Scanning Electron Microcopy Studies on Mango Malformation

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Abstract: Mango malformation disease (MMD) is an economically important disease of *Mangifera indica* globally. *Fusarium subglutinans* has been associated with mango floral and vegetative malformation although confusion still remains regarding the etiology of the disease. In order to determine the *Fusarium subglutinans* penetration site, artificial inoculation of mango seedlings variety Alfonso were conducted. When soil was infested with *F. subglutinans*, the malformation was detected in the buds, three months post inoculation. Symptoms of the disease include loss of the apical dominance and swelling of vegetative buds, proliferation of leaves and flowers, phyllody and hypertrophy of panicle axes. Using scanning electron microscope (SEM), symptoms of vegetative and floral malformation appeared where mycelium of *Fusarium subglutinans* were present in the tissue at high concentrations compared to that of the untreated controls. Studies also revealed the presence of, pin-sized to large holes, disorganised cells and fungal mycelial infection at the base of the malformed buds during bud-inception stages. Moreover, *Fusarium* isolate colonized seedling root systems and became systemic, spreading to above-ground plant tissues include apical and lateral buds. *Fusarium subglutinans* proved to be the dominant fungus. [Nature and Science. 2010;8(4):122-127]. (ISSN: 1545-0740).

Key words: Egypt, *F. subglutinans,* Mango Malformation, *Mangifera indica*.

1-Introduction

Mango (*Mangifera indica* L.) is universally considered one of the most important fruit crop in tropical and subtropical areas of the world. Mango is attacked by various animate and inanimate diseases, which causes 50–80% loss in yield and was described as the abnormal inflorescence (Singh 2006). Malformation is the most notorious malady amongst the animate problems affecting both vegetative and floral parts of mango. Apical or axillary buds turn into deformed and compact structures. In affected panicles, primary and secondary axes are shortened which result in fruit abortion or no fruit setting (Ploetz., 2003). Malformation is noticed on seedlings and saplings organs. Malformation is the most threatening disease causing colossal losses every year (Iqbal *et al.*, 2006). Mango malformation disease (MMD), was first recorded in India in 1891 (Campbell and Marlatt, 1986). Symptoms of the disease include loss of the apical dominance and swelling of vegetative buds, proliferation of leaves and flowers, phyllody and hypertrophy of panicle axes. The vegetative deformation may also affect immature trees and nursery stock, which can lead to the spread of infected plants. More important, however, is the affect of malformation on fruit set: fruit in affected panicles either do not set or abort. Primary and secondary axes on affected panicles are shortened, thickened and greatly branched (Kumar *et al.*, 1993). Mango malformation has been intriguing scientists as to its cause and control for more than 100 years. The earliest hypothesis that mites caused the disorder did not last long as acaricides failed to control the problem (Yadav, 1999). The disease has been associated with physiologic disorders and hormonal imbalances (Tapan *et al.*, 2006) and attacks of an eriophyid mite, *Aceria* (*Eriophyes*) *mangifera* (Doreste, 1984). Similarly, nutrient deficiency or toxicity were discounted (Shah *et al*., 2009). However, *Fusarium subglutinans* [*Gibberella fujikuroi* var. *subglutinans*] appears to have a significant role in malformation. However, the latest citations confirm that a fungus *Fusarium subglutinans* is the cause of mango malformation (Ploetz and Gregory, 1993 and Britz *et al.*, 2002) as the causal agent of malformation. In 2002, a new species, *F. mangiferae*, was established based on nuclear and mitochondrial DNA sequences; it included strains of *F. subglutinans* from Egypt, Oman, Florida, Israel, Malaysia, and South Africa, some of which had been shown to cause MMD by artificial inoculation (Ploetz *et al.*, 2002 , Freeman, *et al.*, 2004 and Kvas *et al*., 2008). Currently, the disease has spread where mangos are grown and causes the most severe damage in Egypt (Ploetz *et al.*, 2002). The scanning electron microscope (SEM) has been shown by Scholefield (1982) and many others to be an excellent tool for presenting detail of plant structure with great depth of focus. Thus whole flowers and floral parts can be photographically presented in more detail than has been possible with light microscopy. The

present paper was further extended include scanning electron microscopy to examine the role and behavior of *F. subglutinans* in incidence of mango malformation under greenhouse.

2-Materials and Methods

2.1.Cultures

Fusarium subglutinans was evaluated for their pathogenic potential of mango seedlings cv El fonse sown in pots -30 cm diam- containing a 50:50 (v/v) mixture of vermiculite and perlite. Seedling of mango was sown into soil inoculated with 10^5 colony forming units | g of soil of pathogenic fungi. Four replications of six seedlings each were evaluated. Sterilized water was used as a control. Transplanted seedlings were monitored for development of wilt and/or foliar malformation. When malformed were developed (after 120 days), ten roots, stem and malformed pieces, each approximately 5 mm in length, from each seedling, were surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite; 1 part standard household bleach in 10 parts water), rinsed in sterile, distilled water, and placed on a selective agar medium for *Fusarium* spp. (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24 ^{0}C for 7-10 days. Emerging fungi were compared with inoculated isolates to determine whether they were the same morphological species.

2.2. SEM procedure.

Apical, lateral buds , root and stem cross section of malformed seedlings were collected and fixed in glutaraldehyde. The samples were dehydrated using a graded ethanol series and critical point dried in CO_2. The pressure was decreased very slowly to prevent tissue damage. Samples were examined by Scanning Electron Microscope using an accelerating voltage of 6 kV and a spot size of 125 nm. With some specimens, up to 30 min observation was possible. All images were computer processed.

3. Results and Discussion

The present studies were aimed to determine the fungus, *Fusarium subglutinans* associated with malformed tissues of inoculated mango seedlings growing under greenhouse conditions. *In vitro*, studies with light and SEM microscopes showed fungal mycelial infection at the base of the fully swollen malformed buds (Fig. 1 A, B and C). As the fungus could not be identified with scanning electron micrographs, healthy and mal formed tissues were cultured *in vitro*. When soil was infested with the fungus, 12 weeks post inoculation, strong colonization of micro and macroconidia of *F. subglutinans* was observed on the malformed lateral and apical vegetative buds (Fig. 2). Also, symptoms of floral malformation appeared where mycelium of *Fusarium subglutinans* were present in the tissue at high concentrations (Fig. 3). White cottony mycelial growth of *Fusarium subglutinans* formed on the fourth day from malformed tissues only. No such mycelial growth was observed in healthy buds. SEM studies also revealed the presence of damage in malformed tissue caused by the fungus, pin-sized to large holes, disorganised cells and fungal mycelial infection at the base of the malformed buds during bud-inception stages. At the same time, morphological and microscopical examination , using SEM in inoculated seedlings with *F. subglutinans* revealed the presence of fungal mycelial and micro and macroconidia infection in the stem and root vessels (Fig. 4). Percent colonization of *Fusarium* was significantly higher in either stem or root sections. In this study we have shown that *F. subglutinans* proved to be the dominant fungus infecting majority of the tissues. Little is know about the epidemiology of the disease, dissemination of conidia, location of infection sites, modes of infection and colonization of plant tissue. This data indicated that the primarily infection via root, completely colonized the seedling root systems and became systemic, spreading to apical plant tissues (apical buds). Apart from competition for nutrients, the fungus may release secondary metabolites, which could create further hormonal imbalance and inhibit the normal growth of the meristematic tissue of the buds (Tapan, *et al.*, 2006). The second infection for long distance dispersal of the pathogen is hypothesized to be via infected nursery stock or by the mango bud mite.

Among other possibilities this may suggest that the fungus, which is closer to vascular channels of the mother plant, competes for the nutrients by acting as a more powerful sink than the buds of the malformed inflorescence and could be a reason for the low uptake of assimilates by the malformed buds as observed in tracer studies (Freeman, *et al*., 2004) . The fungus *Fusarium monilifome* var. *subglutinans* was isolated from malformed parts of mango and its pathogenicity was also proved by demonstrating identical etiology for vegetative and floral malformation, but foliar applications with different fungicides failed in checking malformation (Chakrabarti and Ghosal, 1985) probably due to the fungi is systemic. The results of these studies will be helpful for future statistics, management, forecasting and experimental designing.

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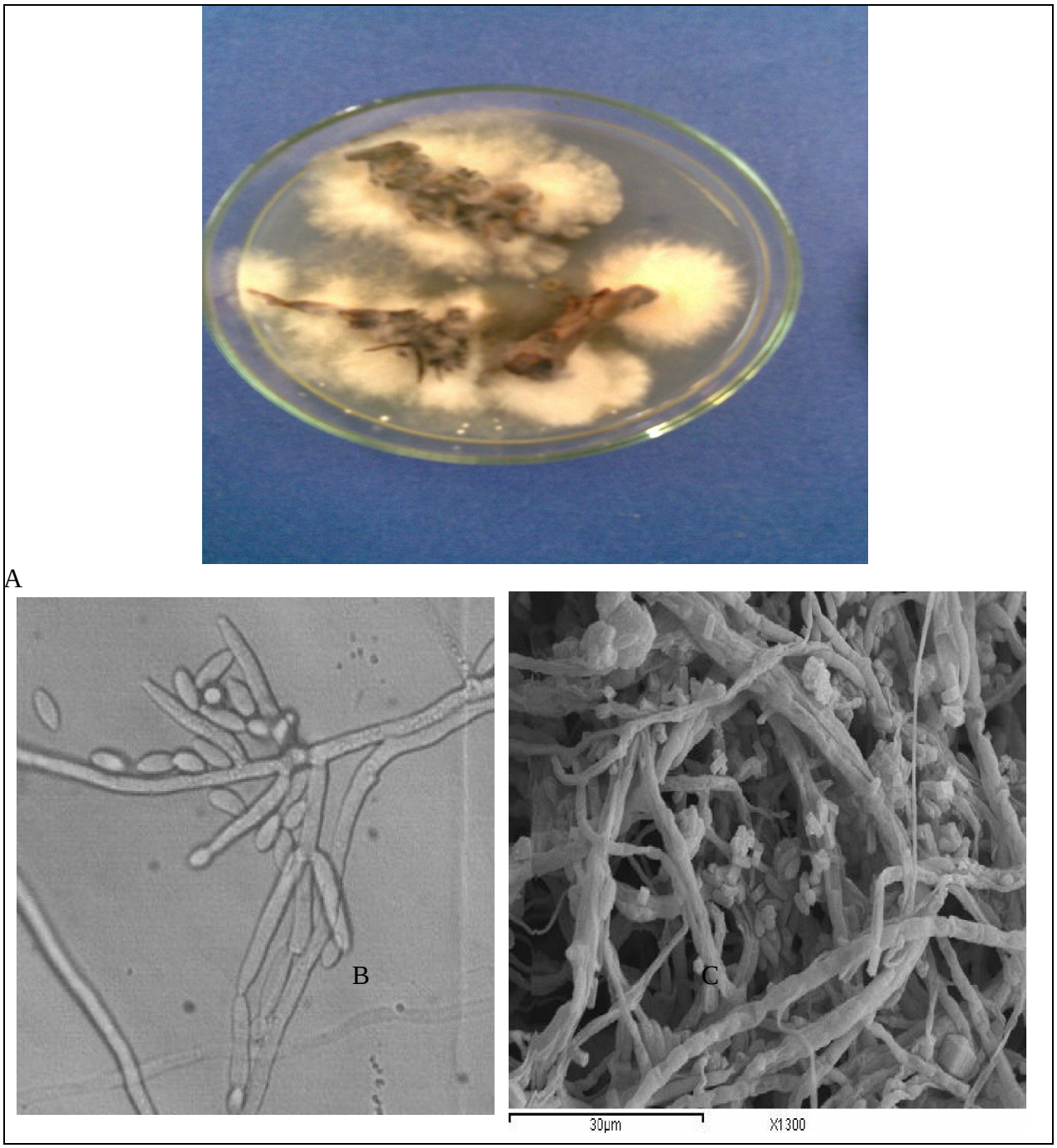
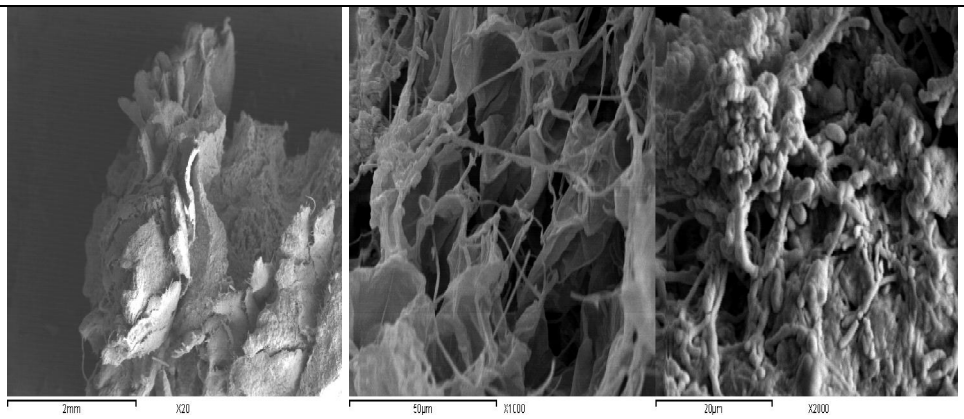
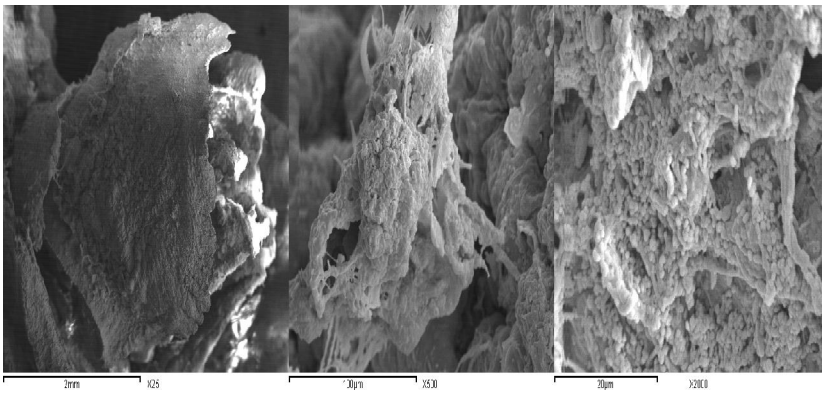


Figure 1. A) Isolation of *Fusarium subglutinans* from buds and stem
B) *In vitro,* Light microscopy of *Fusarium subglutinans*
C) *In vitro,* SEM of *Fusarium subglutinans*

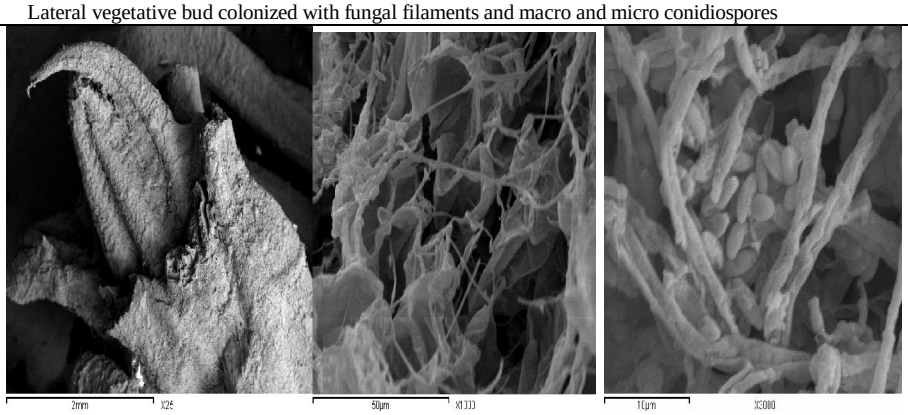


A

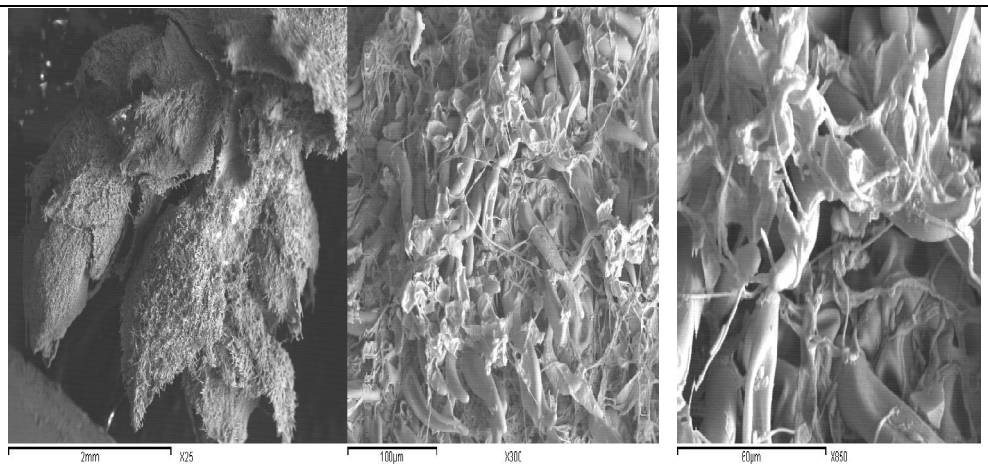


B

Figure 2 . Scanning Electron Microscope of mango tissue seedlings cv.Al-Fonso inoculated with *F. subglutinans*
A) Apical vegetative bud colonized with fungal filaments and macro and micro conidiospores
B) Lateral vegetative bud colonized with fungal filaments and macro and micro conidiospores



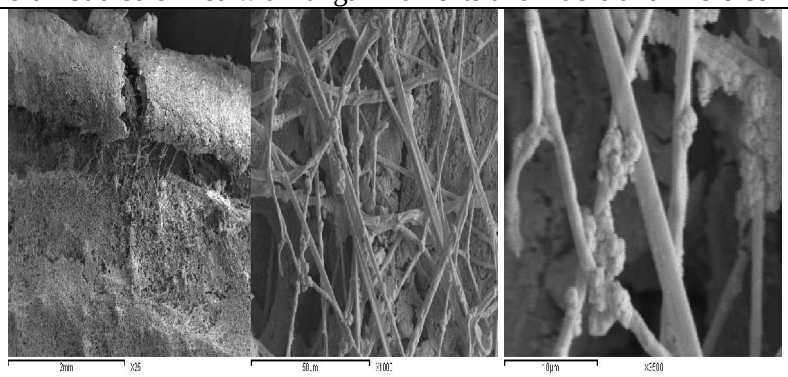
A



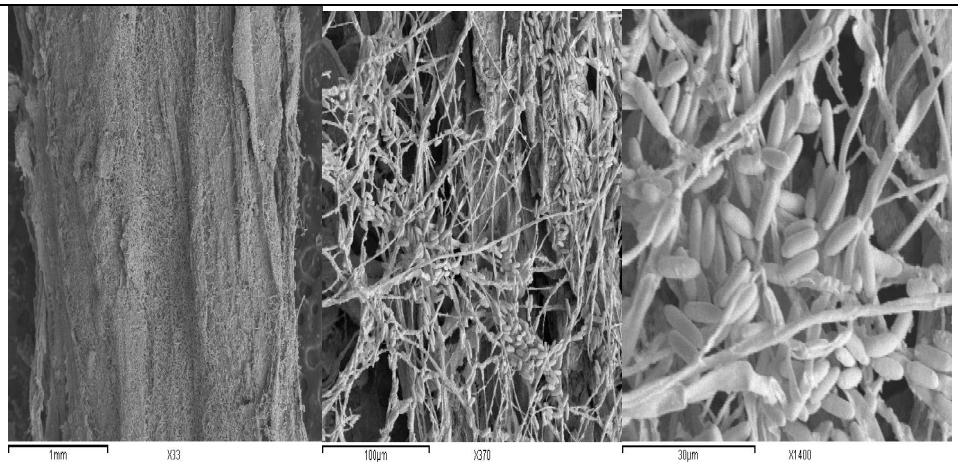
B

Figure 3 . Scanning Electron Microscope of mango tissue seedlings cv.Al-Fonso inoculated wit *F. subglutinans*

A) Apical floral bud colonized with fungal filaments and macro and micro conidiospores
B)Lateral floral bud colonized with fungal filaments and macro and micro conidiospores



A



B

Figure 4 .Scanning Electron Microscope of mango tissue seedlings cv.Al-Fonso inoculated wit *F. subglutinans*

A) Stem vessels colonized with fungal filaments and macro and microconidiospores
B)Tip root colonized with fungal filaments and macro and micro conidiospores

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