops worldwide [6]. In soils, propagules of the *Fusarium* on or close to onion roots germinate usually by forming one or two germ tubes [1]. The mycelium intercellularly advances through the root cortex until it reaches the xylem vessels, which it uses as a pathway for rapid colonization of the host, particularly by means of rapid production of microconidia [7]. Previous reports have provided evidence that pathogen resides in the soil where it infects the onion host through the roots, stem plate (modified stem) and the fleshy leaf bases of the onion plant, in that sequence and root invasion by both direct penetration and/or through wounds was reported.

[1, 2]. First report implicating *F. equiseti* as a fungal pathogen of onion in Nigeria was reported in 2015 [6]. This disease poses a serious threat to the production and biodiversity of this important vegetable crop in Nigeria [4]. Farmers-saved seeds obtained from in Kebbi State had varying degree of fungal infections by *Fusarium* spp., *Aspergillus* spp. and *Mucor irregularis* [5]. This study aims to determine the features of onion tissues infected with *Fusarium equiseti*, a confirmed culture with an accession number (IMI No. 604243) identified at International Mycological Institute (IMI) Egham, London. First isolated by [4] on onions in Nigeria causing die-back disease of onion in Nigeria [6].

Materials and Methods

The inoculum of confirmed *Fusarium equiseti* was adjusted (1×10^6 conidia/ml) and used to inoculate six-week old seedlings using soil drench, root dip and mycelia paste methods as earlier described by [4], to provide descriptions of the distribution of the pathogen in different plant tissues, their colonization and tissue alterations in the plant.

Microscopic examination of tissues from each inoculated method used was conducted using the method earlier described by [8]. Sections of onion leaves and roots were fixed in Formalin 5ml (40%) + Glacial Acetic acid 5ml + Alcohol 90ml (70%) [FAA] and Carnoy fluid composed from Alcohol (60%) + Chloroform (30%) + glacial Acetic acid (10 %) for 24 h respectively. Dehydration was done in Ethanol series (30%, 50%, 75%, 95% and 100% Ethanol) for 2h each and cleared in Chloroform and Ethanol mixtures (1:3, 1:1, 3:1 and absolute Chloroform) for 2h each.

They were subsequently embedded in pure paraffin at 60°C for 24h and 5 μ m of tissue sectioned using Cambridge rotatory microtone (Leitze 1512-West Germany). Staining was performed in Safranin and counter stained with Acid Fast Green before leaf and root sections were mounted for microscopic examination at 400x and digital camera was used for photomicrographs.

Results

Transverse sections of 6-week-old root showed predominance of isodiametric parenchyma cells with thin primary walls and polyhedral shapes in the ground tissue region. These thin walled cells are found between the vascular system and epidermis. Large intercellular spaces were also very conspicuous, while the inner xylem vessels were intact, normal and coherent (Plate 1).

Photomicrographs of transverse sections of inoculated root tissue showed mycelia colonizing the cortical regions as presented in Plates 2, 3 and 4. Cells in the cortex region appear completely clogged with mycelia growth, while adjacent cells

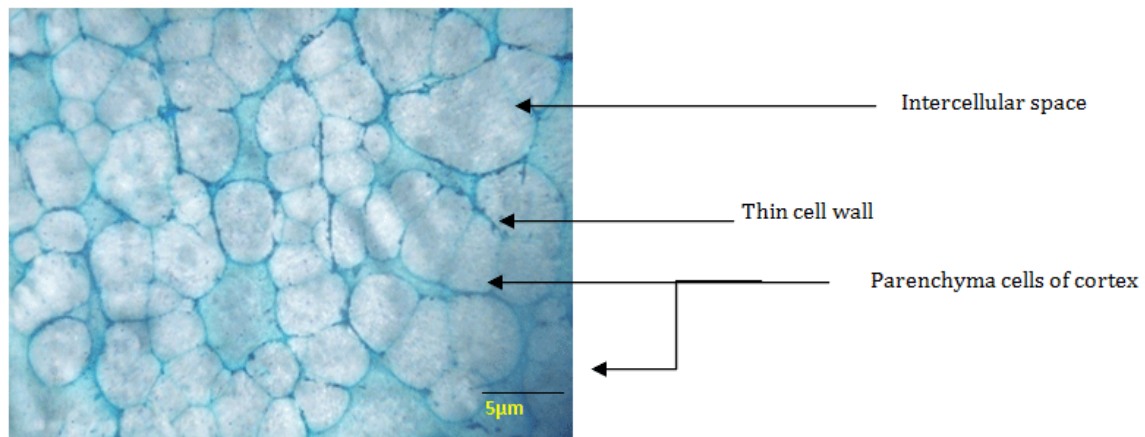


Plate 1: Transverse section through healthy root tissue of *Allium cepa*

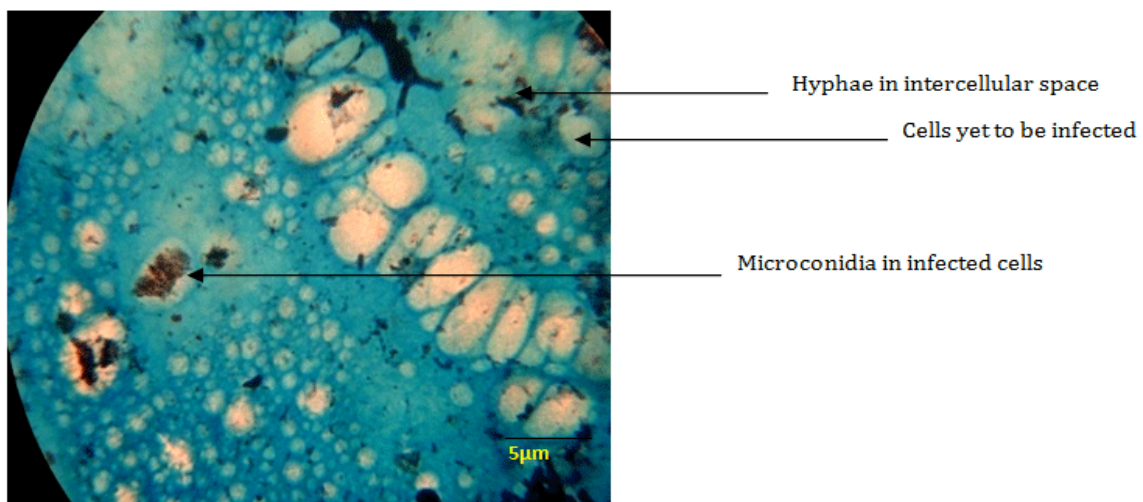


Plate 2: Transverse section through onion root tissue inoculated by mycelia paste

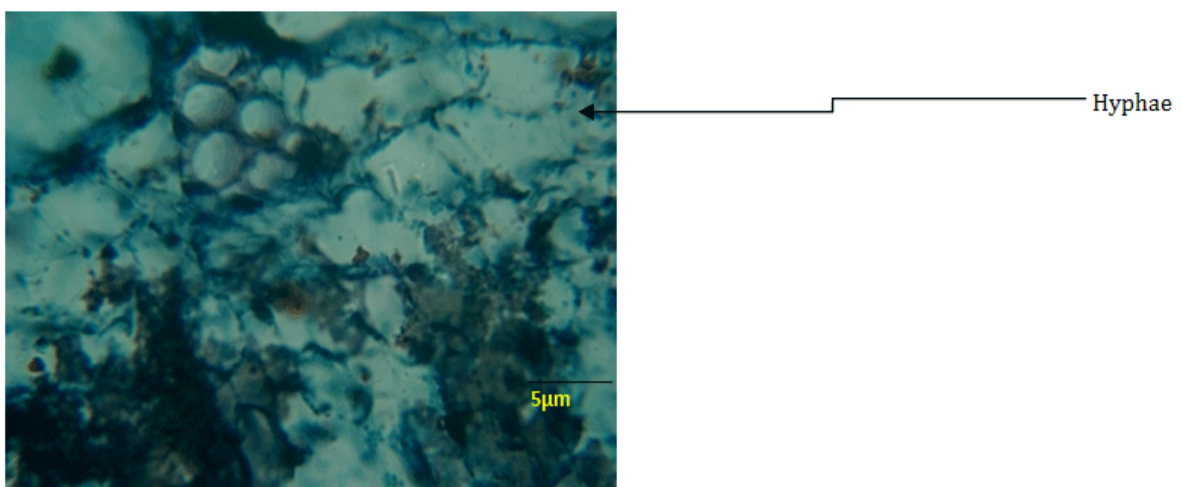


Plate 3: Transverse section through onion root tissue inoculated by root dip

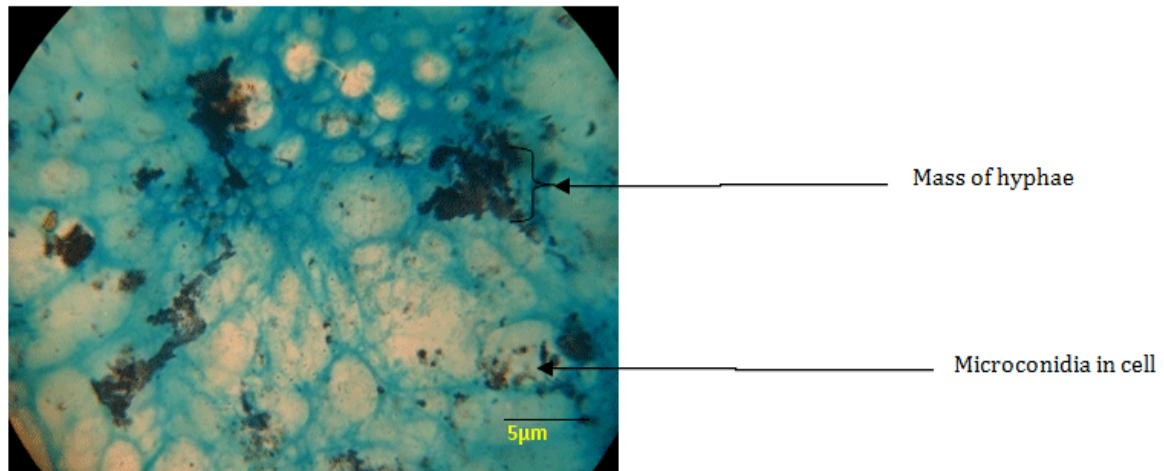


Plate 4: Transverse section through onion root tissue inoculated with soil drench method

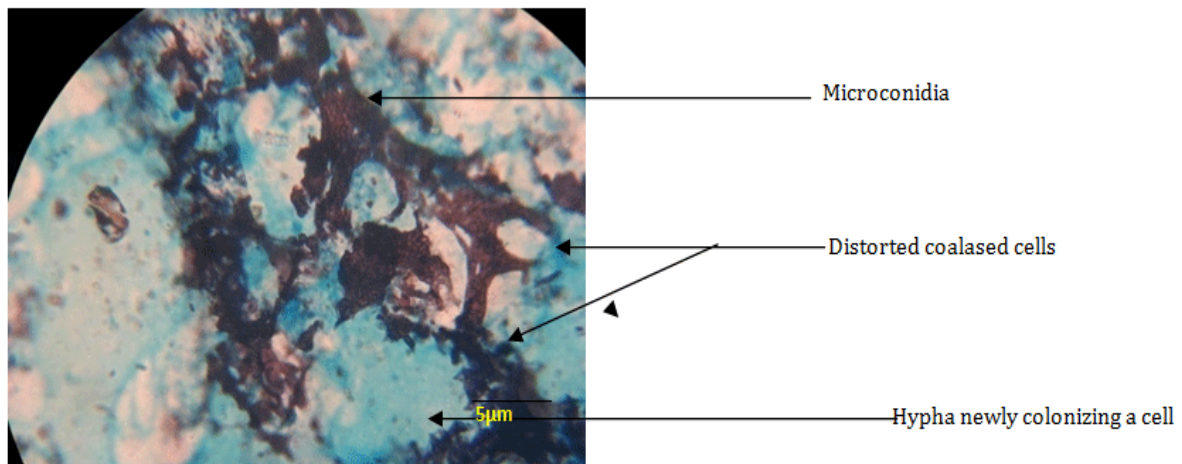


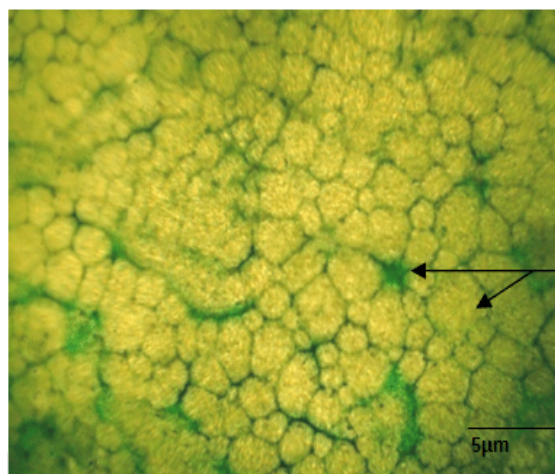
Plate 5: Transverse section through infected onion root tissue from farmers' field

lacked or have a few hyphae. After ingress, hyphae of the pathogen grew mostly within the intercellular spaces of the parenchyma cells. Mycelia structures showed a pattern of progression through the parenchyma, with marked disruption and lack of differentiation (Plate 4). This was observed irrespective of method of inoculation used. Naturally infected samples showed, the root cortex completely disorganized and damaged. Hyphal fragments ramified the entire structure and caused dissolution and clogging of most cells (Plate 5).

The transverse section of healthy leaf showed a predominance of parenchyma cells with chloroplast (stained green) and small intercellular spaces (Plate 6). In infected leaf tissues, there was a generalized disruption of cell arrangement and cell shape loss,

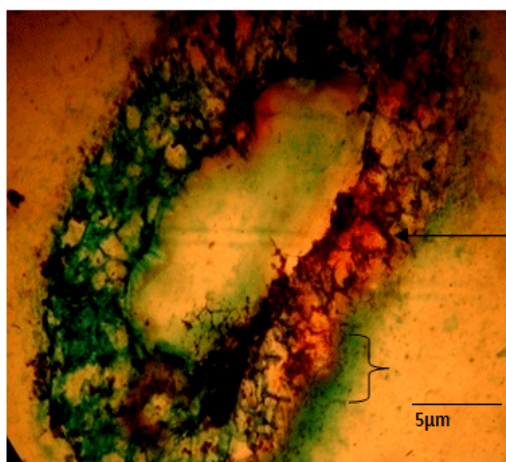
which is associated with hypertrophy (Plate 7). The central leaf cavity and surrounding parenchyma cells were also ramified by the fungal hyphae and microconidia (Plate 7).

The fungus destroyed the walls of the tissue and colonized the entire vascular tissue (Plate 8). There was distortion of the vascular tissues due to the presence *Fusarium* hyphae. Tylose structure was also observed in the parenchyma cells (Plate 9). Advanced late stage of infection, the vessels became disorganized with hyphal fragments being the only structures observed in between the epidermis and endodermis as observed samples from farmer's field with collapsed cells at the edge of cavity (Plate 10).



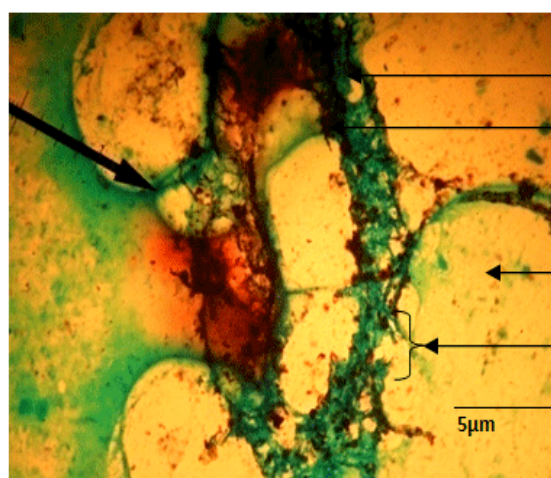
Intercellular spaces

Plate 6: Transverse section through healthy leaf tissue of *Allium cepa*



Parenchyma cell

Plate 7: Transverse section of leaf tissue inoculated by mycelia paste.



Microconidia

Hyphae of *Fusarium equiseti*

Clogged intracellular space

Central leaf cavity

Completely digested tissue wall

Plate 8: Transverse section of leaf tissue inoculated by root dip.

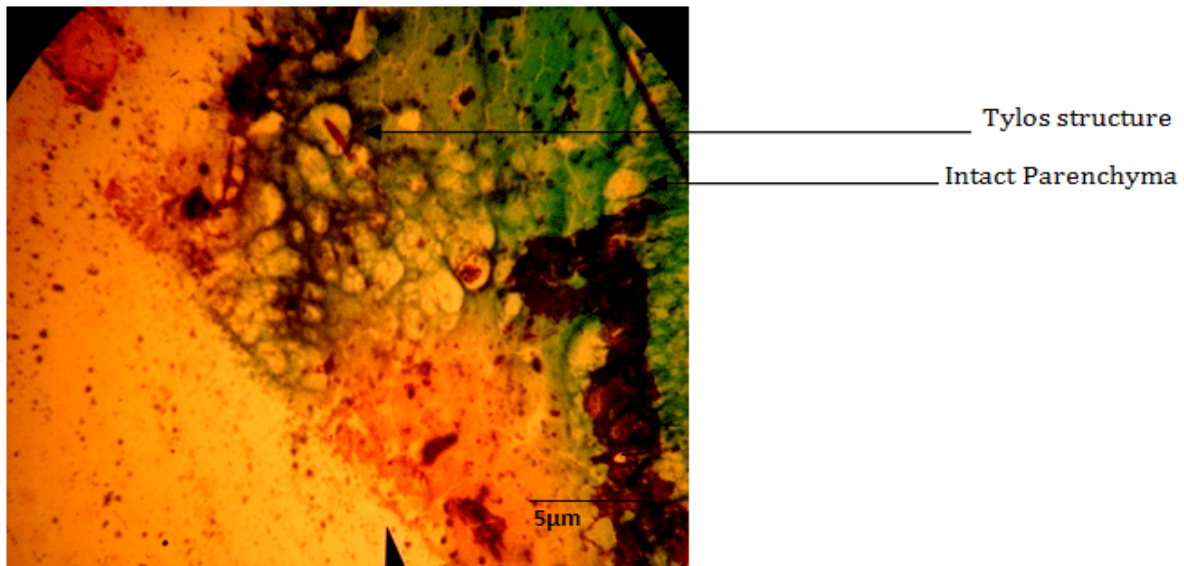


Plate 9: Transverse section of leaf tissue inoculated by soil drench

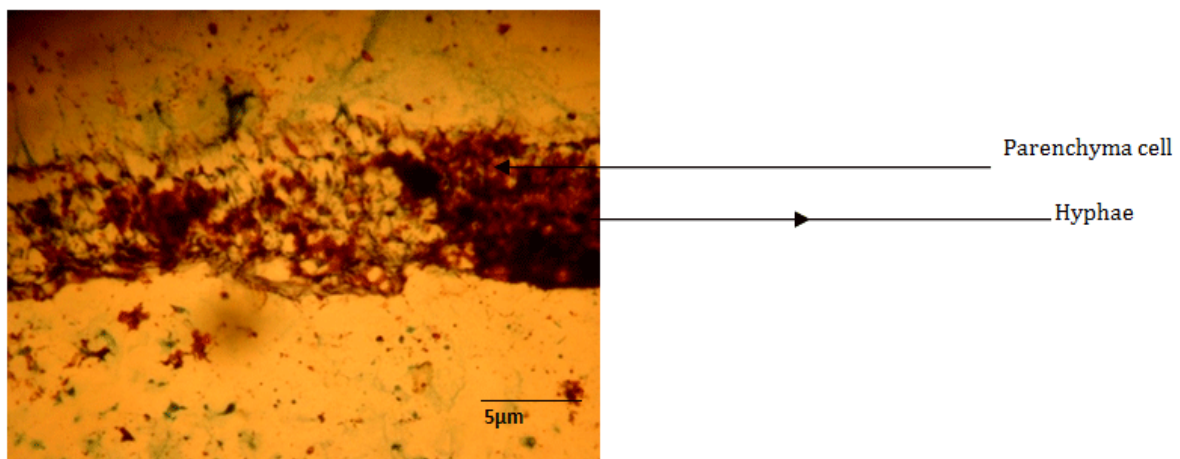


Plate 10: Transverse section through infected leaf from field

Discussions

Tissue colonization was observed for roots and leaves of the analyzed onion plant, which corroborate the earlier reported ability of the pathogen to penetrate the root system and colonize the plant through its vascular system [1]. The mycelium continues growth in the intercellular spaces and within cells of roots, stem pith and xylem. Penetrated xylem reduces ability of the plant to transport water upward leading to plant [5]. It produces mycelium as well as three types of asexual spores: microconidia, macroconidia and chlamydospores.

The fungi dissolved the cell walls of mainly the parenchymatous cells and completely occupied the cells with hyphae and microconidia as observed in the tissues, making the entire vascular system less distinct and distinguishable.

Additionally, the change in the shape of xylem cells and ramification of cells, especially in the root tissue, suggests cellulose degradation processes [1]. The pathogen observed to grow into the intercellular spaces of the root cortex, before its eventual colonization of the vascular system by its hyphae and microconidia, agrees with earlier report of [10] that vascular occlusion being a common feature in disease development incited by *Fusaria*. The colonization of the vascular system has also been reported for tomato and pea susceptible to *F. oxysporium* [2]. Once *F. oxysporium* infection occurred in the roots, the pathogen invaded the shoot system via the vessel elements.

Onion vascular system is very small, endarch and most of the leaf and root tissues are dominated by soft primary walled parenchyma cells of the cortex [10]. Plugging of the xylem

vessel elements by the hyphae and microconidia as well as the disintegration of parenchyma cells of the cortex were observed in all the plants infected with *Fusarium equiseti*. This may account for the initial chlorotic, curved apical leaves and progression of wilted leaves from the tip downward due to restricted translocation activity and slender bulbs from the plants able to survive through the infection.

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