

Histo-pathological evaluation of the *Fusarium* species causing malformation disease of *Mangifera indica*

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Abstract

Mango malformation is a serious disease in tropical and subtropical areas of the world and has been attributed to various *Fusarium* spp. The results of pathogenicity test under greenhouse conditions for 7 different isolates of the fungus *Fusarium* i.e. *F. subglutinans*, *F. solani*, *F. oxysporum*, *F. sterilihyphosum*, *F. proliferatum*, *F. moniliforme*, *F. avenaceum* and *F. chlamydosporum* have recorded that the *F. subglutinans* is the main cause of the mango malformation disease followed by *F. oxysporum*, *F. sterilihyphosum* and *F. proliferatum* while the other isolates appeared the symptoms of root rot when used as inoculated soil with spores suspension. Histological studies of the apical buds in mango cv. Sedekia revealed the presence of disorganised cells, degradative, hyperplasia areas and fungal mycelial infection at the base of the malformed bud during bud-inception stages. Also, large and small degenerated zones were observed in different tissues of cross-section, i.e. cortex, vascular tissues and pith zone. The infection resulted on seriously damaged and degenerated tissues which show, vast and large protolysis areas. Among other possible reasons, tissue damage caused by the fungi may lead to the abnormal development of the malformed tissues. According to the pathological and histological studies, *F. subglutinans*, *F. proliferatum*, *F. oxysporum* and *F. sterilihyphosum* were identified as the causal organisms of mango malformation. *F. subglutinans* is the main pathogen.

Keywords: Histo-pathological; *Fusarium* spp; Mango malformation

Introduction

Mango (*Mangifera indica* "L") is the most important fruit crop in Egypt. Mango ranks third in exports after citrus and grapes. Egypt produces 232,000 tones of mango annually and exports moderate amount (about 1,500 tones) annually to 20 countries in near east and Europe. Although, the area of cultivated region of new land is 42,870 feddan whereas the area of cultivated region of old land is 140,290 feddan, the productivity of new land is greater than the old land (6.46 & 4.32, respectively); FAO 2007. The area under this economic crop was about 85,972 feddans (36,108.24 hectare) and average of mango production reached 287,226 tons during 2000 [2].

Mango suffers from several biotic and a biotic stresses at all stages of its life right from the plants in the nursery to the fruits in storage. Mango malformation is one of the most destructive mango diseases [11]. Vegetative malformation is most serious on seedlings, small plants and also occurs on mature trees [13,14]. Apical and axillary buds produce misshapen shoots with shortened internodes and dwarfed leaves that are brittle towards the supporting stem. Shoots may not expand fully; resulting in bunched appearance (i.e. the 'bunchy-top' symptom of the disease). Floral malformation is most important. Affected inflorescences usually do not set fruit or fruits are aborted. Primary or secondary axes on affected panicles are shortened, thickened and highly branched. Malformed panicles produce up to three times the normal number of flowers, range from one-half to two times the normal size, and have an increased proportion of male to perfect flowers. Malformed panicles may also produce dwarfed and distorted leaves (exhibit phyllody) [12].

A second species, *Fusarium sterilihyphosum* Britz, Wingfield and Marasas, was described originally four isolates from a small area in South Africa [3]. At least four *Fusarium* species have been associated with MMD worldwide. *Fusarium subglutinans* [*Gibberella fujikuroi* var. *subglutinans*] appears to have a significant role in malformation. In subsequent work, it was detected in Brazil, and was recently shown to cause malformation in Brazil after artificial inoculation [9]. *Fusarium oxysporum* Schlecht emend. Snyder and Hansen were reported in

Egypt and Mexico [7], but these reports appear to be have neither been described nor shown to cause the disease [11,1]. *Fusarium* spp. nov. [3] and *F. proliferatum* (*Gibberella intermedia* (Kuhlman) Samuels, Nirenberg and Seifert) were recovered from malformed mango trees in Malaysia [8], but their pathogenicity has not been determined. During a survey of mango plantations in Sindh for investigating the association of fungi with mango malformation disease (MMD), six fungal species viz., *Fusarium nivale* (Fr.) Ces, *F. oxysporum*, *F. moniliforme*, *F. semitectum*, *Alternaria alternata* and *Aspergillus niger* were isolated and identified on the basis of their colony characteristics and conidial morphology. *F. nivale* (Fr.) Ces, was predominantly isolated from malformed tissues of infected inflorescence; it produced white colonies and some discoloration of the agar medium was noticed around the growing mycelium. This is the first record of *Fusarium nivale* (Fr.) Ces. From Pakistan and also the first report of its association with mango malformation in Sindh, Pakistan [3]. *F. sterilihyphosum* and *F. proliferatum* are recorded as association with mango malformation in Egypt [4]. To date, Koch's postulates have not been completed with them.

Thus, objective of the present research is to determine the pathogenicity of different fungi associated with malformed tissues and establish the cause agents of mango malformation in Egypt.

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Materials and Methods

Seven *Fusarium* specie i.e. *F. oxysporum*, *F. proliferatum*, *F. subglutinans*, *F. sterilihyphosum*; *F. moniliforme*, *F. avenaceum* and *F. chlamydosporum* isolated from malformed mango blossom tissue were tested for their ability to cause malformation. Mango seedling cv. Sedekia (two years old) was inoculated with 10^5 colony forming units of *Fusarium* spp. as inoculated soil. Four replications of six seedlings each were evaluated. Sterilized water was used as a control. Transplanted seedlings were monitored for development of malformation. At the end of the experiment (120 days), all surviving seedlings were examined for apical disease symptoms. Data were recorded on symptoms manifestation as diseases incidence.

Anatomical studies

Samples for anatomical studies were taken from shoot apex one-two cm below the tip of young seedlings of *Mangifera indica* "L". at the age of 3 years old grown in soil inoculated with 10^5 colony forming units/g soil with pathogenic fungi. Samples were killed and fixed in F.A.A solutions (formaldehyde :acetic acid: 50% ethanol; 5: 5: 90) dehydrated in ascending concentrations of ethyl alcohol according to [6] then cleared by soaking in series of xylene-absolute ethanol, and embedded in paraffin wax (M.P.55-58Co). Using rotatory microtome (Binko-LTD-Japan), serial of both longitudinal and transverse sections (15-20 μ m) were obtained and stained with saffranin-light green combination [15] and mounted in Canada Balsam. The sections were examined by Nikon light microscope and photographed by Olympus digital camera E-330.

Statistical analysis

For data analysis, the statistical computer application package SPSS 10.0 will be employed. Data will be subjected to analysis of variance (ANOVA) and the means will be compared for significance using Duncan's Multiple Range Test (DMRT; $P = 0.05$).

Results and Discussion

Seven fungi viz. *F. subglutinans*, *F. oxysporum*, *F. sterilihyphosum*, *F. proliferatum*, *F. moniliforme*, *F. avenaceum* and *F. chlamydosporum* isolated from mango malformed tissue were tested using susceptible Sadekia cultivar as inoculated soil (Table 1). Data pertaining to artificial inoculations revealed that effort to produce disease by soil inoculation with spores suspension. Four *Fusarium subglutinans* proved to be the dominant fungus with 100% sample's infection in inoculated soil. Fungi *F. oxysporum*, *F. sterilihyphosum* and *F. proliferatum* showed moderate infection in induced typical malformation symptoms in inoculated mango seedling and were re-isolated. Other *Fusarium* spp. give grown and root rots symptoms. The present investigation deals with histological features of the shoot apex of the stem one cm below the tip of *Mangifera indica*. L. cv Sedekia, of the normal and malformed seedlings of 2 -years old infested with *Fusarium* spp. The anatomical study involved the histopathological differences between the normal

Treatment	Disease severity	Disease incidence %
<i>F. subglutinans</i>	4.0	100.0
<i>F. oxysporum</i>	1.3	50.0
<i>F. sterilihyphosum</i>	2.3	50.0
<i>F. proliferatum</i>	1.6	50.0
<i>F. moniliforme</i>	0.0	0.0
<i>F. avenaceum</i>	0.0	0.0
<i>F. chlamydosporum</i>	0.0	0.0
LSD	0.5	25.0

mango cv. Sedekia seedlings

Values with the same letter in the same column are not significantly different at $P < 0.05$.

Table 1: Comparative virulence of selected *Fusarium* isolates on inoculated.

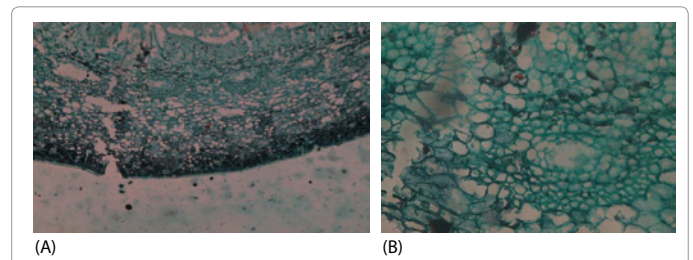


Figure 1: Histological Studies of the main axis one cm below apical apex of mango seedlings cv. Sedekia infected by different *Fusarium* spp. 1 A) *F. oxysporum*.

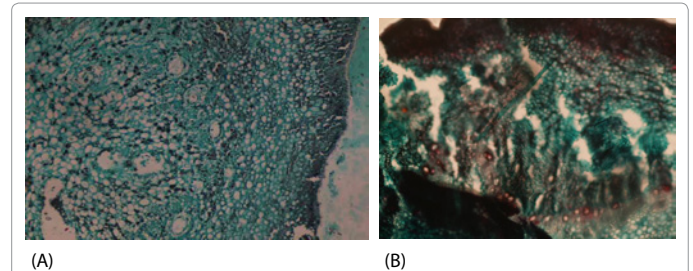


Figure 2: *F. Proliferatum*.

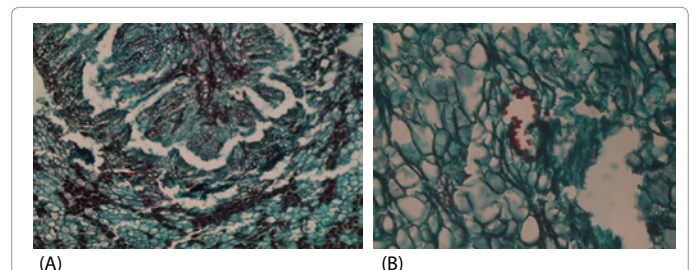


Figure 3: *F. subglutinans*.

and malformed stems shoots apex infected with different strains of *Fusarium* (Figures 1-8).

Anatomical studies

The present studies were aimed to study the behavior of fungus, *Fusarium* spp. of inoculated mango seedlings cv. Sedekia growing under greenhouse conditions. When soil was infested with the fungus, 12 weeks post inoculation, strong colonization of micro and macroconidia of *F. subglutinans*, *F. proliferatum*, *F. oxysporum* and *F. sterilihyphosum* was observed on the malformed apical vegetative buds. Also, symptoms of apical and lateral floral malformation appeared where mycelium of *Fusarium* spp. were present in the tissue at high concentrations. The anatomical observations of the cross-section of the main axis of seedlings infected with *F. oxysporum*. Histological examination of cross-section revealed the presence of large and small degenerated areas with protolysis symptoms on the epidermal and outer cortical layers in hypertrophy and hyperplasia shapes of cortical cells was also recorded (Figure 1A). Thin and dark colored fungal hyphae observed through cortical zone. Vascular tissues, which were associated with degenerated and plasmolysis effect through vascular cylinder as presented in Figure 1A and B.

Transverse-sections through main axis below the apical apex of *Mangifera indica*.L .cv sedekia seedlings infected with *F. proliferatum* showed that, both inner and outer cortical layers were infected with

dark colored fungal spores (Figure 1A) rounded shape and large size of degenerated and protolysis areas were observed on the inner cortical cells was also recorded. The data presented in Figure 1 B showed that, the infection with *F. proliferatum* seriously damaged the vascular tissue especially xylem vessels. The fungal hyphae of the pathogen were observed in two phases one as compacted and dark colored and the other as clear and thick as presented in Figure 2A and 2B .The presence of different developmental stages of pathogen growth such as compacted dark colored hyphae, clear ad thick fungal hyphae and the spores of the pathogen could be explained the harmful effects recorded through histological examination of the cross-section.

Under infection with *F. subglutinans* histological examination of cross-section of the main axis one cm below the apical apex of *Mangifera indica*.L. cv Sedekia seedlings showed that, both fungal hyphae and spores were detected (Figure 3A and B). Also, large and small degenerated zones were observed in different tissues of cross-section, i.e. cortex, vascular tissues and pith zone. The infection resulted on seriously damaged and degenerated tissues which show, vast and large protolysis areas. Such effect was also recorded on the area occupied by vascular tissues as in Figure 3.

Fungal hyphae were presented in dark colored and compacted shape. This lead to the concluded that, the infected pathogen was more active and such type of activity increased protolysis and degenerated effect on *Mangifera indica*.L. cv Sedekia tissues. In additional, the longitudinal -section from main axis below the apical apex one cm of

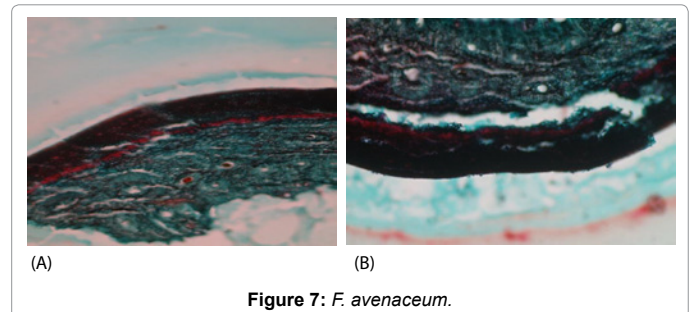


Figure 7: *F. avenaceum*.

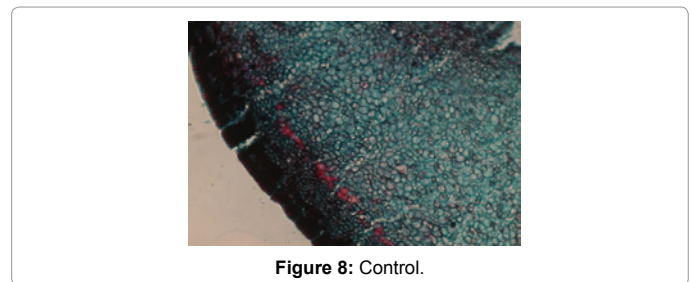


Figure 8: Control.

seedlings infected by *F. subglutinans* showed that, the degenerative cells and tissues resulted in vast and large protolytic zone (Figure 4A).

Fungal hyphae were thick in size and dark colored in shape, which was also detected through the vascular tissues. The histological investigation was also revealed the presence of clear shape and thick size of fungal hyphae extended through different localization of protolysis areas as presented in Figure 4B. Fungal spores was also detected in different areas occupied by longitudinal - section from above results it could be concluded that, the large and vast degenerated area as well as the detection from dark colored and clear shape of thick fungal hyphae and clear shape of thick fungal hyphae and occurrence of fungal spores, were resulted from higher activity of the *F. subglutinans* which was in different developmental stages.

Transverse-sections of the main axis below the apical apex of the *Mangifera indica*. cv. Sedekia infected with *F. sterilihyposium* were concerned as degenerated areas which may be due to protolytic of cells (plasmolysis).such effect was clearly detected on the area occupied be outer cortical layer as presented in Figure 5A. Hypertrophy of cortical cells was also detected through the inner cortical layers. Also, dark colored fungal hyphae were run through intercellular spaces of cortical cells which formed a net shape (Figure 5B).

Histological examination of the main axis one cm below apical apex of seedlings infected by *F. moniliforme* showed the following longitudinal section, the fungal hyphae were more developed with thick and clear shape through the vascular tissues. The infected area by the pathogen *F. moniliforme* showed both dormant and germinated spores. Also, both hypertrophy and hyperplasia cells were detected as in Figure 6. The occurrence of the clear thick fungal hyphae and both dormant and germinated fungal spores, could be due to the fact that, the fungus was in more developed stages of growth.

The anatomical observations of the cross-section of the main axis of seedlings infected with *F. Avenaceum* showed that, the epidermal and outer cortical layers were compacted in shape and dark colored. However, pronounced and vast lacunae extended through the outer cortical layer which was also detected through the inner cortical layer (Figure 7). Such response could be due to the hyperplasia and plasmolysis of the tissues. Some cortical cells disappeared bigger in size

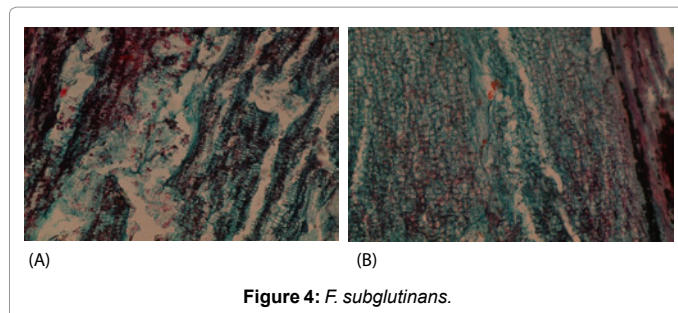


Figure 4: *F. subglutinans*.

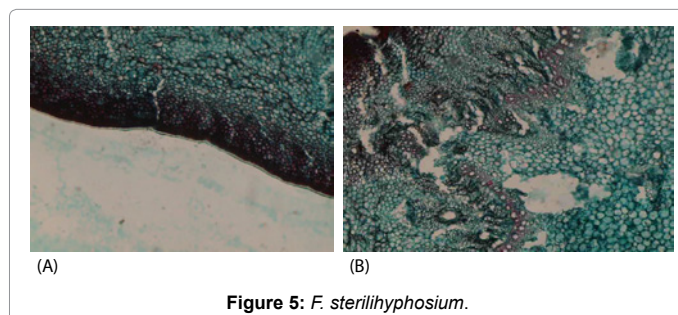


Figure 5: *F. sterilihyposium*.

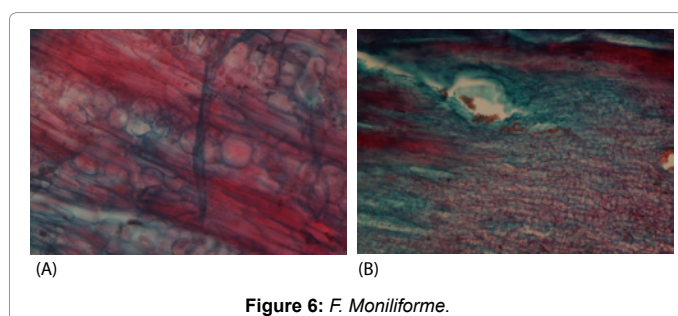


Figure 6: *F. Moniliforme*.

from the normal size of the cortical cells, which were predominately in inner cortical layers. This response may be related to hypertrophy effect. In this regard Narasimhan [10] suggested that, both hypertrophy and hyperplasia may be responsible for malformation of mango inflorescence. The mostly evident histological features of infection detected on the present investigation were the detection from thick and dark colored fungal hyphae run in paralleled rows through the area occupied by cross-section as presented in Figure 7. Also the spores of infected pathogen were spread in various tissues of the transverse-section (Figure 7) even on the plasmolysis zones.

The histological aspects of the main axis of the normal (control) seedlings are illustrated in Figure 8. It realized that epidermal cell was covered by thick cuticle, below the epidermal layer one row of scleredies elongated in shape was detected (Figure 8). The cortical layer was thicker and consists of multilayer (about 26 layers). The cortical cells were differ in shape and size with narrow intercellular spaces. The outer cortical layers was occupied by smaller cortical cells than the inner cortical layer, which were compacted, tightly packed and dark coloured. The vascular tissues and pith appeared also normal in shape and proved the avoidance of any infection and the absence of any abnormality in the structure. such type of results were in complete accordance with the data obtained by Ibrahim and Foad [5] who could not detected from histological differences between the malformed and healthy seedlings. The development of hyperplastic and hypertrophied cells is also observed. The vascular tissues are poorly developed and palisades are absent [5]. In floral malformation the surface of bud turns a warty and puckered and dense growth of epidermal hairs appears on the surface. Other anatomical structures remain almost the same, except affected ones contain much larger number of cells with brownish fluid. The cortex and rachis of panicle may develop hyperplastic cells [10]. The number of cells per unit area of cortex, xylem vessels and pith was one and half times less in malformed panicles than in healthy ones. The thickness of rachis and smaller number of cells per field in the rachis of malformed panicles was due to enlarged cell size. Symptoms of vegetative and floral malformation appeared where mycelium of *Fusarium* species was present in the tissue at high concentrations. According to the histo-pathological studies, *F. subglutinans*, *F. proliferatum*, *F. oxysporum* and *F. sterilihyphosum* were identified as the causal organisms of mango malformation. *F. subglutinans* is the main pathogen. The results of these studies will be helpful for future statistics, management, forecasting and experimental designing.

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