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# HISTOPATHOLOGICAL STUDIES ON INFECTED MANGO ROOTSTOCKS WITH SOME SOIL-BORNE PATHOGENS

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**ABSTRACT: Light microscope used in histopathological studies of transverse and longitudinal root sections of mango (*Mangifera indica* L.) after 14 days from artificially infected, 6 month old rootstock cv. G3 (highly susceptible), by the tested pathogenic fungi revealed that, the cortex layer was completely colonized by *Botryodiplodia theobromae* Pat., *Fusarium solani* (Mart) Sacc., *Rhizoctonia solani* Kühn. and *Macrophomina phaseolina* (Tassi) Goid. This colonization was associated with disorganized epidermal and cortical cells in addition to a dark brown color consisting of crushed and necrotic cells and tissues. Such disorders were not observed in case of *Pestalotia* sp. or *Phytophthora* sp. Thirty days after artificial inoculation of tissues the necrotic were observed in both xylem parenchyma and xylem vessels.**

***Key words: Histopathological, mango, rootstock, soil-borne pathogens***

## INTRODUCTION

Although soil-borne diseases of mango (*Mangifera indica* L.) are relatively less important than the foliar and floral diseases, they are still capable of causing significant damage to seedlings, stocks and mature trees (Kore and Mane, 1992; Tsao *et al.* 1994; Ploetz and Prakash, 1997; and Abd- El. Ghany, 2001). Rots of mango rootstock considered one of the most important soil-borne diseases affecting mango

production and causing a great losses in the nurseries. Under local conditions and according to available literature, no research work was done in this respect. Several fungi including *B. theobromae, F.solani, R. solani, M. phaseolina, Pestalotia* sp. and *Phytophthora* sp. were frequently isolated from mango rootstock suffering root-rot disease (Kore and Patil, 1985; Saxena and Rawal, 1989; Verma *et al.* 1991; Kore and Mane, 1992; Das, 1993; Tsao *et al.* 1994, Al-Adawi *et al.* 2002 and Tohamy *et al.*, 2004). In case of *Pestalotia* sp Das (1993) isolated *Pestalotia sapotae* from wilted guava trees. In apricot seedling roots, Fouad *et al.* (1985) found that, after 48 h of inoculation with *F. solani,* invading hyphae were intra and intercellularly dispersed in the inner cortical layers. Host cells were killed after colonization and the cortex was completely colonized after 60 h. The cortical cells collapsed after 72 h and had many chlamydospores. The fungus was restricted by the endodermis and foliage symptoms appeared after 48 h. Nemec *et al.* (1986) stated that, in rough lemon citrus fibrous, *Fusarium solani* principally directly invaded the broken epidermal cells, root-hair and/or cell walls. Dwivedi (1990) reported that, *Macrophomina phaseolina* hyphae were detected in the xylem vessels of infected guava roots. Ploetz *et al.* (1996) noticed that, necrosis, gummosis, and vascular discoloration of mango tissues following infection with *Botryodiplodia theobromae.*

Abd El-Ghany (2001) revealed that, *B. theobromae* caused plasmolysis and disorganization with a dark brown discoloration of epidermal and cortical cells of mango seedlings. The necrotic areas in both xylem parenchyma and xylem vessels colonized by the hyphae, and dark inclusions inside the xylem as well as abundant production of gummosis and the pathogen spread to all various tissues causing complete breakdown. In case of *R. solani*, the infecting hyphae extended to the spaces between the outer layer of cortical cells and epidermis. *F. solani* completely colonized the root tissues within the cortex. Fungus chlamydospores were found inside the collapsed cortical cells and in xylem vessels. The fungus spread intra and intercellularly. The vessels were colonized by the hyphae and tylosis were observed in xylem vessels of root.

Atia *et al.* (2003) in histopathological investigations of *Botryodiplodia theobromae* and *Fusarium solani* on artificially inoculated grapevine, shoots revealed the induction of various cytological and histological defects. Seven days after inoculation (dai), *B. theobromae* induced disorganized epidermal and cortical cells with a dark brown color consisting of plasmolized cells and tissues. Such disorders were not observed in case of *F. solani.* The above mentioned pathogenic fungal hyphae were clearly observed 21 dai in both xylem parenchyma and xylem vessels causing necrosis for these tissues and colonized them with dark inclusions bodies as well as abundant production of tyloses were also noticed.

The present investigation was carried out to study the effect of artificial inoculation with the pathogenic fungi (*B. theobromae, R. solani, M. phaseolina, F. solani, Phytophthora* sp. and *Pestalotia* sp.) on the structure of mango rootstock root tissues.

## MATERIALS AND METHODS

Virulent pathogenic isolates of *B. theobromae* Pat., *R. solani* Kühn., *M. phaseolina* (Tassi) Goid., *F. solani* (Mart) Sacc., *Phytophthora* sp. and *Pestalotia* sp. were employed in all the following experiments. In previously research work these pathogens were found to be the most important fungi causing mango rootstock rots on basis of their pathogenicity tests in pot experiments, the (highly susceptible) 6 months old seedlings cv.G3 were inoculated with each of the previously mentioned virulent fungi using soil infestation (Tohamy *et al.* 2004). Soil infestation was carried out using barley meal medium inoculated with each of the previously mentioned virulent fungi. Pots of 25 cm. diameter were sterilized with 5 % formalin solution and filled with clayey : sandy soil, 1:1 (w/w). The soil was infested with each single fungus at a rate of 5 % soil weight. Main and lateral root samples were taken after 0, 14 and 30 days from the different treatments, washed in three changes of sterilized water and dried between folds of sterilized paper towels. The inoculated and non inoculated roots were cut into small specimens (5-10 mm. long), killed and fixed in formalin-acetic acid alcohol solution (FAA) according to (Sass 1940). Specimens were then dehydrated and embedded in

wax according to (Johanson 1940). Cross and longitudinal sections 15 and 20 µm thick were microtomed. The sections were fixed on a series of glass slides with Hoples adhesive. The sections were passed through a regular xylol-alcohol concentrations down to alcohol. Staining of sections was done using crystal violet and erythrosin pigment (Johanson 1940). Sections were cleared in xylol and mounted in Canada-balsam then microscopically examined using light microscope and photographed.

## RESULTS AND DISCUSSION

Histopathological investigations of the artificially inoculated roots of mango rootstock cv. G3, with *Botryodiplodia theobromae, Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina, Pestalotia* sp. and *Phytophthora* sp. were carried out. Immediately after inoculation (ai), the investigated sections of non inoculated (healthy) as well as inoculated (diseased) samples at zero time were similar, consisting of normal intact epidermal, cortical, xylem vessels, phloem and pith cells. No hyphae and tylosis were obseved in the uninoculated tissues (Fig.1A,B,C and D).

Light microscope investigations of the sections taken after 14 days of inoculation with *B. theobromae* showed that, the fungus can parasitize epidermal cells and the hyphae extended not only under cuticle layer but also into the intercellular spaces of epidermal, cortical, resin duct (r.d) and parenchymatous cells of phloem (Fig.2A). The same figure also revealed that, most of phloem and cambial cells are destroyed. It also indicate that, parenchymatous rays of xylem tissue were suitable pass-ways for rapid and easy extension of the hyphae (Fig.2B), the chemical and structural constituents of parenchymatous cells of xylem tissues may be play an important role in susceptibility tissues to infection. Thirty days after inoculation with *B. theobromae,* transverse and longitudinal sections show that the disease had progressed and three different reactions were manifested by the various tissues of infected roots (Fig.2C,D and E). It is also clear from the same figures that, necrotic area appeared in xylem parenchyma and xylem vessels associated with dark inclusion bodies, these compounds may be phenolic or lignin-like material, that might accumulated at the site. The results were also similar like

those obtained by Cruickshank, (1980) who notice phenolic compounds accumulate rapidly during host-parasite interactions. Also Gado, (1997) found abnormal accumulation of electron dense materials at the site of fungal hyphae out the sites of plant cell walls.

Microscopic examinations of transverse and longitudinal sections of mango rootstock root inoculated with *R. solani* 14 days after inoculation showed, obliterated and crushed of epidermal and cortical cells with abundant tannic sacs inside it, as well as distortion of most phloem and cambial cells as shown in Fig. 3A and B. Thirty days after inoculation fungal spread in healthy tissues was limited to the xylem, gum plugs being found in the xylem vessels. Dark inclusions appeared in the epidermal and cortical layers and the fungus hyphae advanced more rapidly in the cortex of the lateral root than in that of the main root. The cortex was separated from the rest tissues and pith colonized by hyphae. The hyphae extended inter and intracellular of pith (Figs.3C-E). These results are similar to those recorded by Davis *et al.* (1987); Abd-El.Ghany (2001) and Atia *et al.* (2003). The hyphal growth of *R. solani* along the transverse and longitudinal sections was observed. Walls of the epidermal cells was similar to those reported by Nakayama (1940) and Khadga *et al.* (1963). The cuticle was often separated from epidermis by the invading hyphae and the cells in advance of the invading hyphae were often discolored, this discoloration might be due to the increasing of oxidative enzymes and production of toxic substance. Similar results were obtained by Boosalis (1950) and Gonzalez and Owen(1963). The discoloration of the cells probably resulted from the production of toxic material as mentioned by Wyllie (1962) and Bateman (1963).

Cross and longitudinal sections in diseased roots infected with *F. solani* 14 days after inoculation revealed that the fungus caused discoloration in all walls and eliminate the red reaction in sclerotic cells presented in cortex layer as shown in Fig.4A,B,C and D. These figures revealed that, deep brown vascular discoloration as well as tyloses was observed in the active xylem. Mycelium fragments of the fungus were presente in the pith intra and intercellular as shown in (Fig.4.D). Thirty days after inoculation revealed that, defense structures

(D.S) as a response of plant infection (host-parasite interaction) as shown in (Fig.4.E-F), it is clear from the same fig., mycelium mass colonized and extending longitudinally in vessels elements.

Transverse and longitudinal sections of the roots of mango rootstock (G3) inoculated with *M. phaseolina* confirmed that, through 14 days after inoculation the fungus completely colonized the cortex where the cortical cells undistinguished may be defense structures (DS) and epidermis cells seemed like smut appearance and it might be sclerotic bodies (Fig.5A). The hyphae advanced more rapidly in xylem parenchyma (Fig.5B and C).

After the elapse of 30 days from artificial inoculation with *Phytophthora* sp. the examined diseased roots showed the presence of *Phytophthora* sp. mycelia intra and intercellular in all examined tissues particularly in epidermal and cortical cells. Transverse and longitudinal sections revealed that, the diseased tissues became undistinguished. The reaction on cortex invaded with *Phytophthora* sp. showed deterioration of epidermal cells with necrotic area in the phloem (Fig.6 A,B and C). It also seemed that, the xylem parenchyma were suitable pass way for rapid and easy extension of the hyphae to destroy the pith (Fig.6A and B). Neither hyphae nor necrotic area were observed 14 days after inoculation. Section microscopically appeared without any visible changes in this histological structure.

Transverse and longitudinal sections of root infected with *Pestalotia* sp. after 30 days (Fig.7A,B and C) showed, necrotic area in cortical cells and partial necrosis in xylem vessels and the mycelium inter and intracellular concerted in cortical cells. Neither hyphae nor necrotic area were noticed 14 days after inoculation. Fouad *et al.* (1985) revealed that , the mycelium of *F. solani* develops in cortex of the invaded roots mostly intercellular causing cell deterioration after colonization. As a result of root infection the vegetative growth was also affected. This might be attributed to the enzymatic activity and toxic effects during pathogenesis. These results are in agreement with those obtained by Chatterjee (1958) and Abd-El. Malek (1982).

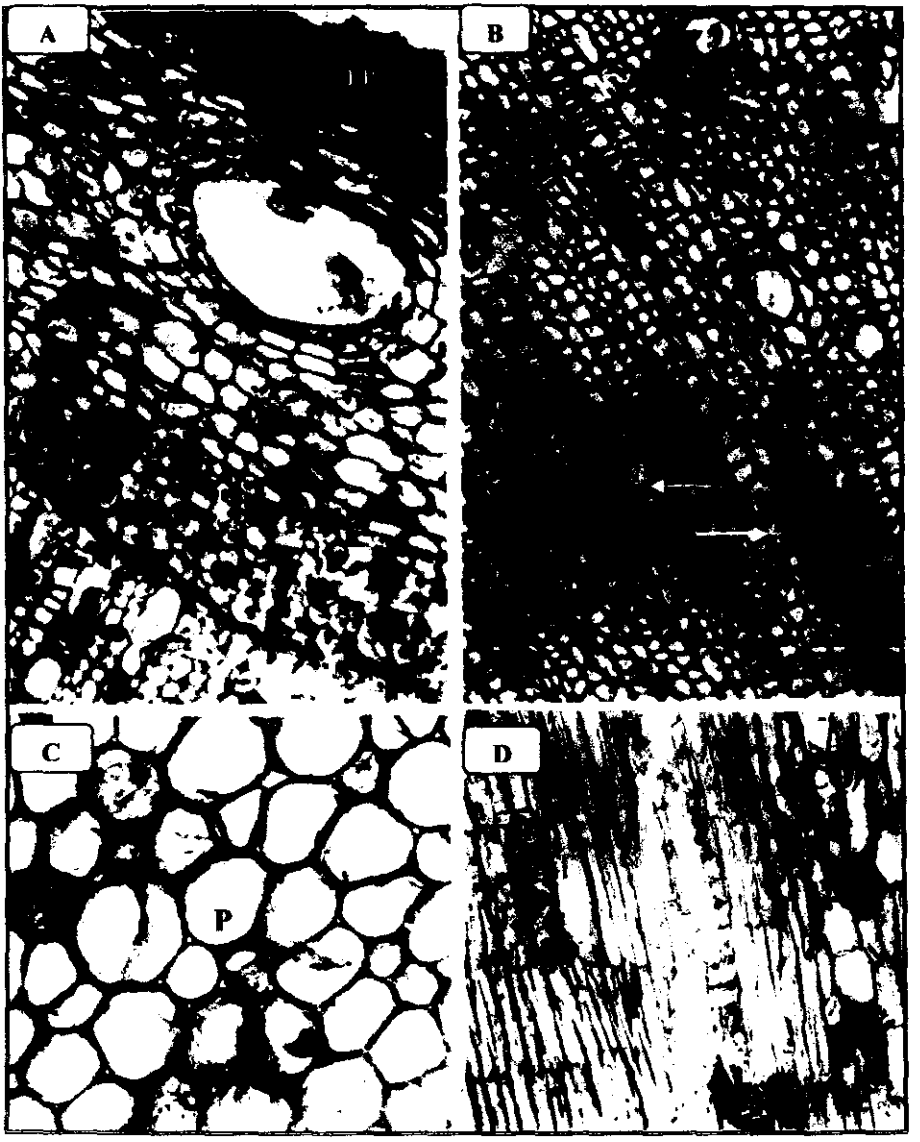


**Fig.1.A-D.** Light micrographs of healthy mango rootstock sections. A, B, and C, cross section in non-inoculated roots, showing normal EP (Epiderm), CO (Cortex), R.D (Resin Duct), T.S (Tannic Sacs) and Ph (Phloem) in Fig.A.Fig.B. show normal X (xylem) and R (Rays). Fig.C. indicate normal P (Pith) (X200). Fig.D. A longitudinal section show normal Cortex cells (X100).

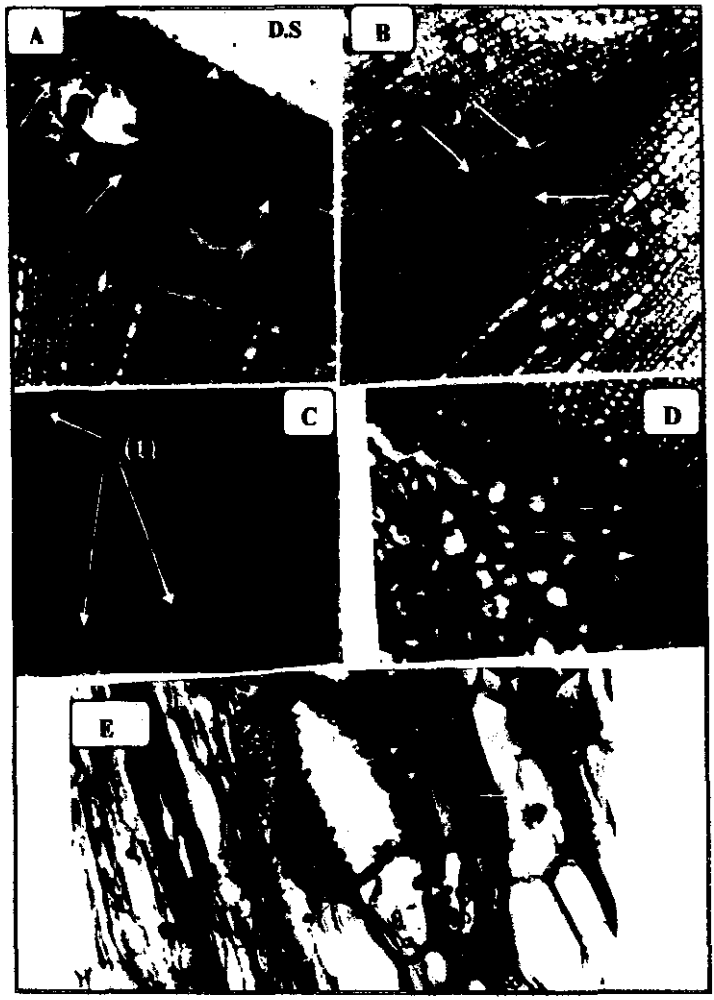


**Fig. 2.** A-E. Histological responses of mango rootstock to *Botryodiplodia theobromae.* A-B, transfer sections 14 days after inoculation (dai).A, show collapsed, necrotic cortical cells and hyphae in cortex indicated by arrows (X100). B, show the hyphae extending through xylem rays towards pith indicated by arrows (X100). C-D, transverse section 30 days after inoculation (dai). C, show numerous tyloses (t) in xylem indicated by arrows (X200). D, show necrotic area in xylem parenchyma indicated by arrows(X200). E, longitudinal section of diseased root infected by *B. theobromae* after 30 days. Notice mycelium colonized xylem vessels indicated by arrows (X400).

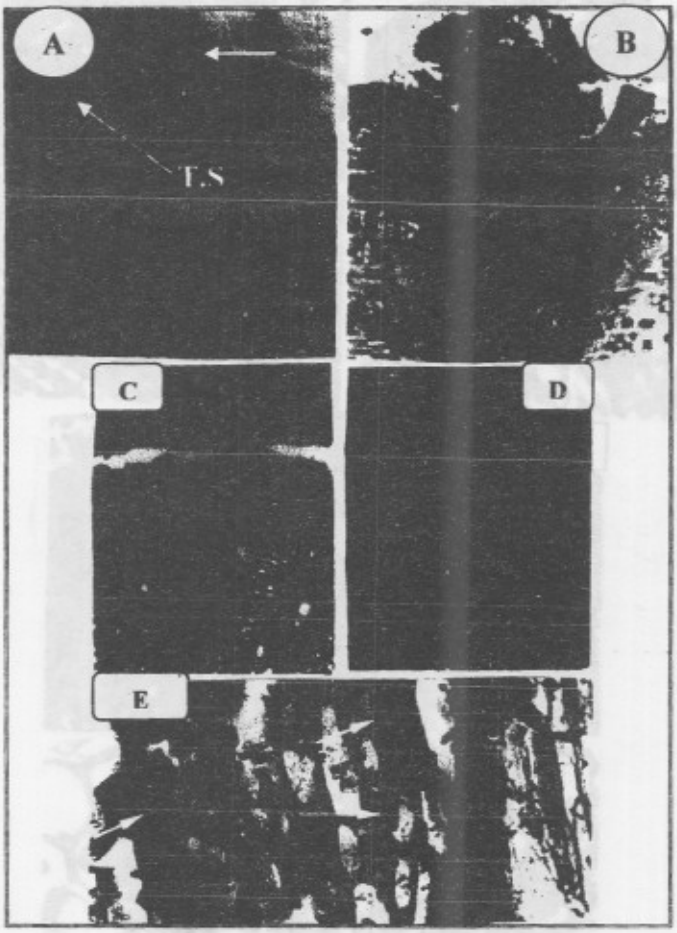


**Fig. 3. A-B.** Light photographs of mango rootstock variety, G3 after inoculation with *Rhizoctonia solani.* A, transverse section of infected main root 14 days after inoculation showing, plasmolysis and diorganisation with dark brown discoloration of epidermal and cortical cells, notice T.S and D.S (X100). B, cross section of diseased root pass with secondary root 14 days after inoculation notice, the cortex tissues as well as phloem and cambium were destroyed and the vessels were pluged with gum-like (X100). C-D. Microscopical photographs of infected tissues 30 days after inoculation. C, show the cortex was separated from the rest tissues (X100). D, show pith colonized by hyphae. The hyphae extended inter and intracellularly of pith (X100). E, longitudinal section 30 days after inoculation where xylem vessels and pith are filled with undistinguished granules and fungal mycelium as shown by arrows (X200).

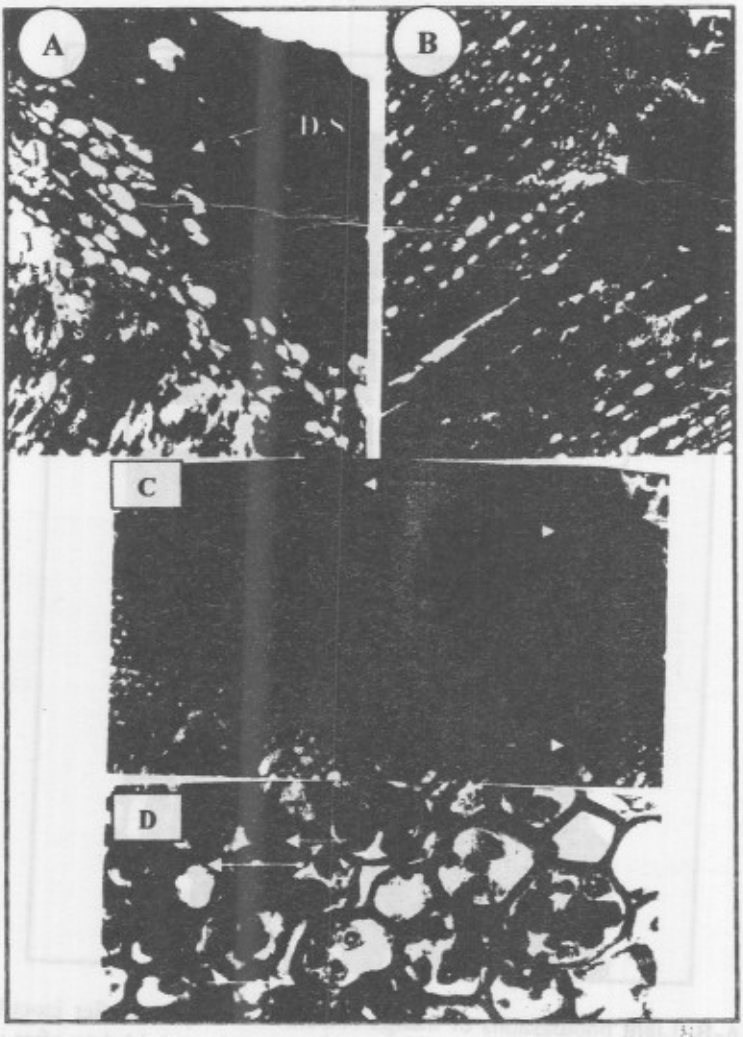


**Fig. 4. A-B.** Histological responses of mango rootstock root infected with *Fusarium solani.* A., transverse section 14 days after inoculation showing the breakdown cortex and Defense structures (D.S) as showen by arrow (X200). B, transverse section 14 days after inoculation showing necrotic area in R colonized by fungus (X100). C-D. Microscopical photographs of transverse section 30 days after inoculation. C, show, the crack and irregularly shaped cavities, cortex, cambium and phloem were break down , showing also necrotic area in xylem vessels and xylem paranchima indicated by arrows (X200). D, show pith colonized hyphae notice, destruction of pith cells as shown by arrows (X400).

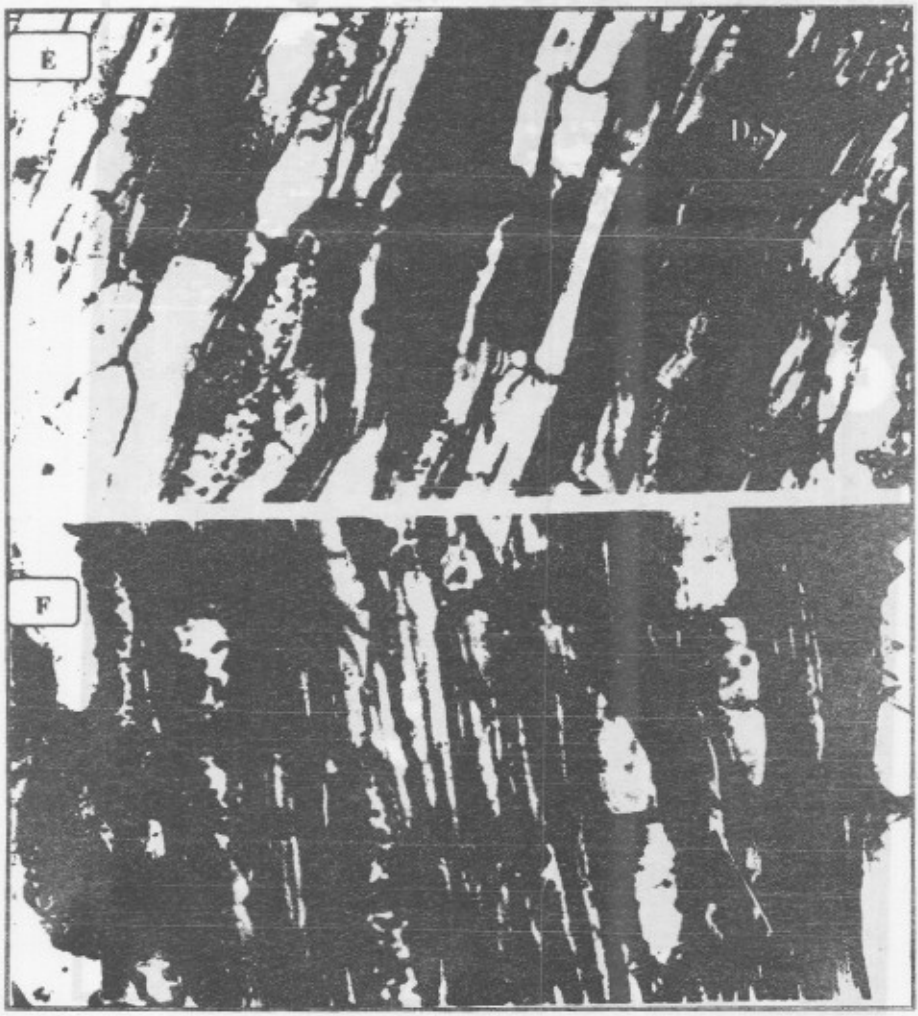


**Fig. 4. E-F.** Histological responses of mango rootstock infected with *Fusarium solani*. Microscopical photographs of longitudinal sections. E, show the response of cortex and phloem, (the crack and irregularly shape with dark inclusion inside tissues after 30 days from inoculation) notice Defense structure layers (D.S). F, show mycelium mass colonized and extending longitudinally in vessels elements after 30 days from inoculation.(X400)

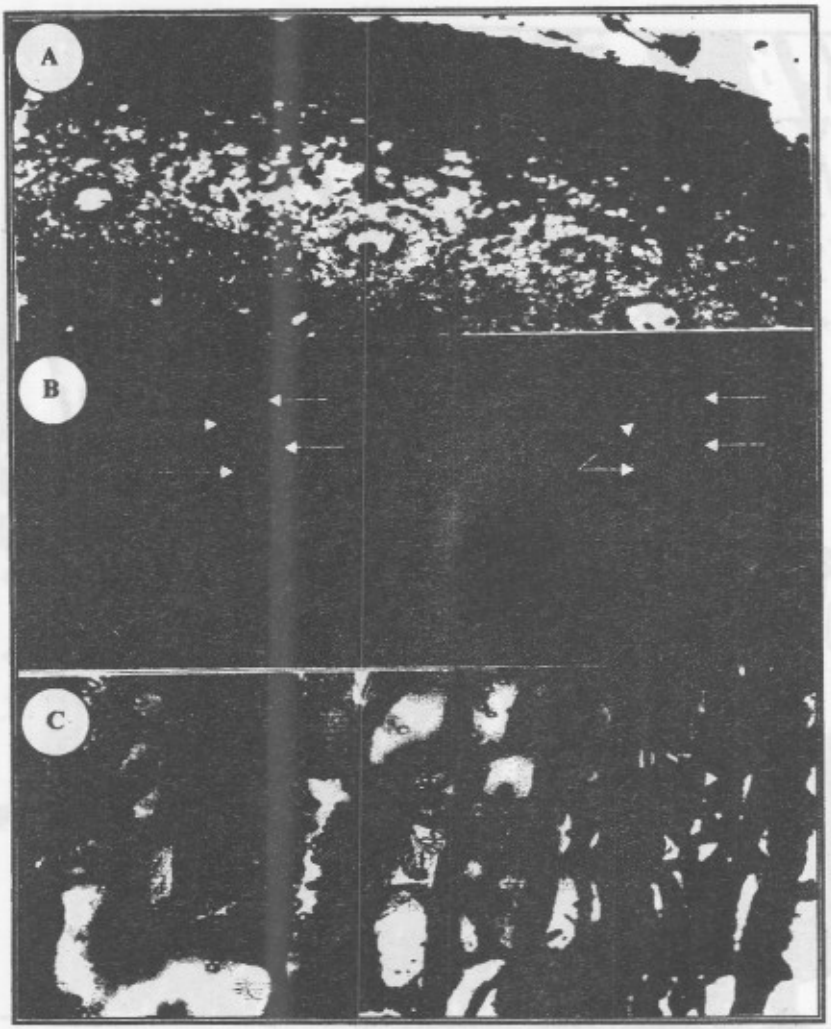


**Fig. 5. A-C**. Histological responses of mango rootstock infected by *Macrophomina phasealina* after 14 and 30 days from inoculation. A. microscopical photograph of transverse sections after 14 dai showing, epiderm and cortex cells break down, most of phloem and cambial cells are destroyed, filled with gum (X40). B, showing mycelium extension through R ( indicated by arrows), notice necrotic area in xylem parenchyma, and no visible changes in pith (X100). C, longitudinal section notice, mycelium colonized cortex layer with irregular shape of cells and Defense Structures as shown by arrow (X400).

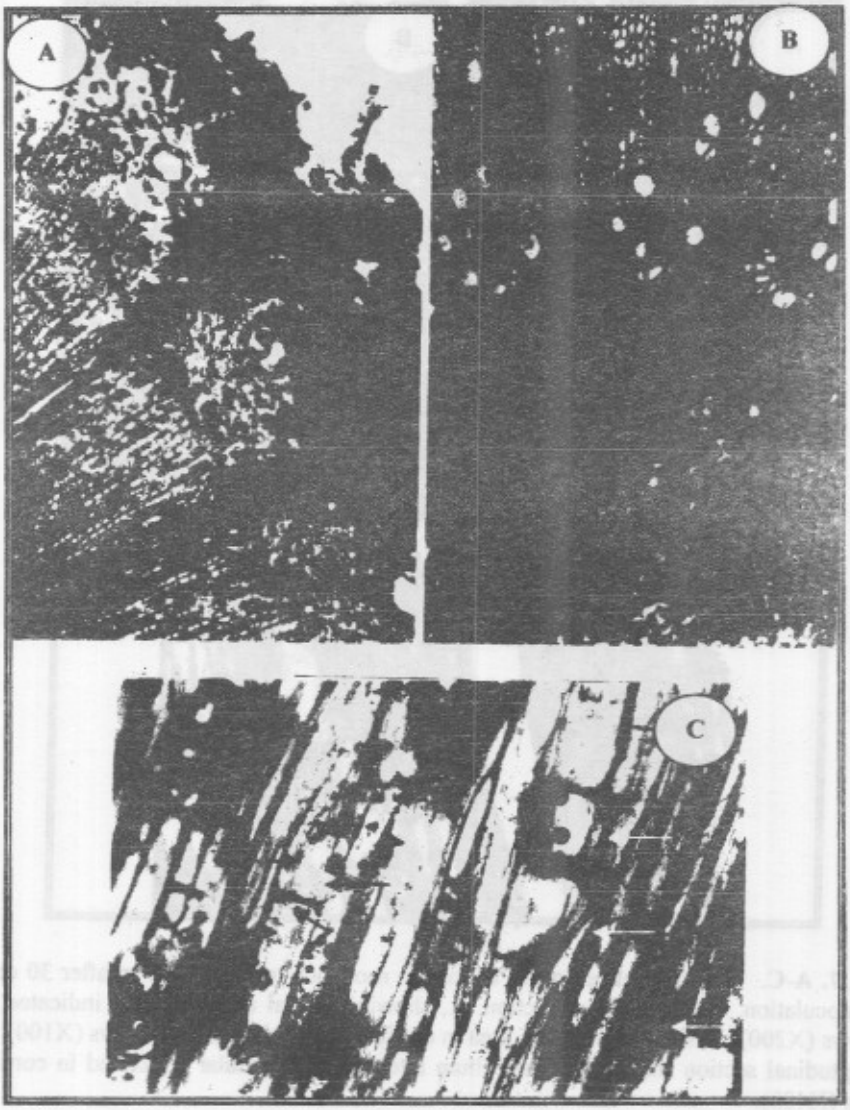


**Fig. 6. A-C.** Histological responses of mango rootstock to *Phytophthora* sp. after 30 days from inoculation. A, transverse section showing plasmolysis and disorganization with dark brown discoloration of epidermal cells; cortical and R (X40). B, transfer section notice, pith cells are destroyed (X100). C, longitudinal section showing extension of mycelium inter and intra cellular from cortical cells to xylem parenchyma and xylem vessels indicated by arrows (X400).

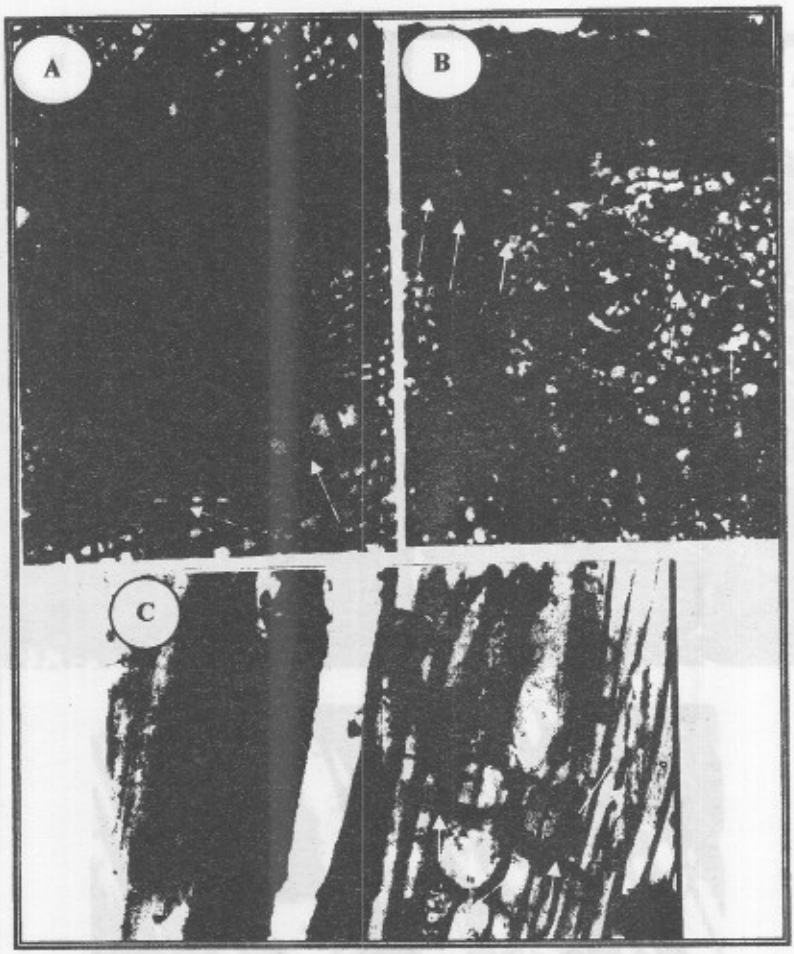


**Fig. 7. A-C.** Histological responses of mango rootstock to *Pestalotia* sp after 30 days of inoculation. A-B transverse section. A, showing partial necrosis in X indicated by arrows (X200). B, showing necrotic area in cortical cells indicated by arrows (X100). C, longitudinal section showing the mycelium inter and intracellular concerted in cortical cells (X400).

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## دراسات مرضيه تشريحية على أصول المانجو المصابة ببعض مسببات أمراض النباتات المحمولة بالتربة.

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أظهرت الدراسات التشريحية المرضية لجذور أصول شتلات المانجو (صنف G3القابل للاصابة) عمر ٦ أشهر (سبق عدواها بفطريات أثبتت قدرتها المرضية) قدرة هذه الفطريات على غزو أنسجة الجذور المختلفة (قشره، لحاء، خشب). حيث أظهر الفحص بالميكروسكوب الضوئى لقطاعات طوليه وعرضيه بعد ١٤ يوم من العدوى الصناعية أن طبقة القشرة قد استعمرت تماماً وذلك في حالة الاصابة بالفطريات بتروديبلوديا ثيوبرومى، فيوزاريوم سولانى، ريزوكتونيا سولانى وماكروفومينا فاصيولينا مع بلزمه وعدم تمييز لخلايا البشرة والقشرة وتلونها باللون الأسود وهو عبارة عن الخلايا والانسجه المتكرنزة والمحطمة . أيضا شوهدت مساحات ميته في طبقة القشرة بعد ١٤ يوم من العدوى الصناعية. في حين لم تلاحظ هذه التغيرات في حالة الاصابه بفطري فيتوفثورا و بستالوشيا وذلك بعد ١٤ يوم من العدوى الصناعية. أما بعد ٣٠ يوم من العدوى الصناعية فقد شوهدت مساحات ميته في كل من بارانشيما وأوعية الخشب . مع ملاحظة وجود عدد وفير وكبير من التيلوزات داخل الأوعية الناقلة في حالة الاصابة بفطرى بتروديبلوديا ثيوبرومى و فيوزاريوم سولانى.