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Histopathological studies of sesame (*Sesamum indicum*) seedlings infected with *Fusarium oxysporum*

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Abstract

Sesame (*Sesamum indicum* L.) is one of the most important and oldest oil seed crops, and vascular wilt caused by the soil-borne fungus *Fusarium oxysporum* is the most destructive disease of sesame worldwide. Histopathological studies were conducted on sesame plants after artificial inoculation with *F. oxysporum*. Characteristic disease symptoms such as chlorotic and wilted leaves appeared 15–20 days after inoculation. Light microscopic studies revealed the presence of pathogen in xylem vessels during initial stages. The pathogen then moved to adjacent cortical and epidermal cells intercellularly causing disintegration of the cells. Formation of cavities and plugging of xylem vessels with gum was observed, which retards water and nutrient supply to plants, thus resulting in wilting and ultimately leading to death of plant.

Key words – disease – histopathology – inoculation – pathogen – sesame

Introduction

Sesame (*Sesamum indicum* L.) is one of the most important and oldest oil seed crops (Bedigian et al. 2010) belonging to the family Pedaliaceae (Falusi & Salako 2001). The crop is usually cultivated in tropical and subtropical regions of Asia, Africa and South America (Ashri et al. 1998, Anilakumar 2010). It has been cultivated in Asia for over 5000 years (Bisht et al. 1998) but its origin and evolution is still unclear (Ashri et al. 1998).

China and India are the world's largest producers of sesame, followed by Myanmar, Sudan, Uganda, Ethiopia, Nigeria, Tanzania, Pakistan and Paraguay (FAO et al. 2011). In 2009, the world production of sesame seed was 3,976,968 tonnes, and the major production areas were Asia (2,489,518 tonnes) and Africa (1,316,690 tonnes), constituting about 62.6% and 33.1% of the total world production, respectively (FAO et al. 2008). Pakistan ranks fourteen among major sesame producing countries producing about 32,000 tonnes with an average yield of 4000 Kg/Ha (FAO et al. 2014).

Diseases are major constraints in sesame production worldwide and are thought to account for losses of 7 million tonnes annually. Sesame seed is susceptible to a variety of pathogens, and is attacked by eight fungal pathogens (Kolte et al. 1985) and 65 species of insects (Ahuja & Bkhetia 1995). The soil-borne fungus *Fusarium oxysporum* is the causal agent of vascular wilt disease

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resulting in sudden death of sesame plants. This pathogen may cause heavy yield losses ranging from 50–100% (Gaber et al. 1998). It affects the vascular system of plants resulting in hyphal proliferation in the xylem vessels. This results in appearance of characteristic disease symptoms, such as vein clearing, leaf epinasty, wilt, and defoliation. The pathogen invades the parenchymatous tissue and sporulates on the plant surface, thus completing its life cycle (Di Pietro et al. 2003).

Histopathological studies have been carried on various plants infected with *Fusarium* species including passion fruit (Ortiz et al. 2014), mango (Haggag et al. 2011), bean (Pereira et al. 2013), staghorn sumac plant (Ouellette et al. 2005), grapevine (Atia et al. 2003), and sweet corn (Lawerence et al. 1981). Histopathological studies have been carried out on sesame infected with *Pseudomonas syringae* pv. *sesame* (Firdous et al. 2014) and *Macrophomina phaseolina* (El-Wakil et al. 2012). No histopathological studies of *Fusarium* on sesame have been reported from Pakistan. Therefore, the present study was conducted to understand the mechanism of fungal infection and histological changes in sesame cells infected with *Fusarium oxysporum*.

Materials & Methods

Fungal culture and slide preparation

Fusarium oxysporum (Accession no. 511) was obtained from the First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. The isolate was grown under aseptic conditions on potato dextrose agar (PDA) media and incubated at 30°C. After fungal colonies became visible, they were inspected carefully for their morphology. The fungal slide was prepared using lactophenol cotton blue, observed using a light microscope and photographs were taken with a NIKON eclipse 80i (Fig. 1).

Seed samples collection

Three varieties of sesame seeds (TS-3, TS-5 and SG-27) were obtained from the Oil Seed Program of National Agriculture Research Council (NARC), Islamabad. Seeds were surface sterilized by treating with 2% sodium hypochlorite (NaOCl) for 2 minutes and then rinsed three times with distilled water.

Preparation of potting mixture

Pot experiments were conducted for artificial inoculation of plants with pathogen. Potting mixture was prepared containing sand, clay and farmyard manure (2: 1: 1). Before filling, pots were sterilized with bleach, while commercial formalin (37%) was used to sterilize the potting mixture. Eight to 10 sesame seeds of each variety were sown and watered regularly.

Inoculation of seedlings

Inoculum was prepared from 15-days-old culture of *Fusarium* by adding sterilized water to each culture plate and scraping the surface with a sterilized needle. The suspension was collected and concentration was adjusted to 105 spores per ml using a hemocytometer (Kroes et al. 1998). Plants were inoculated 20 days after sowing by pouring 50 ml of the spore suspension over the soil surface in each pot, close to the sesame seedling roots. Distilled water was used for control plants.

Histopathology method

The following steps were performed to prepare diseased tissue for histopathological studies (Firdous et al. 2014).

Fixation and embedding

Healthy and infected stem and root sections (1–2 mm) of all three varieties of sesame were fixed in 4% formaldehyde in 50 mM phosphate buffer with pH 7.2 at 4° C for 48 hours. Sections were then washed in fresh buffer for 5 minutes.

Sections were dehydrated in an ethanol series, 30, 50, 70, 95 and 100%, each for one hour at 40° C. After that the tissues were dehydrated with ethanol and xylene in ratio of 3:1, 1:1 and 1:3, each for one hour and then kept in pure xylene overnight at room temperature.

Excessive xylene was removed by adding a few paraffin chips until saturation and kept in oven at 40º C. Xylene wax solution was replaced by pure wax overnight at 60˚ C (Ruzin et al. 1999).

Melted paraffin wax was poured into steel molds and then sections were placed in steel mold. Tissues were arranged and oriented with the help of a needle and molds were placed on the surface of ice cold water immediately. After solidification the blocks were stored at 4° C before staining.

Sectioning and staining

For sectioning, a paraffin wax block was placed in a rotary microtome (LEICA RM2125RT) in such a way that edge of razor blade just touched the block and thin sections (11–15 µm) were made. After cutting, the ribbon pieces were removed from the microtome with the help of a needle and placed on glass slides. After drying, slides were stained with 0.5% toluidine blue-O containing 0.5% H_3BO_4 and 2% Na_2CO_3. Sections were observed using a microscope and photographs were taken with a NIKON eclipse 80i.

Results

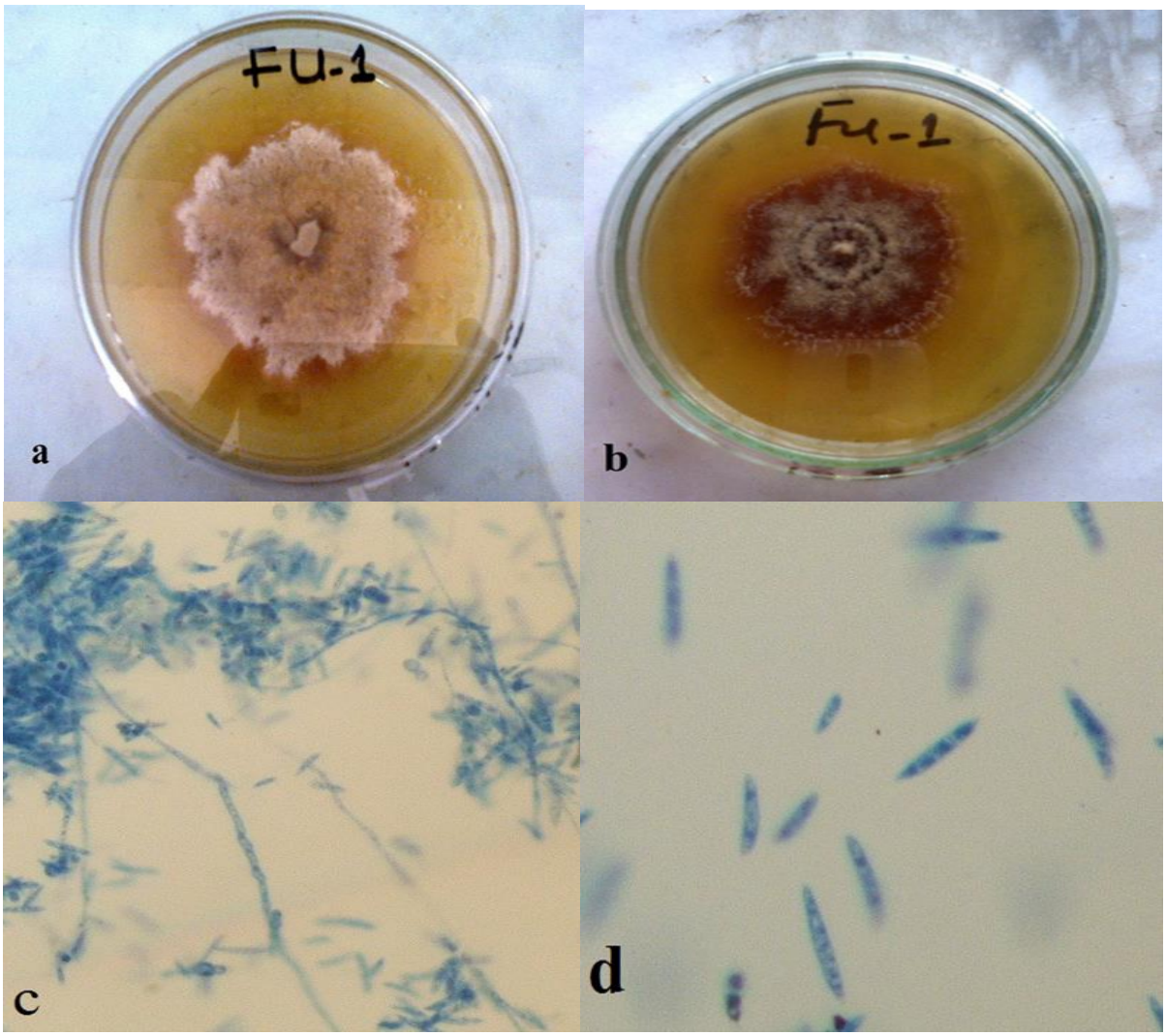

Fig. 1 – Colony characteristics of *Fusarium oxysporum* on PDA. a) Colony after 7 days. b) Colony after 15 days. c) Hyphae, microconidia and macroconidia (20 ×). d) Macroconidia (40 ×).

Macroscopic symptoms

Symptoms were apparent after 15 days of infection showing slight yellowing of lower leaves, thus indicating chlorosis of leaves. After 25 days of inoculation approximately 50% of leaves and branches showed wilting and chlorosis, followed by complete wilting and defoliation eventually leading to death of plant after 30 days of inoculation.

Histopathology

Anatomy of healthy stem

Healthy stems of the three varieties showed three distinct regions of epidermis, cortex and vascular bundles. The epidermis or outermost layer surrounds the stem and it is composed of a single layer of closely packed living cells. Multicellular stem hairs or trichomes are present on the surface of the epidermis (Fig. 4a). Two layers of hypodermis composed of collenchymatous cells are found beneath epidermis. Cortex is the major portion of stem represented by loosely arranged parenchyma cells. Endodermis is the innermost layer of cortex composed of closely packed cells. Next to endodermis is stele which forms central cylinder of plant and consists of pericycle, vascular bundles and pith (Fig. 2). Vascular tissues consist of xylem, which transports water and minerals and phloem which transports food. Xylem is present towards the center and phloem is present outside. Protoxylem is present towards center and metaxylem towards outside (Fig. 3). Large pith is present in the center and is composed of parenchymatous cells (Fig. 4b).

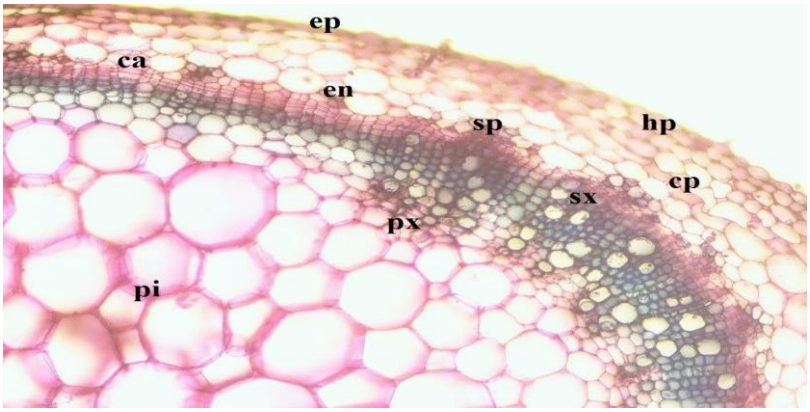

Fig. 2 – Cross section of healthy stem of *Sesamum indicum* (TS-3); epidermis (ep), hypodermis (hp), cortical parenchyma (cp), endodermis (en), cambium (ca), secondary phloem (sp), secondary xylem (sx), primary xylem (px), pith (pi).

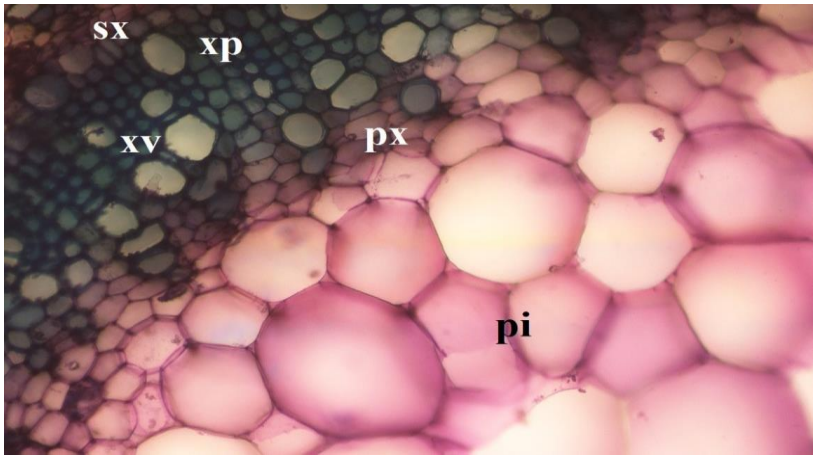

Fig. 3 – Cross section of healthy stem of TS-5; secondary xylem (sx), xylem parenchyma (xp), xylem vessel (xv), primary xylem (px), pith (pi).

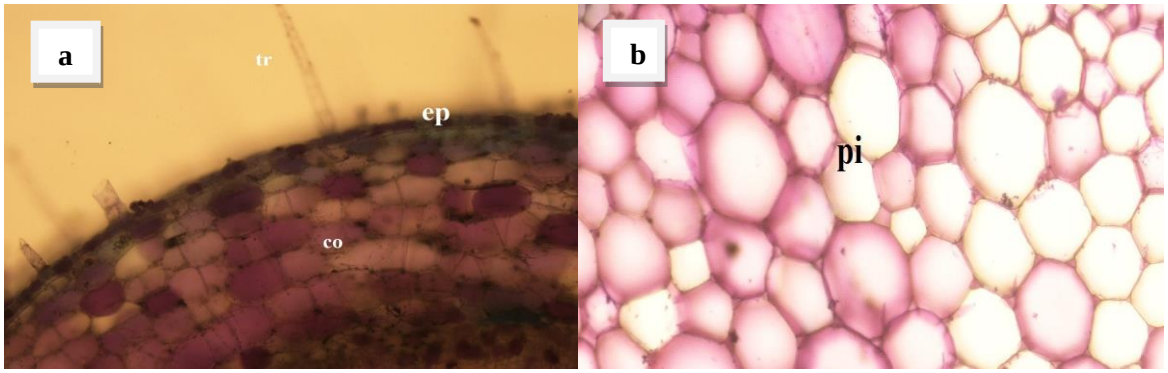

Fig. 4 – Cross section of SG-27 a) showing trichomes (tr), epidermis (ep), cortex (co). b) Cross section of TS-3 stem showing pith (pi).

Anatomy of infected stem

All three varieties of sesame showed a similar response towards the pathogen and hence each variety is not discussed separately. Transverse sections of sesame infected with *Fusarium oxysporum* showed infection of xylem elements. The darkly stained fungal mycelium was present in both primary and secondary xylem vessels (Fig. 5). Cambium was also infected with fungus. The walls of cambium and phloem were lined with fungal hyphae (Fig. 6a). Vessels were partially filled with occluding material (Fig. 6b).

After 20 days following inoculation the fungus moved to adjacent cortical cells. The walls of cells became slightly swollen due to deposition of gums and pectinaceous material. Xylem vessels were heavily colonized by fungal hyphae and mycelium (Fig. 7). Cell walls of parenchyma cells were dilated showing distortion of cortical parenchyma cells (Fig. 8). Movement of *Fusarium* towards cortex was also recorded at this stage. After 25 days following inoculation vascular tissue was completely occupied by fungal mycelium and disintegration of vascular tissue was observed. Epidermis and cortical parenchyma was heavily colonized by fungus and most of the cells were degraded (Fig. 9). Fungus was also observed to be present in most of the cells of cortex. After 30 days, all the tissues of stem were completely invaded with fungus. Complete destruction of cortex was observed. Xylem vessels were completely blocked with fungal mycelium and hyphae. Phloem and vascular cambium were also fully destroyed. Large degenerated areas were observed which show deterioration of tissues (Fig. 10).

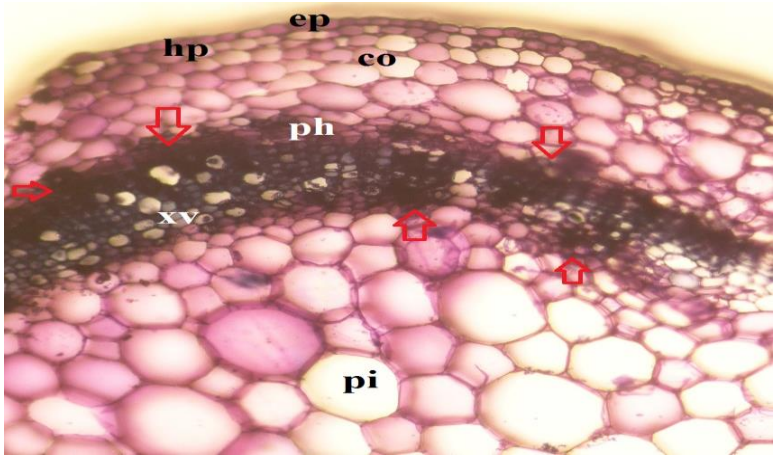

Fig. 5 – Cross section of infected TS-3 stem, Arrows showing invasion of xylem elements by *F. oxysporum*. Dark stained masses of fungal hyphae in vascular tissues. Epidermis (ep), hypodermis (hp), cortex (co), phloem (ph), pith (pi).

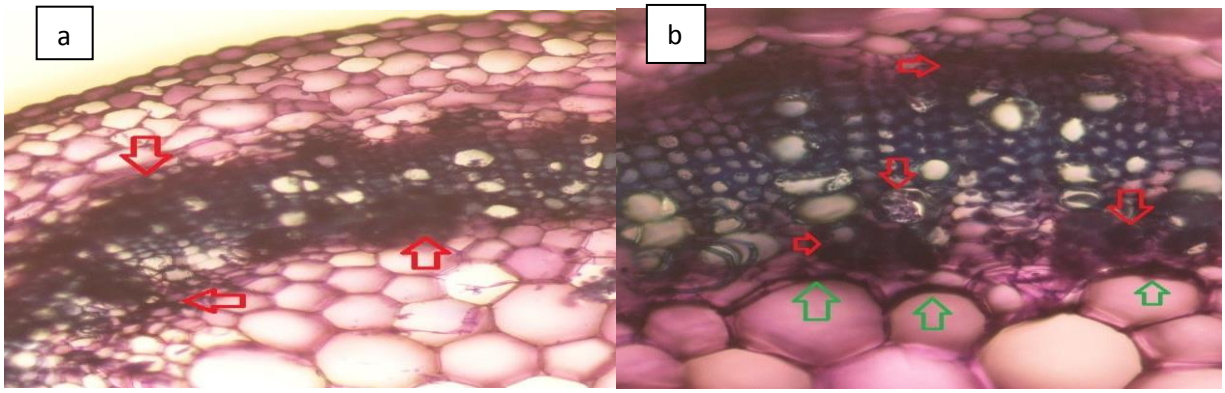

Fig. 6 – Cross section of infected TS-5 stem 20 days after inoculation. Arrows showing the xylem vessels invaded by fungal hyphae.

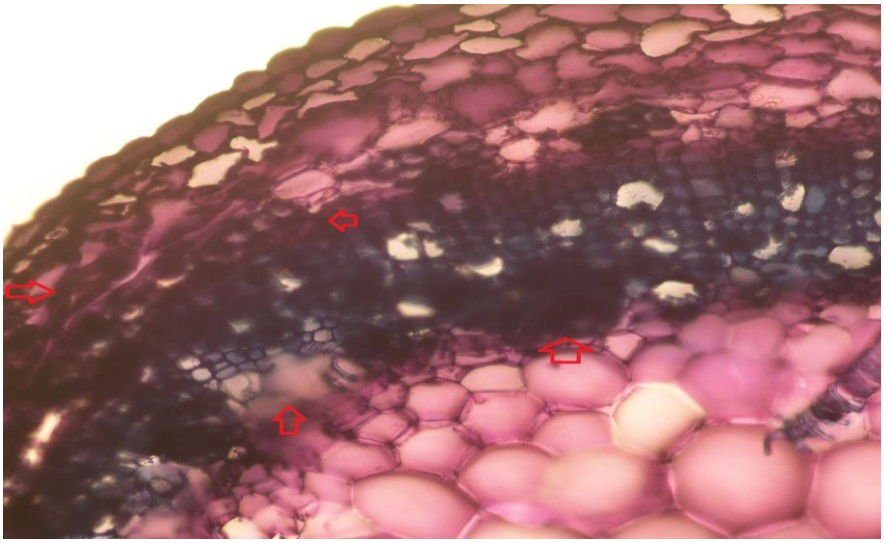

Fig. 7 – Cross section of SG-27 stem after 20 days after inoculation, Arrows showing massive colonization of xylem vessels and distortion of cortex.

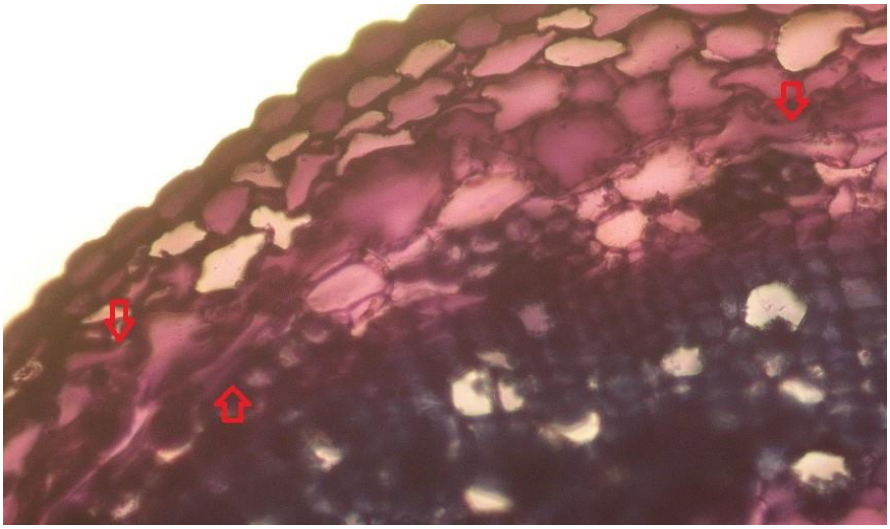

Fig. 8 – Image of SG-27 showing abnormal shape and distortion of cortical parenchyma cells.

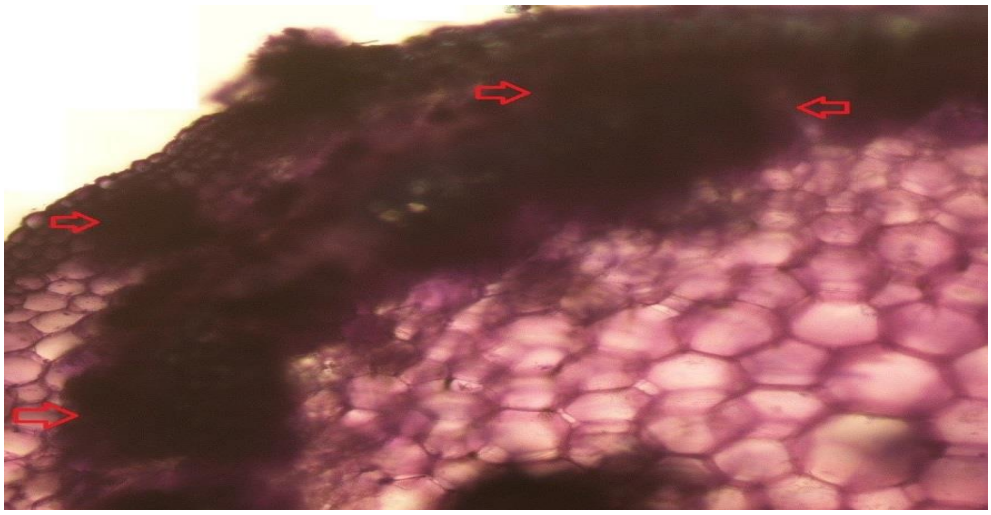

Fig. 9 – Section of TS-3 stem showing massive colonization of vascular tissues, cortex and epidermis and movement of fungus towards pith.

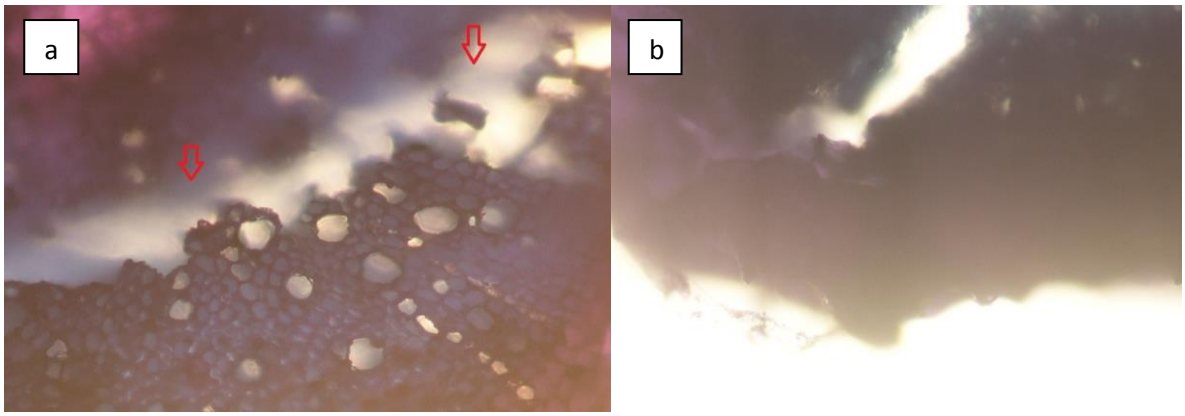

Fig. 10 – Cross section of TS-3: Arrows showing the a) formation of cavities b) degradation of xylem.

Discussion

Similar studies have been reported in *Mangifera indica* infected with different *Fusarium* species. Thick and dark coloured hyphae were detected throughout the vascular tissues and cortical tissues along with presence of degenerated areas (Haggag et al. 2011). Another study on infection process of *F. oxysporum* f. sp. *phaseoli* on bean cultivars reported presence of hyphae in the xylem vessel (Pereira et al. 2013). Presence of fungal hyphae and mycelial fragments in the xylem vessels was reported after 21 days of infection (Atia et al. 2003). The fungus moved intra- and intercellularly in all tissues and vessels were plugged with gum as well as formation of tyloses was reported. Xylem vessels were plugged with resins and xylem and parenchyma cells become hypertrophied before degradation (Broaddus & Dwinell 1983). The pathogen attacked parenchyma cells and created gaps in cortex (Broaddus & Dwinell 1983). Resins were accumulated before disintegration of cells. Xylem vessels were occluded, vascular parenchyma cells disintegrated and fungus spread throughout the tissue (Lawerence et al. 1981). These results are in accordance with studies carried out on Chrysanthemum cultivar infected with *Fusarium oxysporum* f. sp. *chrysanthemi* in which plugging of xylem vessels, hyperplasia and hypertrophy of parenchyma cells and formation of cavities were reported which resulted in collapse and disintegration of the cells (Emberger & Nelson 1981).

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