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Factors affecting epidemiology and conidial germination of *Fusarium mangiferae*, the causal agent of mango malformation

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Dedicated to my Parents

and my Husband

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List of Abbreviations

Conidia/ml	Number of conidia per milliliter
CFU	Colony forming unit
CM	Centimeter
CRD	Completely randomized design
DW	Distilled Water
g	Gram
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
L	Liter
LSD	Least significant difference
mg	Milligram
ml	Milliliter
mm	Millimeter
µM	Micro molar
MGR	Mycelium growth rate
PPM	Part per million
RH	Relative humidity
SDW	Sterile distill water

Factors affecting epidemiology and conidial germination of *Fusarium mangiferae*, the causal agent of mango malformation

Abstract:

Mango malformation, caused by the fungus *Fusarium mangiferae* is one of the most destructive diseases of mango crop, occurring in most mango producing regions worldwide. The disease is characterized by malformation of vegetative growth and inflorescence, causing serious yield loss since malformed panicles do not bear fruit. The present study investigated the spread of the disease in North of the West Bank, the influence of several physical and chemical factors on germination of conidia and growth parameter *in vitro*, and finally disease management options including biological control *in vitro*. Disease survey proved the presence of Mango Malformation in North of the West Bank; *F. mangiferae* isolates were all collected from Qalqelia. The optimum temperature for germination and growth of *F. mangiferae* was 25°C. Germination and growth were stimulated by sugars (sucrose, glucose and fructose), amino acids (Glutamic acid, proline, alanine and aspartic acid), the cations (Ca^{+2}, Mg^{+2} and K^{+}) and nitrate form of nitrogen. Germination and growth were, however, retarded by the cations (Fe^{+2}, Cu^{+2} and Zn^{+2}), and the oxidants (hydrogen peroxide and menadione). Concerning Mango Malformation management, *T. harzianum* (Jn14 and Jn58) fungitoxic metabolites were able to reduce mycelium growth of *F. mangiferae* significantly by 69% and 58% respectively, compared to the control. In addition, *T. harzianum* was more tolerant to the fungicide Fosetyl-Al than *F. mangiferae* who showed considerable sensitivity to the chemical *in vitro*. .

1. INTRODICTION

1.1. Mango Malformation

Mango malformation disease was first reported in India in 1891. Since then, it has been reported from several places in Asia (Palestine, Malaysia, and Pakistan), Africa (Egypt, South Africa, Sudan, Swaziland, and Uganda), and the Americas (Brazil, El Salvador, Mexico, Nicaragua, the United States, and Venezuela) (Britz, 2002; Freeman, 1999; Kumar, 1993; Noriega, 1999; Ploetz, 2003). Two types of malformation on vegetative and floral parts have been characterized with similar etiology (Schlosser, 1971a; Kumar & Beniwal, 1987a). The disease affects vegetative shoots of juvenile plants causing severe damage in nurseries. It also affects floral panicles causing deformation and hypertrophy (Kumar & Beniwal, 1992; Ploetz, 1994). Mango malformation can be a destructive disease with yield losses ranging from (80-100%) (Ginai, 1965; Ploetz et al., 2002). During the last two decades, the problem of malformation has assumed an alarming magnitude. It is the most threatening malady affecting mango cultivation. It has a serious impact on the propagation of trees and commercial fruit production.

1.2. Disease Etiology

The factors causing the abnormal behavior in shoots and panicales of mango tree has been of great concern. Despite completion of the Koch`s postulate for vegetative and floral forms of the disease by artificial inoculations of the fungus, still other factors have been attributed as its cause. These include hormonal imbalance, nutritional problems (Kanwar and Kahlon, 1987), physiological abnormalities (Sattar, 1946; Khader, 1986), viral infection (Das 1989; Kausar, 1959), phytoplasma, and the mite (e.g. *Aceria mangiferae*) which

was hypothesized as the causal agent of mango malformation for over 40 years mainly due to high numbers of mites observed in malformed trees (Westphal and Manson 1996). Despite the fact that the fungal theory was well established following the Koch's postulate with several fungi, certain members of the genus *Fusarium* have been shown to cause the disease (Summanwar et al., 1966; Varma et al., 1974; Noriega-Cantu´ et al., 1999; Kvas et al., 2008; Rodrı´guez-Alvarado et al., 2008). All members of the genus *Fusarium*, have been associated with this disease. *Fusarium mangiferae* (previously recognized as *F. moniliforme* J. Sheld. and later as *F. moniliforme* var. *subglutinans* Wollenw. & Reinking) has the largest geographic distribution (Lima et al, 2008). Koch's postulate was completed with this species for the first time in 1966 (Britz et al, 2002; Chakrabarti and Ghosal, 1989; Freeman et al,1999; Marasas et al, 2006), and also recently with *F. sterilihyphosum* Britz, Marasas & M. J. Wingf. (Lima et al, 2008), which proved to be distributed in Brazil and South Africa (Britz et al, 2002; Marasas et al, 2006). In addition to that a new pathogenicity lineage that is closely related to *F. sterilihyphosum* and is known only from Brazil was added (Lima et al, 2008). Another recent study from Mexico reported the successful completion of Koch's postulate with local strains of *Fusarium* sp., which were different from *F. mangiferae* and *F. sterilihyphosum* (Rodriguez-Alvarado et al, 2008). Pathogenicity tests were not conducted for two other suspected taxa, *Fusarium* sp. and *F. proliferatum* Samuels, & Nirenberg reported to affect mango in Malaysia (Britz et al, 2002; Marasas et al, 2006). It is also clear now that the isolation from malformed mango parts have displayed the dominance of *F. mangiferae* (Ploetz, 1999; Britz. et al., 2002; Ploetz et al., 2002; and Freeman et al., 2004).

1.3. Symptomology

Malformation comprises two forms of sumptoms, vegetative and floral (Kumar and Beniwal, 1987a). Both are considered to be the expression of mango malformation.

1.3.1.Vegetative malformation

Young mango plants in nursery are more vulnerable to vegetative malformation (Kumar and Beniwal, 1992). On young seedlings, the disease appears at quite an early stage. Even 3-4 months-old seedlings, have been found to be affected. Vegetative buds swell and produce small shootlets bearing small scaly leaves with a bunch like appearance on the shoot apices. Leaves are dwarfed, and are narrow, brittle and bend back towards the supporting stem and get transformed into abnormal tiny leaflets borne on very much shortened axis. Additional secondary shoots also develop with shortened internodes and scale like leafy structures to form compact masses of malformed foliage (Kuhlman et al., 1978). The multi-branching of shoot apex with scaly leaves is misshapen and have dramatically short internodes (Bhatnagar and Beniwal, 1977; Kanwar and Nijjar, 1979;Ploetz, 2004). Apical dominance can be severely affected and the shootlets and their branches are not distinguishable. Vegetative malformation is also manifested on mature trees. Axillary buds get swollen and produce small shootlets bearing numerous scaly leaves giving a bunchy appearance (Kumar, 1983). The seedlings, which become malformed early, remain stunted and die young while those getting infected later resume normal growth above the malformed areas (Singh et al., 1961; Kumar and Beniwal, 1992). Trees of ages, 4 to 8 years suffer the most (90.9%) from vegetative malformation (Singh et al., 1961). Furthermore, the disease seriously debilitates seedlings used as rootstock

and complicates the safe national and international movement of germplasm (Ploetz, 2001).

1.3.2. Floral malformation

Floral malformation that appears in the panicles significantly reduce fruit production since affected inflorescences usually do not set fruit. Thus, floral malformation is more serious problem than vegetative malformation (Mahrous, 2004). It appears with the emergence of inflorescence on shoots. Normally, the healthy panicles are greener and heavier with increased crowded branching, possess numerous flowers that remain unopened, are male and rarely bisexual (Singh et al., 1961; Schlosser, 1971; Hiffny et al., 1978). Malformation increases the number of male flowers in an inflorescence and the ovary of malformed bisexual flowers is exceptionally enlarged and non-functional with poor pollen viability or either sterile or, if fertilized, eventually abort (Mallik, 1963; Shawky et al., 1980; Ploetz, 2004). The proportion of staminate to hermaphrodite flowers is extremely high in malformed inflorescences as compared with healthy ones (Kausar, 1959; Majumdar and Sinha, 1972). The diameters of malformed buds and flowers are greater than normal as the individual flowers are greatly enlarged. Malformed panicles showing severe infection produce far greater number of flowers compared to healthy ones, although most of them remain unopened. The ovaries in such flowers are either nonfunctional, or the fruits if borne, are shed off before attaining the pea stage (Kumar and Beniwal, 1992). Primary and secondary axes on affected inflorescences are shortened and thickened. At maturity such panicles appear hypertrophied. The floral parts are bunched together to make compact structures (Praksah and Srivastava, 1987; Singh and Dhillon, 1990c; Ploetz and Prakash,

1997). Affected panicles either do not set fruit or abort fruit shortly after they have set; yield can be reduced by as much as 90% (Ploetz, 2001).

Healthy vegetative tissue

Healthy floral inflorescence

Malformed vegetative tissue

Malformed floral inflorescence

Malformed vegetative tissue

Malformed floral inflorescence

Figure 1. Symptoms of mango malformation disease on vegetative growth and inflorescences.

1.4. The Causal agent

1.4.1. Vegetative growth and propagules

The colonies of *F. mangiferae* are mostly dense and tinged with purple and rosey color (Britz et al., 2002; Iqbal, 2004). The undersurface of the potato dextrose agar (PDA) medium petridishes was orange with purple midpoint at first, which after 14 days led to a prominent purple pigmentation spreading across the whole surface. Aerial mycelia were usually abundant on PDA with no developed chlamydospores. The sporulation started quickly after two days in the aerial mycelium as microconidia; after two days, macroconidia started to appear. The macroconidia are slender, falcate, borne on monophialides and fairly abundant. Three to four-septate macroconidia were always found, while microconidia is slightly sickle shaped to straight with dorsal and ventral surface almost parallel. The size of macroconidia was 3.5-5×45-60µm. The microconidia were abundant, fusiform, oval to elliptical, and sometimes spindle shaped (Nelson et al., 1983; Iqbal, 2004).

Figure 2. Microconidia and microconidia of *F. mangiferae*

1.4.2. Taxonomy

Taxonomy of the causal fungus of mango malformation disease has passed through different phases. Summanwar et al. (1966) and Varma et al. (1969) in India were the first to report that the floral and vegetative malformition in mango was caused by *Fusarium moniliforme* (recognized later as *F. subglutinans*). Later on, it was well cited and confirmed that the fungus *Fusarium moniliforme (Gibberella fujikuroi)* var. *subglutinans* is the dominant causal agent of mango malformation (Campbell and Marlatt, 1986; Salazar-

Garcia, 1995; Kumar et al., 1997; Ploetz and Gregory, 1993; Britz et al., 2002). Total confusion resulted for many years because the fungi that cause this array of different plant diseases, including mango malformation disease, were all called "*F. subglutinans*". 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 mango malformation disease by artificial inoculation (Britz et al., 2002; Ploetz et al., 2002, Freeman, et al., 2004; Kvas et al., 2008). Subsequently another new group of fungi causing malformation were described, which shown to be phylogenetically distinct from the *F. mangiferae* in South Africa (Britz et al. 2002) and was subsequently also reported to occur in Brazil (Zheng and Ploetz, 2002) . *F. mangiferae* and *F. sterilihyphosum* are members of the *Gibberella fujikuroi* species complex, but do not form a *G. fujikuroi* teleomorph (Leslie, 1995; Steenkamp et al., 2000; Ploetz et al., 2002). Iqbal, et. al., (2010) studied the assay of malformed parts of mango varieties in Pakistan and revealed the association of four fungi viz., *F. mangiferae*, *F. pallidoroseum*, *F. equiseti* and *Alternaria alternata* while *F. mangiferae* proved to be the major infecting and dominant in association with malformed tissues of diverse origins.

Fusarium mangiferae is currently classified (Britz, 2002; Wingfield and Marasas, 2002) as:

Domain: *Eukaryota*

Kingdom: *Fungi*

Subkingdom: *Dikarya*

Phylum: *Ascomycota*

Subphylum: *Pezizomycotina*

Class: *Sordariomycetes*

Subclass: *Hypocreomycetidae*

Order: *Hypocreales*

Family: *Nectriaceae*

Genus: *Fusarium*

Specific descriptor: *mangiferae*

Scientific name: - *Fusarium mangiferae*

1.5. Epidemiology and Disease cycle

1.5.1. Sources of inoculum and infection courts

Disease epidemiology is poorly understood in terms of dissemination of conidia, location of infection, modes of infection and colonization of plant tissues (Ploetz, 2001). Most of infection studies were performed by wounding the plant tissue, assuming that a wound is necessary for fungal penetration and infection (Manicon, 1989; Ploetz, 2001; Ploetz and Gregory, 1993; Summanwar and Raychaudhuri, 1968). The primary mechanism of infection is hypothesized to be via infected nursery stock or by the mango bud mite, *Aceria mangiferae* (Figure 1), vectoring fungal conidia. Single celled microconidia are produced in abundance and carried on sympodially branched conidiophores bearing mono-

and polyphialides, while macroconidia are usually three to five cells, borne on sporodochia. No sexual stage is known for *F. mangiferae* species (Britz et al. 2002; Leslie and Summerell, 2006). Conidia are considered the main inoculum, and the fungus being a weak pathogen invades the host via soft plant parts, vegetative, floral buds and flowers (Chakrabarti and Ghosal, 1989). The highest colonization incidence were found in malformed vegetative and floral shoots, decreasing incidence in asymptomatic shoots and rare colonization in branch tissues, even when the branch is supporting malformed inflorescences (Ploetz, 1994). When a whole infected seedling were sectioned, pathogen colonization descended from the top to the lower section (Youssef et al. 2007). This study further demonstrated that survival of conidia decline very rapidly in soil and since the pathogen was not detected in mango seeds, seed coat, or flesh, implying that the pathogen is not soilborne. Furthermore, Ploetz, (1994) and Youssef et al., (2007) indicated that the pathogen is not a typical soilborne pathogen, and that mango buds are apparently the primary sites for infection. Freeman et al., (2004) as well showed that *F. mangiferae* is not seedborne and seedlings cultivated in a disease-free environment should remain disease-free. Furthermore, it was shown that the pathogen does not infect systemically and that the inoculum does not originate from the seed, although minor infections may occur from affected debris buried in the soil (Ploetz, 2003). Since the pathogen was detected in malformed panicles but was rarely detected in branches (Ploetz,1994), it was postulated that vegetative, and floral buds are probably the primary sites for infection (Ploetz, 2003).

1.5.2. Role of the mango bud mite *Aceria mangiferae*

The putative role of the mango bud mite *Aceria mangiferae* (Figure 1) was partly based on the fact that eriophyiod mites are known to cause bud proliferation, and gall symptoms of inflorescences in other plants (Westphal and Manson, 1996). In addition, herbivores may facilitate fungal infection by two main mechanisms, either by vectoring pathogen propagules, or by creating wound sites for fungal penetration (Agrios, 2005; Hatcher and Paul, 2001), *A. mangiferae*, is commonly found within closed vegetative mango buds, in both malformed and healthy trees (Sternlicht and Goldenberg 1978). These mites disseminate by wind from opening buds, land passively on a random tree, and actively find their way into mango buds. Thereafter, the mite settles and begins feeding by penetrating its stylet into the epidermal cell wall, creating shallow wounds of approximately 2-5 µm in depth (Westphal and Manson 1996). *A. mangiferae* was identified in both healthy and diseased trees, and in the absence of a direct correlation between the mite and mango malformation. It was proposed that mango malformation might result from an interaction between the mite and *F. mangiferae* (Prasad et al. 1972; Sternlicht and Goldenberg 1976). Ploetz (2001), indicated that the bud mite serves as a vector for the fungal conidia. Summanwar and Raychaudhuri (1968), recovered the pathogen from *A. mangiferae* body, when sampled from diseased trees. Manicom (1989), indicated that spraying the pathogen`s conidia on apical buds did not yield symptoms, but when mites were added, 8% of apical buds were malformed, concluding that the presence of mites may enhance infection.

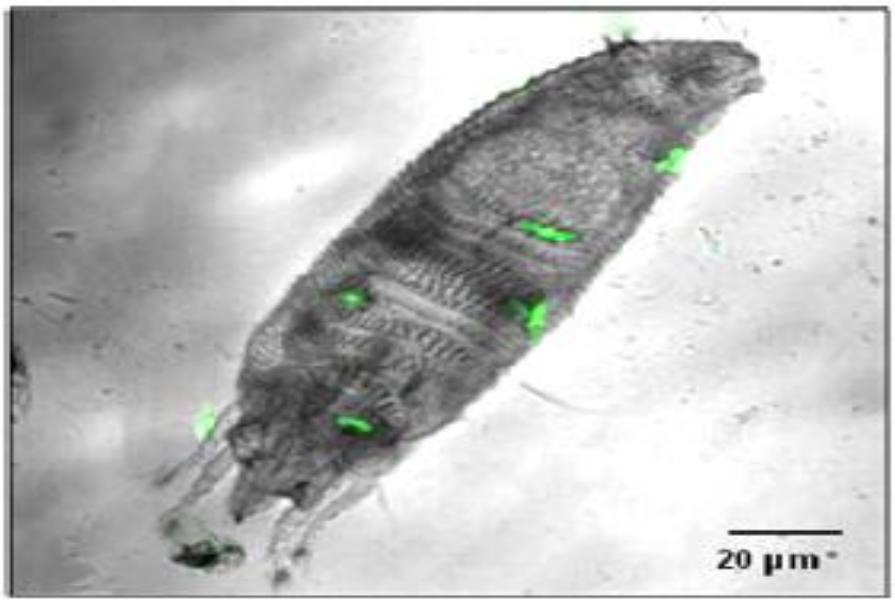

Figure 3. Mango bud mite, *A. mangiferae* (Gamliel-Atinsky et al, 2008)**.**

1.5.3. Disease cycle

Disease cycle for mango malformation caused by *F. mangiferae* is shown (Figure 2). Malformed inflorescences and malformed vegetative growth serve as sources of inoculum. Inoculum from infected panicles and malformed vegetative tissues disseminate passively in the air as conidia or fall from dry, malformed inflorescences as dry debris (Gamliel-Atinsky et al, 2009). Most of the conidia that fall on the mango canopy reach infection sites by at least three different routes: falling by chance on the apical bud, vectored on the body of the bud mite *A. mangiferae* (Gamliel-Atinsky et al, 2009), or via conidia in dry debris falling into the funnel-like structure of the apical buds. Other possible routes, could also assist conidia in reaching the apical bud (e.g., transport of conidia in dew droplets or rain splash dispersal of conidia from leaves to buds) (Gamliel-Atinsky et al, 2008 and 2009). Conidial germination and infection of

apical buds may occur if appropriate conditions are met: temperatures of 5 to 41°C accompanied by at least 2 h of wetness (Gamliel-Atinsky et al, 2008). Moderate temperatures of 15 to 30°C and longer durations of wetness (>3h) may accelerate the infection process (Ploetz, 2001; Gamliel-Atinsky et al, 2009). Presence of *A. mangiferae* inside the buds assists fungal penetration and increases frequency and severity of infection (Gamliel-Atinskyet al, 2008). After penetration, the pathogen colonizes the bud tissue but does not progress beyond this point. Apical buds could either differentiate into a reproductive inflorescence following appropriate exposure to cold temperatures or remain vegetative and develop into a young shoot (Nunez-Elisea, 1996). Inflorescences from a colonized bud may emerge malformed, probably due to a build-up of the pathogen until an infection threshold is met (Ploetz, 2001 and 2003). Alternatively, when a young shoot emerges from an infected apical bud, the pathogen may colonize the apical or lateral buds of the young shoot, but remain localized and dormant in buds until bud break. This young shoot may show symptoms of vegetative malformation or bear the pathogen within bud tissues without showing typical disease symptoms.

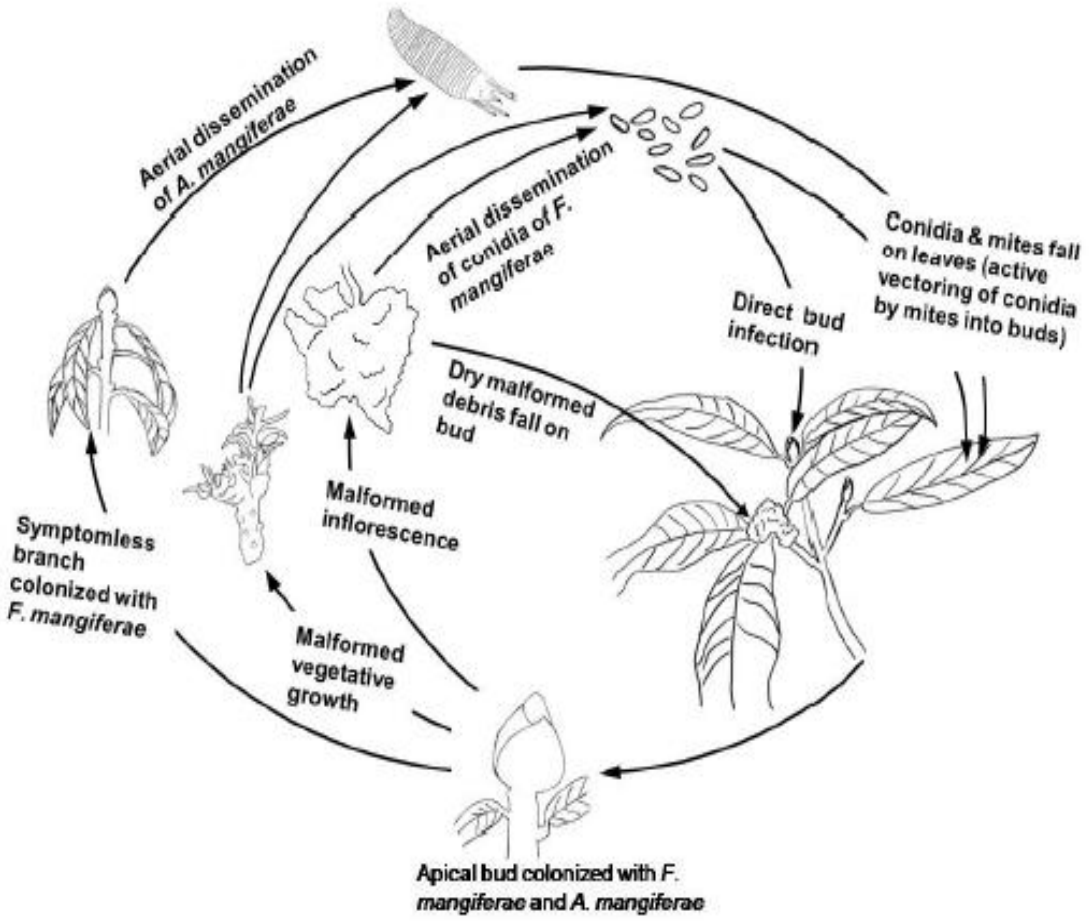

Figure 4. Proposed disease cycle of Mango Malformation (Gamliel-Atinsky et al, 2009).

1.6. Histopathology

Malformation may be dispersed by grafting infected bud wood, which is a common mean by which the disease is moved to new areas (Kumar et al, 1993). Spread on a small scale has also been demonstrated in nurseries (Prakash and Srivastava, 1987), infected nursery stock (Haggag, 2010) and mango bud mite. Most reports indicated, however, that the disease moves slowly in infected orchards (Kumar and Beniwal, 1992). Macro- and micro conidia of *F.*

mangiferae are most likely the infective propagules since they are the only propagules that are produced by the fungus and form profusely on various malformed tissues (Freeman et al., 2004). It appears that the pathogen does not behave as a typical soilborne fungus since conidia of the pathogen declined rapidly in soil under controlled and outdoor conditions (Freeman et al., 2004). Isolates of *F. mangiferae* from mango that were transformed and consequently used to artificially inoculate mango, verified that bud and flower tissues of the host are the primary infection sites, and that wounds may provide sites of entry for the pathogen (Freeman et al., 1999). Haggag et.al. (2010) observed *F. mangiferae* colonizing seedling root systems and became systemic, spreading to above-ground plant tissues including apical and lateral buds. In contrast, Darvas (1987) could not detect the pathogen in roots of malformed trees.

(Freeman et. al., 2004) detected no infection on the seed and seed coat of the fruits harvested from infected trees, as well, suggesting that the pathogen is not seed-borne. However, inoculum of the pathogen was isolated from the surface of these fruits, indicating that there is a possibility of survival and transmission of the pathogen on the surface of fruits picked from infected orchards but not through seeds (Youssef et al. 2007).

F. mangiferae was detected in 97% of seedlings apical meristems, declining gradually to 5% colonization in roots and concluded that inoculum of the pathogen originates from infected panicles and affects seedlings from the meristem, with infections descending to lower stem sections and roots (Freeman et. al., 2004). Minor infections of roots may occur from inoculum originating from infected panicles.

1.7. Conidial germination of *F. mangiferae*

Very little or no information has been published regarding conditions affecting germination and growth of *F. mangiferae* (Gamliel-Atinsky, 2009) The seasonal variation in population of the pathogen on mango shoots indicated that the fungal density reach its peak in February-March, when min/max temperature ranges from 8-27°C and relative humidity (85%). Warm and drier conditions coincided with decline in fungal population (Shawky et al., 1980; Campbell, 1986). Both conidial germination and mycelial growth required temperatures >5°C in order to commence the germination and growth processes. Low temperatures (< 5°C) did not permit conidial germination and colony growth (Leslie, 2006; Rossi, 2002). Optimal temperatures for growth and germination vary between different *Fusarium spp*. (Leslie, 2006; Rossi, 2002). The optimal temperature for germination and growth of *F. mangiferae* conidia was 28 and 25°C, respectively. Mycelial growth was better observed at temperatures between 25-30ºC and pH 7.0 on PDA medium (Akhtar et al., 1999). Accordingly, it is assumed that, temperatures in this range does not appear to be a limiting factor in seasonal disease development of mango malformation in mango cultivation areas worldwide. In most fungal pathogens, successful infection depends on a minimal duration of wetness (Huber, 1992) provided in the form of rain or dew (Carisse, 2000; Luo, 2001; Rotem, 1994; Webb, 1997). A minimum of 2-h wetness period was required for the onset of *F. mangiferae* conidial germination at 25°C; this increased with increasing exposure to wetness, and reached a peak after 8 h of wetness. Therefore, moisture is also not expected to be a restricting factor for infection under field conditions, where these requirements are routinely obtained during the rainy season (October to

March) or during heavy dew events commonly occurring during the dry season of May to August (Berkowicz, 2004; Goldreich, 2003).

1.8. Disease management

The control measure of mango malformation tested have shown inconsistent results because a reduction in the incidence of the disease was observed in some orchards but not in others (Chakrabarti, 1996).

1.8.1. Cultural Control

New plantings should be established with pathogen-free nursery stock. Scion material should never be taken from an affected orchard, and affected plants that are observed in the nursery should be removed and destroyed. Nurseries should not be established in orchards affected by malformation (Ploetz, 2001).

Breeding resistant cultivars to malformation is important and in epidemic prone areas, alternate bearing and late flowering varieties should be grown (Pandey, 2003).

Pruning: Moderate pruning of 20 cm shoots bearing malformed panicles in the month of January at panicle emergence stage can be effective in suppressing the incidence of malformation (Sirohi, et al., 2009), which is usually very high in early emerging flower buds and panicles, (Singh, et al., 1974). Pruning of shoots probably removes malformation inducing principle (Kumar et al.,1993) which accumulate at the shoot tip. Conventionally, affected terminals and the subtending three nodes are cut from trees, removed from the field and burned. If these measures are practiced for 2 or 3 consecutive years, the disease can be reduced to insignificant levels. Thereafter, the disease can be kept in check by removing symptomatic tissues every other year (Muhammad et al., 1999; Ploetz, 2001).

Combination of pruning , insecticides, fungicides and growth regulators may control mango malformation (Ploetz, 2001; Varma et al., 1974).

Integrated management package which includes sanitary pruning, incorporation of organic matter to the soil, control of vectors, irrigation management, balanced chemical fertilization, protection of new buds, weed control and promoting anticipated blooming (Giim, 1998; Noriega et al., 1999) may keep the disease severity below economic level.

1.8.2. Chemical Control

Fungicides provide a potential tool to control mango malformation disease where other measures prove ineffective. The effectiveness of fungicides depends on its innate toxicity and permeation. Different protectants and systemic fungicides have been reported effective *in vitro* against the fungus *F. mangiferae.* Kumar (1983) found Carbendazim as highly effective fungicide *in vitro* against *Fusarium* sp. isolated from malformed mango tissues. Akhtar et al. (1999) determined the sensitivity of *F. mangiferae* to six fungicides (Benlate, Antracol, Topsin-M, Dithane M-45, Annvil and Nordox). These fungicides inhibited fungal growth at low concentrations. Benlate has been found effective by different workers to reduce the intensity of malformation (Hafeez et al., 1989). Application of Carbendazim as spray has been reported to be effective in reducing severity of floral malformation (Kumar and Beniwal, 1992). Diazinon, Captan and Benomyl were also reported to result in satisfactory control of malformation (Khurana and Gupta, 1973; Ibrahim et al., 1975). Bavistin proved very effective giving disease reduction of 95 % (Mehata et al., 1986). Benlate, Carbendazim and Topsin-M belong to the same Benzimidazole group. They are systemic and are readily absorbed to reach the target part. Benlate and

Carbendazim have the same active ingredient Methyl 1-2 Benzimidazole carbamate (MBC). Great similarity in fungitoxic spectrum, mode of action and their chemical structures has been reported. They interfere with mitosis in cells and fungal hyphae and have good correlation between *in vitro* efficacy and disease control (Singh, 1984).

The use of other chemical substances as foliar application proved to be effective in reducing mango malformation disease, because they may delay or advance the beginning of flowering (Shawky et al., 1978 and Nunez et al., 1986). In addition, the application of plant growth regulators such as gibberellic acid (GA_3) at 50 ppm reduced flower malformation of Taimour mango trees (Shawky et al., 1978; Azzouz et al., 1980 and 1984). Foliar sprays of Naphthalene acetic acid (NAA) at 100-200 ppm in October reduced the incidence of malformation in the following season particularly at the higher rate (Majumder et al., 1970, 1976 and Majumder and Diware, 1989; Mahrous, 2004). Singh and Dhillon (1986) showed, that the incidence of floral malformation was reduced most by using NAA at 100 ppm and also by Indole-3-butyric acid IBA at 200 ppm prior to flower bud differentiation.

Partial control of mango malformation can be accomplished by spraying the diseased parts with mangiferin Zn^{++} and mangiferin Cu^{++} chelates since, mangiferin metal chelates reduced the abnormally high concentration of mangiferin in the malformed tissues (Chakrabarti and Ghosal, 1989). Mangiferin treatment also increased the contents of chlorophyll, carbohydrates and total nitrogen (Chakrabarti and Ghosal, 1989).

1.8.3. Biological Control

Trichoderma sp. has been studied as a biocontrol agent against soil–borne and foliar plant pathogenic fungi (Inbar and Chet, 1994). Many researchers have reported that several strains of *Trichoderma* had a significant reducing effect on the plant diseases caused by *Fusarium* sp. (Dubey et al. 2007; Gupta et al. 2010). *Trichoderma* spp. grew considerably faster than pathogenic *Fusarium* under the same conditions. The rapid growth of *Trichoderma* has an advantage to compete with plant pathogens for the space and nutrients. *Trichoderma* also develops its arsenal of mycotoxins (Simon and Sivasithaparam, 1988) and contributes in controlling many crop diseases. Strains of *Trichoderma* proved to produce antifungal metabolites which check the growth of various fungi, act as competitors (Kucuk and Kivanc, 2003) and promote plant growth (Inbar et al., 1994). Volatile compounds released by these bioagents have also been found effective against different pathogens (Hutchinson and Cowan, 1972; Tapwal et al., 2004; Gupta et al., 2010). Bhatnagar and Beniwal (1977) reported that mango malformation is systemic in nature and research findings indicated that vegetative malformation in nurseries may spread through soil. Hence, application of bioagents in nursery may be advocated, which may help in the management of vegetative malformation in nurseries along with other control measures. Application of *T. harzianum* may also control or suppress other soil-borne diseases of mangoes in nurseries.

The growth of *F. moniliforme* var. *subglutinans* was antagonistically inhibited by the bioagents of *Trichoderma* spp. by a significant production of non-volatile antibiotic substances (Kumar et al., 2012). Maximum inhibition was observed using the culture filtrate of *T. harzianum* followed by *T. virence* and *T. viride*. Kumar and Dubey (2001) reported similar results using *F. solani* f. sp. *pisi* through the production of non-volatile substances. Hajieghrari et al. (2008) also

reported that *T. harzianum* was able to inhibit *F. graminearum* through production of non-volatile substances under controlled conditions.

Kumar et al. (2012), showed that *T. viride, T. virens* and *T. harzianum* isolates significantly inhibited the radial growth of *F. moniliforme* var. *subglutinans* by releasing volatile compounds. Similar reports for *T. viride* and *T. harzianum* as the best antagonists for the growth inhibition of *F. oxysporum* f. sp. *ciceris* in dual culture and through production of volatile inhibitors were reported by Dubey et al. (2007).

1.9. Study Objectives

The current study was conducted to

1. Survey the disease on Mango trees distributed in North of the West Bank.
2. Improve the selective media for the isolation of *F. mangiferae*.
3. Evaluate the influence of various physical and chemical factors on conidial germination of *F. mangiferae* including time, conidial concentration, temperature, sugars, amino acids, pH, inorganic nitrogen form (NO^{-3} and NH^{+4}), cations and oxidants.
4. Evaluate disease management options :
    - ✓ Fungicides (Aliete).
    - ✓ Biological control (*Trichoderma herzianum*).

Chapter 2

2. Materials and Methods

2.1. Disease Survey

2.1.1. Collection of diseased samples:

Twenty four bud samples were collected from five mango orchards in North of the West Bank (Qalqelia and Tulkarm) during the flowering period of mango trees (May/ 1-9, 2010 / 2011) (Table 2.1). Suspected bud samples were collected from fields of mango trees having the symptoms of vegetative mango malformation (e.g. thicken young shoots, short internodes and dwarfed scaly malformed leaves). Floral malformation visible symptoms included (short thick floral panicales, large flowers and hermaphrodite flowers with no fruits). Suspected samples were placed in a plastic bag and saved in the refrigerator in the lab until sampling.

Table 2.1. Mango orchards screened for mango malformation in North of the West Bank

Orchard Number	Location	No. of Samples
1	Qlaqelia	5
2	Qlaqelia	9
3	Qlaqelia	3
4	Tulkarm	4
5	Tulkarm	3

2.1.2. Isolation of *Fusarium* species from infected mango buds:

Suspected samples were cut into small pieces (4-5 mm), washed in sterilized distilled water, surface-sterilized by immersion in 1% sodium hypochlorite solution for 3 minutes, and washed again with sterilized distilled water. Three pieces were placed in each 90 mm Petri dish containing *Fusarium* selective medium Nash (Nash and Snyder, 1962), and other Petri dishes containing (PDA) medium with 500mg/L chloramphenicol. Plates were kept in an incubator at 25ºC ± 1ºC for 8 days until fungus growth was visible. The tips of fresh fungal growth from plates were transferred to plates with PDA. Finally, every suspected *Fusarium* isolate was further purified by subculturing single-spores on PDA plates.

2.1.3. Identification of *F. mangiferae*

Suspected fungi isolates from each malformed plant sample were grown on growth medium (Nash selective medium and PDA), and incubated at 25ºC for 12 days with continuous light to promote typical coloration and maximum micro and macroconidial production. *F. mangiferae* was identified morphologically according to (Britz et al., 2002), based on growth features such as colony color, colony growth, no of septation of conidia, presence/absence of polyphialides and chlamydospores. The colony diameter of isolates was observed after 3, 6, 8 and 12 days of incubation.

2.2. Selective media enhancement for the isolation of *F. mangiferae.*

Nash medium was modified as a selective media for the isolation of *F. mangiferae* from soil by adding different concentrations of the fungicide Dodine (inert). The Nash medium is composed Per litter of 20g agar, 15g peptone, 1g KH_2PO_4, 0.5g $MgSO_47H_2O$ and 0.5g Chloramphenecol. Different concentrations of Dodine (0, 0.5, 1, 1.5 and 2.5 ppm) were added to the medium. *F. mangiferae* conidia were harvested from 7 day- old cultures and the number of conidia was determined using a haemocytometer. The concentration of the conidial suspension was set at $7.5*10^6$ conidia/ml and 200 µl of this suspension was added to 100 ml DW. The final spore suspension was then added to 100 g autoclaved soil (sand and clay soil, from Hebron University). A total of 10 g of the inoculated soil were added to 100 ml of DW. The mixture was shaked for 30 min on a mechanical shaker. A volume of 200 µl were then removed and added onto a plate containing the selective medium and incubated at 25 ± 1ºC for 7 days under continuous light. The number of spores germinated was then determined as CFU\gm soil. The experimental design was completely randomized with three replicates per treatment.

2.3. Mycelia growth rate (MGR) of *F. mangiferae* isolates

Mycelium growth rate of *F. mangiferae* isolates were assessed on plates containing (PDA). Four Petri dishes (90 mm diameter) containing PDA were centrally inoculated with mycelia disks (5mm) of agar plugs from 7-day-old PDA cultures of each *F. mangiferae* isolate to determine the average mycelium growth rate (MGR) for each isolate. Plates were incubated at 25°C under continuous light and inspected daily for three consecutive days. Mycelia growth

rate was recorded every 24 hours during the study period (3 days). The colony diameter was measured as the mean of two perpendicular diameters measured at the third day minus the diameter at first day. Mycelium growth rate (MGR) was calculated by using the formula described by (Barakat and Al-Masri; 2005).

$$MGR\ cm^2/day = \frac{((D_2/2)^2 - ((D_1/2)^2 * \pi)}{T_2\text{-}T_1/24}$$

Where D_1: Culture Diameter (mm) at T_1.

D_2: Culture Diameter (mm) at T_2.

T_1: Time of evaluation D_1.

T_2: Time of evaluation D_2.

Π : 3.14

The experimental design used was completely randomized with four replicates (plates) per treatment.

2.3.1 The effect of temperature on mycelium growth rate of *F. mangiferae* ($Q_{2.4}$).

The effect of temperature on the mycelium growth rate of *F. mangiferae* on selective medium was investigated after 24, 48 and 96 hours. Four Petri dishes (90 mm diameter) containing PDA were centrally inoculated with mycelia disks (5mm) of agar plugs from 8-day-old PDA cultures of each *F.mangiferae* isolate to determine the mycelium growth rate under the temperatures of 5, 10, 15, 20, 25, 30 and 35ºC. Plates were incubated under continuous light and inspected daily for three consecutive days. The colony diameter was measured as the mean of two perpendicular diameters measured at the third day minus the diameter at first day. Mycelium growth rate (MGR) was calculated using the

formula described by (Barakat and Al-Masri, 2005). A completely randomized design was used with 6 replicates (plates)

2.4. Factors affecting germination of *F. mangiferae* ($Q_{2.4}$) conidia.

2.4.1. Incubation time

The effect of incubation time on germination of *F. mangiferae* was determined in sterilized distilled water. Conidia of *F. mangiferae* were harvested from 8 day-old-sporulating cultures grown previously on PDA medium. Ten ml of SDW were added to plates; sterile glass rod was used to harvest conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. Spore concentration was set to $1*10^5$ conidia/ml, and a 20µl of the suspension was placed in the middle of each well of the 24 Sarstedt microtitre plate wells (Sarstedt, Newton, USA), in addition to 480µl of SDW to reach a final volume of 0.5 ml. Plates were then incubated under light at 25ºC±1 and germination percentages were measured after 3, 6, 18, 24 and 36 hours. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used with 4 replicates for each treatment.

2.4.2. Conidial concentration

The influence of conidia concentration on conidial germination percentages of *F. mangiferae* was assessed in a 24 well Sarstedt microtitre plate . Ten ml of SDW were added to PDA plates of an 8-day-old- sporulating cultures; sterile glass rod was used to harvest conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. The concentrations of the conidia suspensions

were determined by a haemocytometer and diluted to the final concentrations of 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7 conidia/ml. Twenty µl of each concentration were placed in the bottom of each well to which 480 µl of 10 mM Fructose solution were added to reach a final volume of 500µl (Doehlemann et al, 2006) . Plates were then incubated under continuous light at 25ºC ±1and the germination of conidia determined after 24 and 48 hours of incubation. Randomly selected conidia (100 conidia) were counted in each of the 4 wells sampled under inverted microscope. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used, with 4 replicates. A conidium was considered germinated when the germ tube length was equal and / or exceeding the conidial diameter.

2.4.3 Temperature

F. mangiferae was grown on PDA medium with 0.5g/ml chloramphenicol. Conidia from 7-day-old culture were harvested with 10ml SDW/plate using a sterile glass rod. Conidial concentration was fixed at 1×10^5 conidia /ml, and 20µl of the conidia suspension were placed in the middle of each Sarstedt microtitre plate well. Conidial germination was then determined after 24 hours of incubation under different temperatures of 5, 10, 15, 20, 25, 30 and 35ºC. Randomly selected conidia (100 conidia) were counted in each of the 4 wells sampled under inverted microscope. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used, with 4 replicates. A conidium was considered germinated when the germ tube length was equal and / or exceeding the conidial diameter.

2.4.4. Sugars

The role of carbon sources in conidial germination of *F. mangiferae* was investigated using three sugars: Fructose, Glucose and Sucrose in six molar concentrations (0.001 mM, 0.01 mM, 0.1 mM, 1 mM, 10 mM, and 100 mM). Sugar solutions were prepared in distilled water and sterilized in the autoclave for 30 minutes at 127ºC. *F. mangiferae* was grown on PAD and incubated at 25ºC±1 with continues light for eight days. Ten ml of SDW were added to plates; sterile glass rod was used to harvest conidia. Spores suspension with bits of mycelia was filtered using cheese cloth. Spore concentration was set to $1*10^5$ conidia/ml, and a 20µl of the suspension were placed in the middle of each of the 24 Sarstedt microtitre plate wells (Sarstedt, Newton, USA), in addition to 480µl of each sugar solution to reach a final volume of 500µl. Plates were then incubated under continuous light at 25ºC ±1and the germination of conidia determined after 24 hours of incubation. Randomly selected conidia (100 conidia) were counted in each of the 4 wells sampled under inverted microscope. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used with 4 replicate for each treatment.

2.4.5. Amino acid

The role of amino acids in conidial germination of *F. mangiferae* was assessed using six amino acids: Glycine, Alanine, Asparagine, Aspartic acid, Glutanic acid and Proline in seven molar concentrations (0.001 mM, 0.01 mM, 0.1 mM, 1 mM, 10 mM, 100 mM and 1000 mM). Solutions were prepared in distilled water and sterilized (SDW) in the autoclave for 30 minutes at 127ºC. *F. mangiferae* was grown on PDA and incubated at 25ºC±1 with continues light for eight days. Ten ml of SDW were added to plates; sterile glass rod was used

to harvest conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. Spore concentration was set to $1*10^5$ conidia/ml, and a 20 µl of the suspension were placed in the middle of each of the 24 Sarstedt microtitre plate wells (Sarstedt, Newton, USA), in addition to 480 µl of each amino acid solution to reach a final volume of 500µl. Plates were then incubated under continuous light at 25ºC ±1 and the germination of conidia determined after 24 hours of incubation. Randomly selected conidia (100 conidia) were counted in each of the 4 wells sampled under inverted microscope. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used with 4 replicate for each treatment.

2.4.6. Inorganic Nitrogen forms

The effect of inorganic nitrogen forms (NO^-_3 and NH^+_4) on conidial germination of *F. mangiferae* was investigated using six concentrations (0.001 mM, 0.01 mM, 0.1 mM, 1 mM, 10 mM and 100 mM) of each N-form. Solutions were prepared in distilled water and sterilized in the autoclave for 30 minutes at 127ºC. *F. mangiferae* was grown on potato dextrose agar and incubated at 25ºC±1 with continuous light for eight days. Ten ml of SDW were added to plates; sterile glass rod was used to harvest conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. Spore concentration was set to $1*10^5$ conidia/ml, and a 20µl of the suspension were placed in the middle of each of the 24 Sarstedt microtitre plate wells (Sarstedt, Newton, USA), in addition to 480µl of each N- form solution to reach a final volume of 500µl. Plates were then incubated under continuous light at 25ºC ±1and the germination of conidia determined after 24 hours of incubation. Randomly

selected conidia (100 conidia) were counted in each of the 4 wells sampled under inverted microscope. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used with 4 replicate for each treatment.

2.4.7. Cations

The effect of the cations Ca^{+2}, Mg^{+2}, Fe^{+2}, Zn^{+2}, Cu^{+2} and K^{+} on germination and germ tube length of *F. mangiferae* was investigated. Ca ($CaCl_2$), Mg ($MgSO_4$), and Fe ($FeSO_47H_2O$), Zn ($ZnSO_4$), Cu ($CuSO_4$) and K (KCl) were prepared in seven concentrations (0.001mM, 0.01mM, 0.1mM, 1 mM, 10 mM, 100 mM, and 1000 mM). *F. mangiferae* was grown on potato dextrose agar and incubated at 25ºC±1 with continuous light for eight days. Ten ml of SDW were added to plates; sterile glass rod was used to harvest conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. Spore concentration was set to $1*10^5$ conidia/ml, and a 20µl of the suspension were placed in the middle of each of the 24 Sarstedt microtitre plate wells (Sarstedt, Newton, USA), in addition to 480µl of each cation solution to reach a final volume of 500µl. Plates were then incubated under continuous light at 25ºC ±1and the germination of conidia determined after 24 hours of incubation. Randomly selected conidia (100 conidia) were counted in each of the 4 wells sampled under inverted microscope. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used with 4 replicate for each treatment.

2.4.8. pH values

The effect of pH values on conidial germination and germ tube length of *F. mangiferae* was investigated in (100 mM) glucose solution. Glucose solutions were prepared and adjusted to pH ranges by using NaOH (3, 4, 5, 6, 7, 8, 9, 10, 11and 12). *F. mangiferae* was grown on potato dextrose agar and incubated at 25ºC±1 with continuous light for eight days. Ten ml of SDW were added to plates; sterile glass rod was used to harvest conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. Spore concentration were set to $1*10^5$ conidia/ml, and 20µl of the suspension was placed in the middle of each of the 24 Sarstedt microtitre plate wells (Sarstedt, Newton, USA), in addition to 480µl of each glucose solution to reach a final volume of 500µl. Plates were then incubated under continuous light at 25ºC ±1and the germination of conidia determined after 24 hours of incubation. Randomly selected conidia (100 conidia) were counted in each of the 4 wells sampled under inverted microscope. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used with 4 replicates for each treatment.

2.4.9. Oxidants

The role of oxidants in conidial germination of *F. mangiferae* was assessed by using hydrogen peroxide and menadion in seven concentrations (0.001 mM, 0.01 mM, 0.1 mM, 1 mM, 10 mM, 100 mM and 1000 mM). *F. mangiferae* was grown on potato dextrose agar and incubated at 25ºC±1 with continuous light for eight days. Ten ml of SDW were added to plates; sterile glass rod was used to harvest conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. Spore concentration was set to $1*10^5$ conidia/ml, and a 20µl of the suspension were placed in the middle of each of the 24 Sarstedt microtitre plate

wells (Sarstedt, Newton, USA), in addition to 480µl of each oxidant solution to reach a final volume of 500µl. Plates were then incubated under continuous light at 25ºC ±1and the germination of conidia determined after 24 hours of incubation. Randomly selected conidia (100 conidia) were counted in each of the 4 wells sampled under inverted microscope. At the same time, the average germ tube lengths of 10 random germinated conidia (replicates) were recorded (µm) using a calibrated inverted microscope eyepiece graticule. A completely randomized design was used with 4 replicate for each treatment.

2.5. Mango Malformation Management

2.5.1. Biological control with *Trichoderma harzianum*.

Effect of *T. harzianum* metabolites on mycelium growth rate of *F. mangiferae* (Q2.4).

The ability of *T. harzianum* isolates (Jn14 and Jn58) to inhibit the mycelium growth of *F. mangiferae* through the production of fungitoxic metabolites at 25^{o}C was tested according to the method of (Dennis and Webster, 1971c*)*. Potato dextrose broth PDB (100ml) was placed in 250 ml Erlenmeyer flask, and incubated with 5 mm mycelia disks from 8 day-old cultures of two *T. harzianum* isolates (Jn14 and Jn58, obtained from the fungal collection of the Plant Protection Research Center at Hebron University). After 10 days of incubation, the cultures were filtered through filter paper (0.45µm). The culture filtrates of *T. harzianum* were then added to PDA plates in two concentrations (20% and 40%). Amended PDA plates were then centrally inoculated with 5 mm mycelia discs of *F. mangiferae,* and incubated at 25^{o}C. The mycelium growth rate of *F. mangiferae* was measured after 24, 48 and 96 hours as

cm^2/day according to the equation described by Barakat and Al-Masri (2005). The experimental design was completely randomized with four replicates (plates) for each treatment.

2.5.2. Chemical Control with fungicides.

Fosetyl-Al (Aliete) fungicide was used for assessing the efficiency of chemical control against *F. mangiferae* ($Q_{2.4}$) *in vitro.*

2.5.2.1. Effect of the fungicide Fosetyl-Al on conidial germination of *F. mangiferae*.

This experiment was conducted to evaluate the effect of the fungicide (Fosetyl-Al) on spore's germination of *F. mangiferae* at the concentrations of (0,1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg ml^{-1}). Fungicide solutions were prepared using sterilized distilled water. *F. mangiferae* was grown on PDA and incubated at 25ºC±1 with continuous light for eight days. Ten ml of SDW were added to plates; sterile glass rod was used to harvest conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. Spore concentration was set to $1*10^5$ conidia/ml and a 20µl of the suspension was placed in the middle of each of the 24 Sarstedt microtitre plate wells (Sarstedt, Newton, USA), in addition to 480µl of each fungicide concentration to reach a final volume of 0.5 ml. Plates were then incubated under continuous light at 25ºC±1 and germination percentages were measured after 24hours. A completely randomized design was used with 4 replicate for each treatment.

2.5.2.2. Effect of the fungicide Fosetyl-Al on mycelial growth rate of *F. mangiferae.*

This experiment was conducted to evaluate the effect of the fungicide Fosetyle-Al on mycelium growth of *F. mangiferae* at the concentrations 0, 100, 200, 300, 400, 500, 600,700 and 800 µg ml^{-1}. A fixed amount of each fungicide concentration was added to each PDA Petriplate and mixed with the medium before solidification according to table 2.2 The control treatment (zero concentration) was represented by using SDW only. Amended Petri dishes were inoculated with 5mm- mycelia disks of 8 day old cultures of *F. mangiferae*. Plates were incubated at 25°C±1 under continuous light. The experimental design was completely randomized with four replicates per treatment. Mycelium growth rate (cm^2/day) was recorded after 24, 48 and 96hours according to the equation developed by Barakat and Al-Masri (2005).

Table 2.2 Fosetyl-Al concentrations used in ppm and the respective amounts (ml) added per 100 ml PDA

Fungicide Conc. (ppm)	Respective amounts (ml) added / 100ml PDA
0 ppm	0 ml
100	0.5
200	1
300	1.5
400	2
500	2.5
600	3
700	3.5
800	4

2.5.2.3. Effect of the fungicide Fosetyl-Al on the conidial germination of *T. harzianum*.

This experiment was conducted to evaluate the effect of the fungicide (Fosetyl-Al) on conidial germination of *T. harzianum* isolates (Jn14 and Jn58) at the concentrations of (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg ml^{-1}). *T. harzianum* was grown on potato dextrose agar and incubated at 25ºC±1 with continuous light for eight days. Ten ml of SDW were added to plates; sterile glass rod was used to harvest *T. harzianum* conidia. Spores suspension with bits of mycelia was filtered using a cheese cloth. Spore concentration was set to $1*10^4$ conidia/ml and 20µl of the suspension was placed in the middle of each of the 24 Sarstedt microtitre plate wells (Sarstedt, Newton, USA) in addition to 480µl of each fungicide concentration were added to reach a final volume of 0.5 ml. Plates were then incubated in light at 25ºC±1 and germination percentages were measured after 24, 48 and 96 hours. A completely randomized design was used with 4 replicate for each treatment.

2.5.2.4 Effect of the fungicides Fosetyl-Al on the mycelia growth of *T. harzianum*.

This experiment was conducted to evaluate the effect of the fungicide Fosetyle-Al on mycelium growth of *T. harzianum* isolates (Jn14 and Jn58) at the concentrations 0, 100, 200, 300, 400, 500, 600,700 and 800 µg ml^{-1}. A fixed amount of each fungicide concentration was added to each PDA Petriplate and mixed with the medium before solidification according to table 2.2 The control treatment (zero concentration) was represented by using SDW only. Amended Petri dishes were inoculated with 5mm- mycelia disks of 8 day old cultures of the *T. harzianum*. Plates were incubated at 25°C±1 under continuous light. The experimental design was completely randomized with eight replicates per

treatment. Mycelium growth rate (cm^2/day) was recorded after 24, 48 and 96hours according to the equation developed by Barakat and Al-Masri (2005)

2.6 Statistical analysis

Data of all experiments were analyzed statistically using one way analysis of variance (ANOVA); fisher least significant difference (LSD) test was used for mean separation (Sigma Stat 2.0 program, SPSS, USA).

3. Results

3.1. Disease Survey

3.1.1 Isolation of *F. mangiferae*

Seven isolates of *F. mangiferae* were isolated from suspected samples all from Qalqelia (orchard 1 & 2). *F. mangiferae* was not isolated from any suspected samples collected from Tulkarm. (Table 3.1).

Table 3.1. *F. mangiferae* isolates recovered from suspected samples collected from different locations in the North of the West Bank (2010/2011).

Orchard No.	Location	No. of samples	No. of *F. magiferae* isolates recovered	% of infected samples out of the total collected from the orchard
1	Qalqelia	5	2 ($Q_{1.1}$ /$Q_{1.2}$)	40%
2	Qalqelia	9	5 ($Q_{2.1}$/ $Q_{2.2}$/ $Q_{2.3}$/ $Q_{2.4}$/ $Q_{2.5}$)	56%
3	Qalqelia	3	0	0%
4	Tulkarm	4	0	0%
5	Tulkarm	3	0	0%

Infected samples were all from Qlaqelia (orchards 1and 2). Out of all the suspected samples, 30% were infected with *F. mangiferae*; 40% of samples collected from orchard one were infected, while 56% of samples collected from orchard two were infected with *F. mangiferae*.

3.1.2 Identification of *F. mangiferae*

Identification to *F. mangiferae* was based mainly on the morphology of produced the micro-and macroconidia (Booth, 1971; Nelson et al., 1983; Kedera et al., 1994). Suspected plant samples cultured on PDA gave dense colonies with abundant aerial mycelium. The under surface of the petridishes was orange with a purple midpoint at first. In 14 days, a prominent purple pigmentation spreaded across the whole surface. Sporulation started quickly in two days giving microconidia first and in another two days, macroconidia started to appear in between. Macroconidia were slender, falcate and borne on monophialides. Three to four-septate macroconidia were always found, while microconidia is slightly sickle shaped to straight with dorsal and ventral surface almost parallel. The size of macroconidia was 3.5-5×45-60µm. The microconidia were abundant, fusiform, oval to elliptical, and sometimes spindle shaped (Nelson et al., 1983; Iqbal, 2004). None of the seven *F. mangiferae* isolates produced chlamydospores in cultures.

3.2 Selective media enhancement for the isolation of *F. mangifera* ($Q_{2.4}$).

In this experiment, Nash medium was amended with several concentrations of Dodine for improving selectivity to *F. mangiferae* (Table 3.2). In the two soil types tested, the number of total fungi decreased significantly after the addition of Dodine, but in different proportions compared to the control. However, the numbers of *F. mangiferae* increased at high concentrations of Dodine (> 1.5 ppm) compared with the control. The best concentration of Dodine which gave positive selectivity for the benefit of *F. mangiferae* towards other fungi was 1.5 ppm. At this concentration (1.5 ppm), total number of fungi was reduced by

65%, while the numbers of *F. mangiferae* were increased by 71%, compared to the control.

Table 3.2. The effect of different Dodine concentrations on the selectivity of Nash medium for the isolation of *F. mangiferae*.

Dodine Conc. (ppm)	**Clay(CFU/gm)**		**Sand(CFU/gm)**	
	*Total Fungi	**F. mangiferae*	*Total Fungi	**F. mangiferae*
CK	49 a	28 c	55 a	23 d
0.5	18.5 e	30 c	16 e	32 c
1	29 c	31 c	31 c	31 c
1.5	17 e	48 a	16 e	47 ab
2.5	18.5 e	41 b	24 d	32.5 c

Means data followed by the same letter in the same column and raw are not significantly different at (LSD= 9.01).

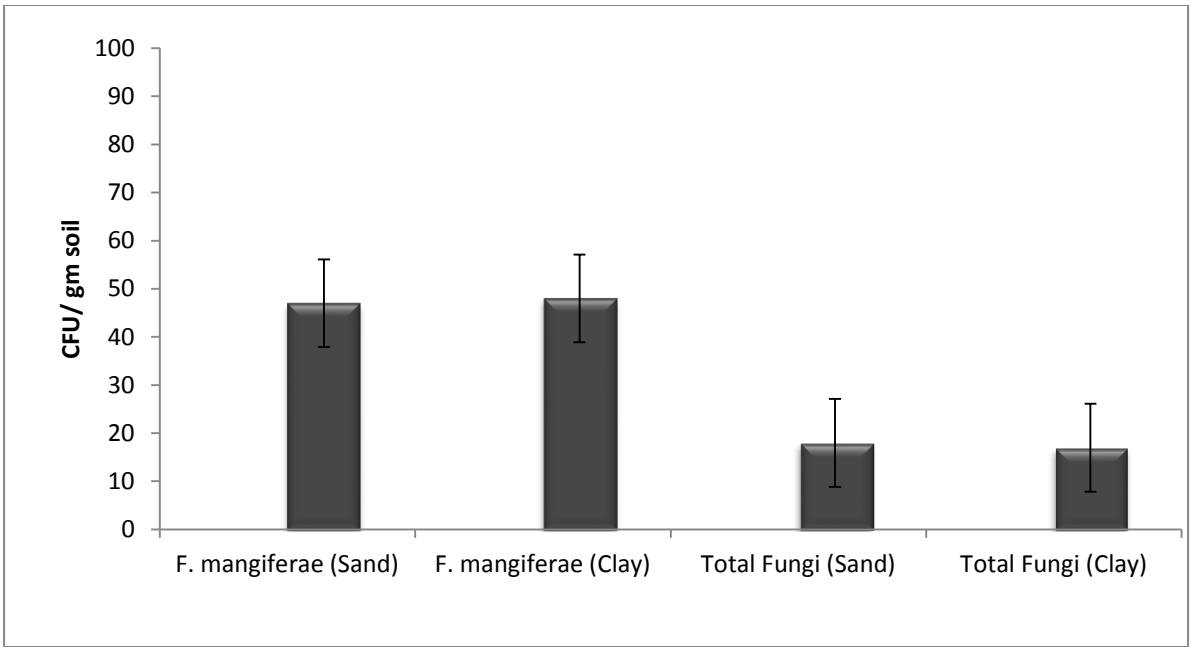

Figure 5. The effect of Dodine at the concentration (1.5 ppm) on the population of *F. mangiferae* and total fungi in two types of soils **(LSD=9.132)**.

3.3 Mycelia growth rate (MGR) of *F. mangiferae* isolates.

The mean of mycelia growth rate for the isolates ranged from 12.2 mm^2/day to 25.3 mm^2 /day. Results of mycelia growth rate of *F. mangiferae* isolates grown on PDA are presented in (Figure 6) . The highest mycelium growth rate (25.3 mm^2 /day) was recorded by the isolate ($Q_{2.4}$), which was significantly different from the other isolates growth rates. Variation in growth rates of the other *F. mangiferae* isolates were not significantly different in general at 25ºC. The lowest growth rate was recorded by the isolate ($Q_{2.5}$).

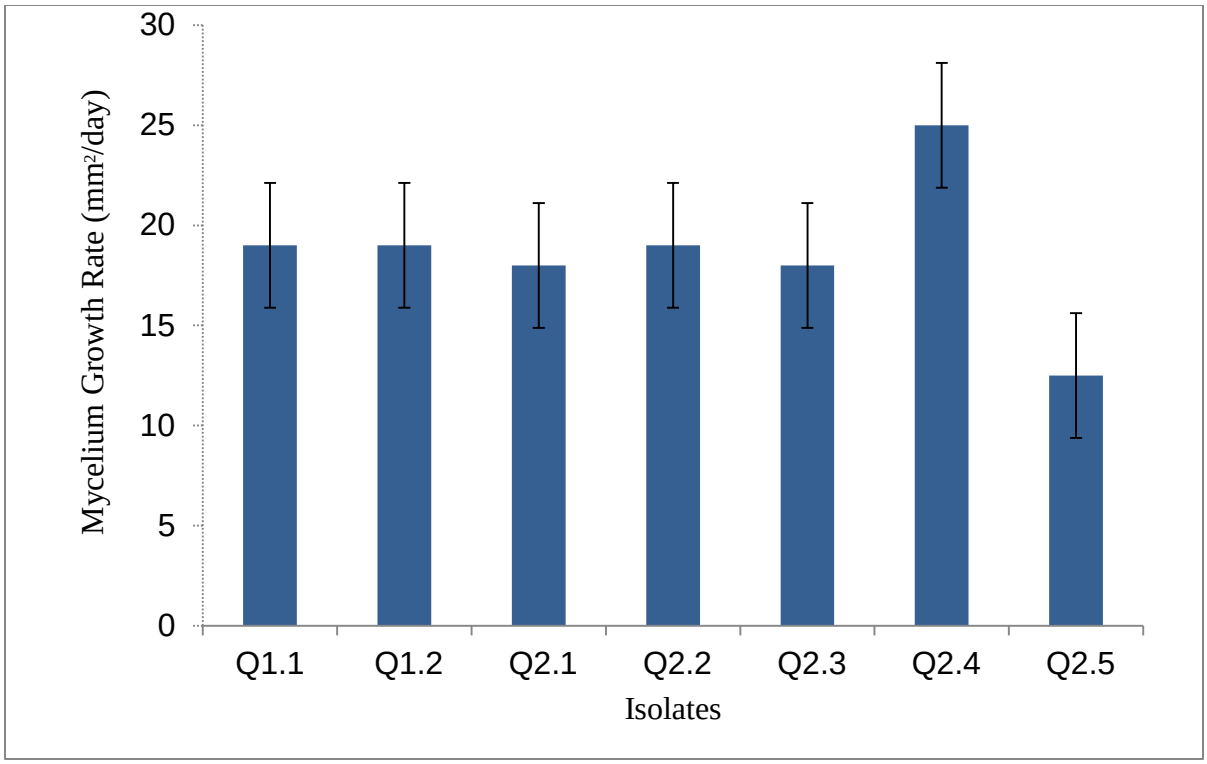

Figure 6. Mycelia growth rate of *F. mangiferae* isolates growing on PDA incubated at 25ºC, and continuous light **(LSD = 6.22).**

3.3.1 The effect of temperature on mycelium growth rate of *F. mangiferae* ($Q_{2.4}$).

The results showed (Figure 7) that mycelium growth rate was significantly affected by the various temperatures. The highest mycelium growth was obtained at 25 ºC; mycelial growth was completely inhibited at 5 and 35 ºC.

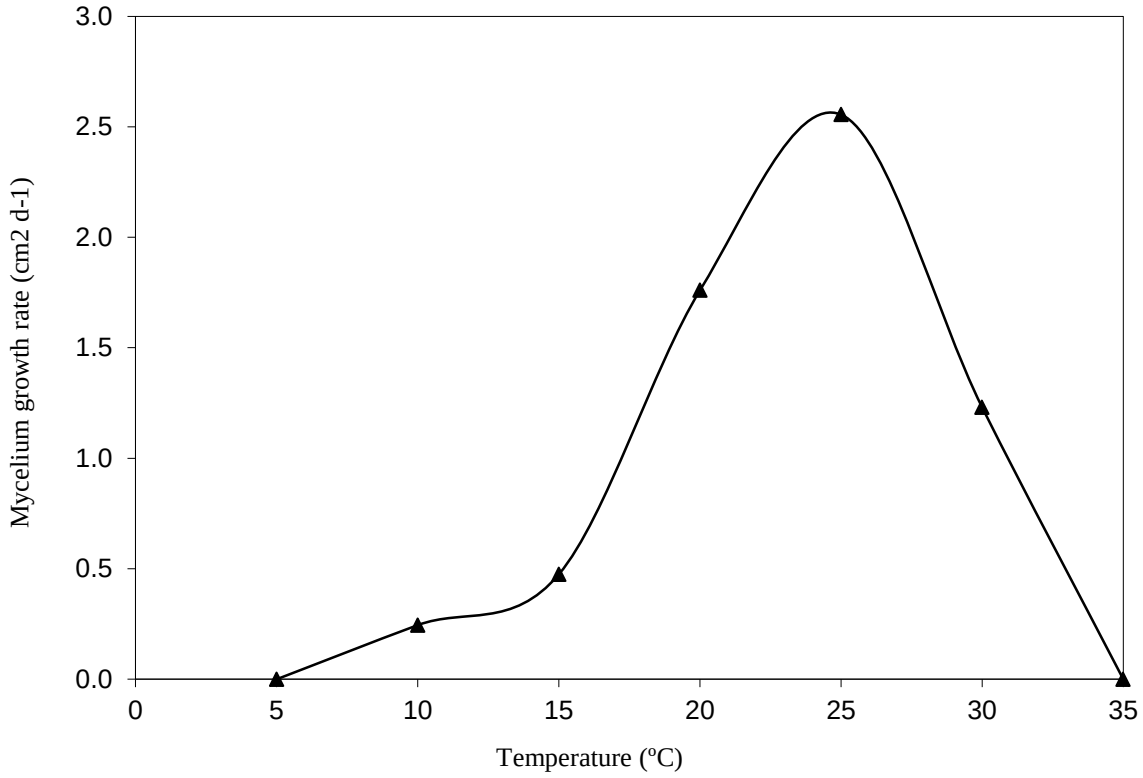

Figure 7. Effect of temperatures on *F. mangiferae* ($Q_{2.4}$) mycelia groth rate (cm^2/day) grown on PDA, after 24 h of incubation **(LSD=0.45)**

3.4. Factors fecting germination of *F. mangiferae* ($Q_{2.4}$) conidia

3.4.1. Incubation time.

The germination of *F. mangiferae* ($Q_{2.4}$) conidia was quantified with time in SDW (Figure 8). Conidial germination started after 3 hours of incubation. The germination of conidia and germ tube growth were significantly affected by time after 3, 6, 18, 24 and 36 h. The highest germination percentage (90%), and germ tube growth (28µm) were recorded after 36 hours.

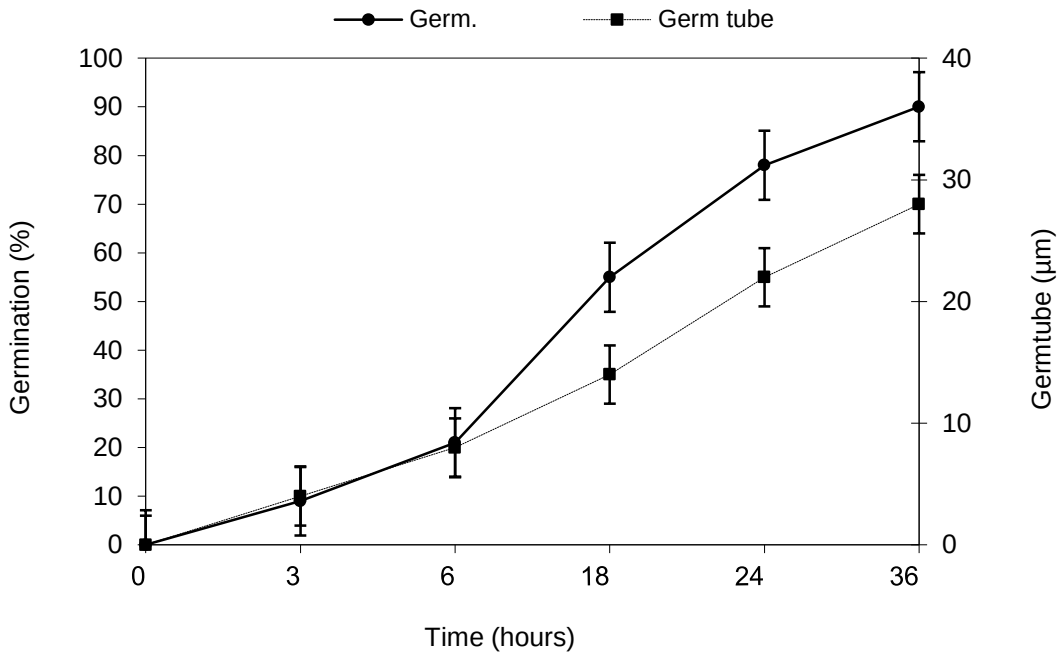

Figure 8. Conidial germination (LSD=7.1) and germ tube growth **(LSD=2.4)** of *F. mangiferae* isolate $Q_{2.4}$ in SDW at 25ºC, and continuous light.

3.4.2. Conidia Concentration

The effect of spore concentration of *F. mangiferae* ($Q_{2.4}$ isolate) on conidial germination was determined in SDW and 10 mM fructose (Figure 9). Results showed that conidial germination rates decreased with increasing spores concentration. The highest germination rate was recorded at the lowest spore concentration tested (10^2 conidia/ ml). Furthermore, there was no significant differences in germination rates between SDW and fructose at both incubation times. The germ tube growth reduce with increasing spore concentration compared with the control with no significantly different between all spore concentration.

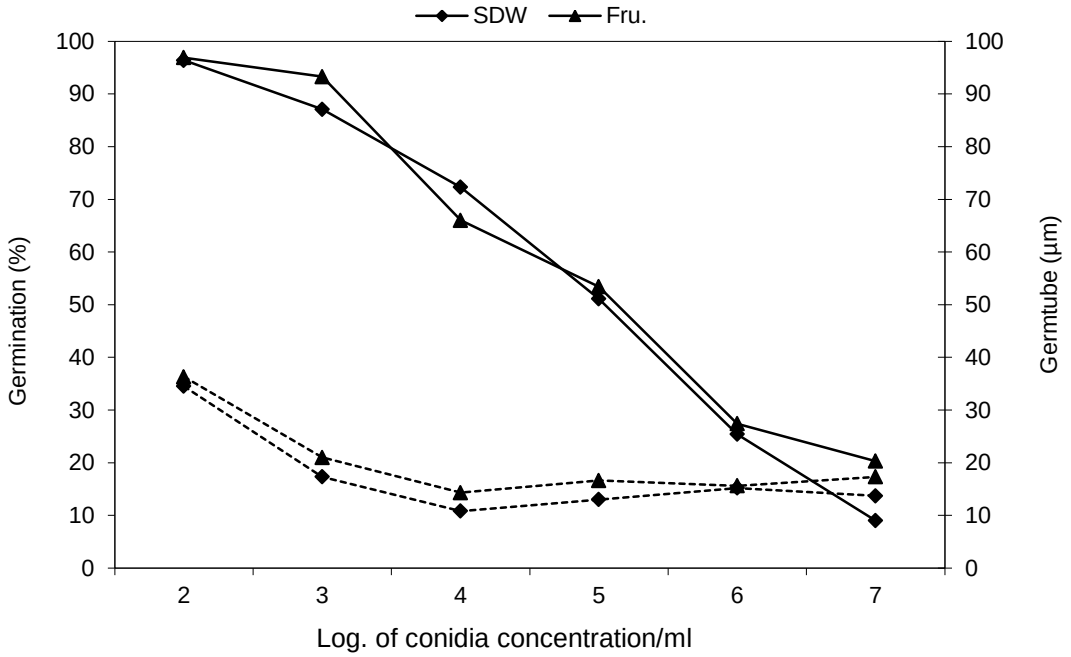

Figure 9. Effect of conidial concentration on conidial germination (%) (—) **(LSD=8.16)** and germ tube length(µm)(….) **(LSD= 2.6)** in SDW and fructose after 24 ad 48 hours of incubation at 25ºC**.**

3.4.3. Temperature

The effect of temperature on germination of conidia and germ tube growth of *F. mangiferae* ($Q_{2.4}$) was assessed under the range (5-35 ^{o}C) in SDW (Figure 10). The results showed that conidial germination and germ tube growth were significantly affected by the various temperatures. The highest germination rate of conidia and germ tube growth were obtained at 25 ^{o}C; germination and germ tube growth were completely inhibited at 5 and 35 ^{o}C.

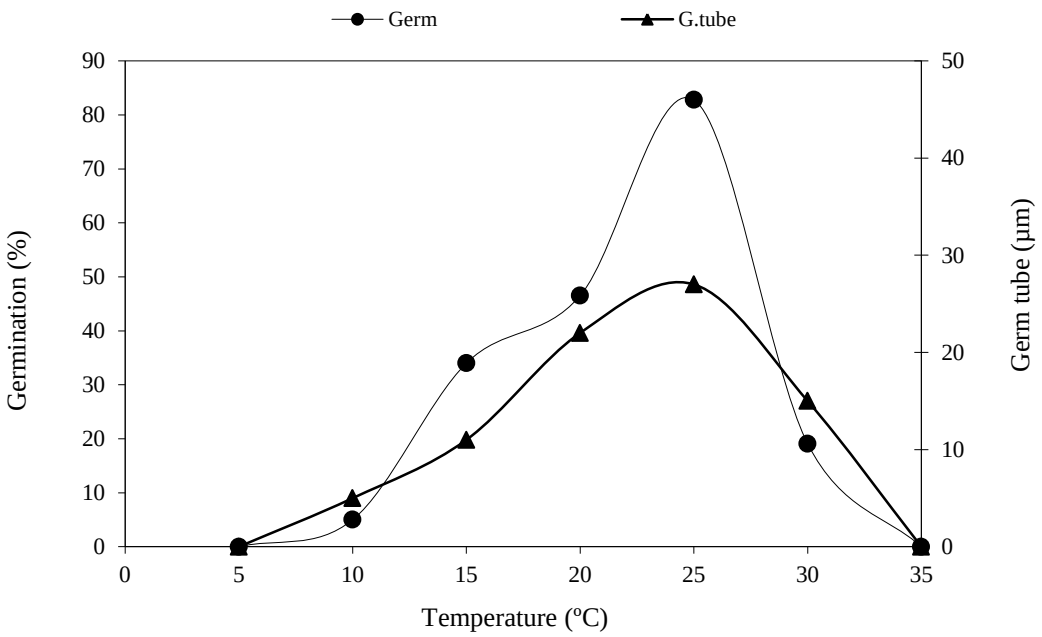

Figure 10. Effect of temperatures on *F. mangiferae* conidial germination (%) **(LSD=12.22)**, and germ tube length (µm) **(LSD=5.2)**, in wells containing SDW after 24 h of incubation

3.4.4. Sugars

The effect of the sugars (Fructose, Sucrose, and Glucose) on conidial germination (Figure 11) and germ tube growth (Figure 12) of *F. mangiferae* was tested in various concentrations under 25 ºC. The results showed that germination of conidia and germ tube growth were stimulated in all sugars in various proportions. Glucose stimulated the highest germination rate (92%) after 24 hours of incubation at the highest concentration used (100mM) compared with sucrose (86%), and fructose (84%). Sucrose, however, stimulated the highest germ tube growth rate (25µm) after 24 hours of incubation at the highest concentration used (100mM) compared with glucose (24µm), and fructose (21µm).

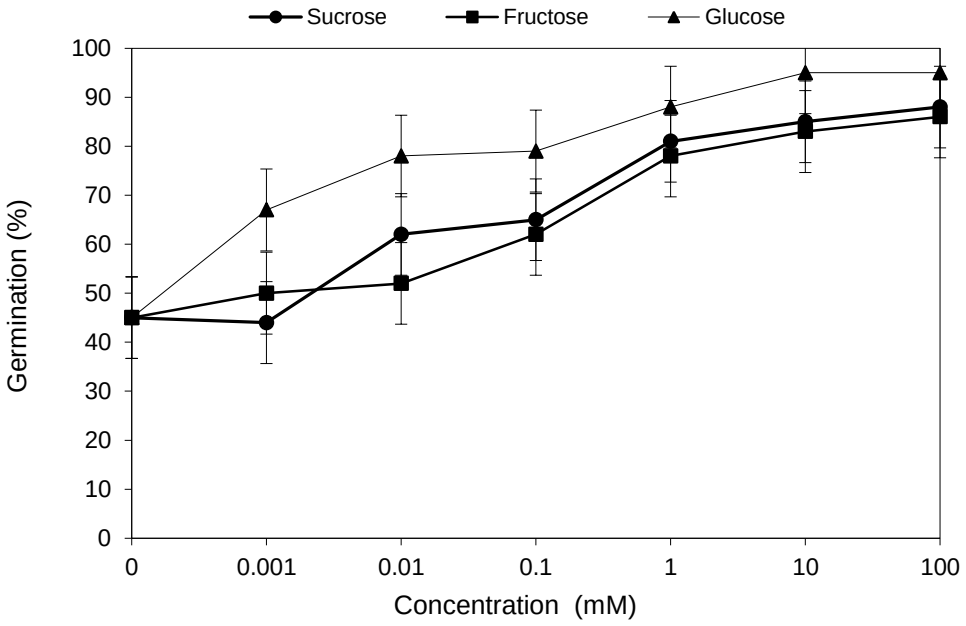

Figure 11. Effect of the sugars Sucrose, Fructose, and Glucose on conidial germination of *F. mangiferae* in wells after 24h of incubation at 25°C **(LSD=8.34).**

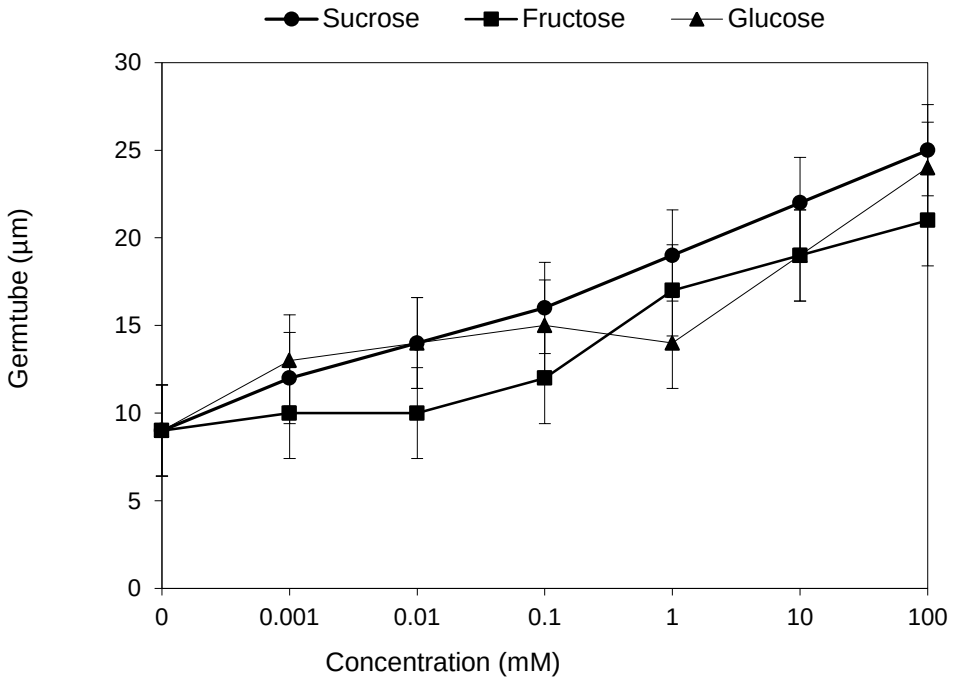

Figure 12. Effect of the sugars Sucrose, Fructose, and Glucose on germ tube growth of *F. mangiferae* in wells after 24h of incubation at 25°C **(LSD=2.6).**

3.4.5. Amino acids

The effect of amino acids on the germination rate of conidia and germ tube growth of *F. mangiferae* was inspected. All amino acids in general increased conidia germination rates and germ tube growth with increasing concentration but in different proportions. Glutamic acid, however, enhanced the highest germination rate (100%) but at the highest concentration used (1000 mM). Alanine on the other hand was effective at lower concentrations and at the highest concentration was able to increase germination up to 96%. Aspatic acid came third and increased germination up to 90% at the highest concentration used (1000 mM) and proline (80%).

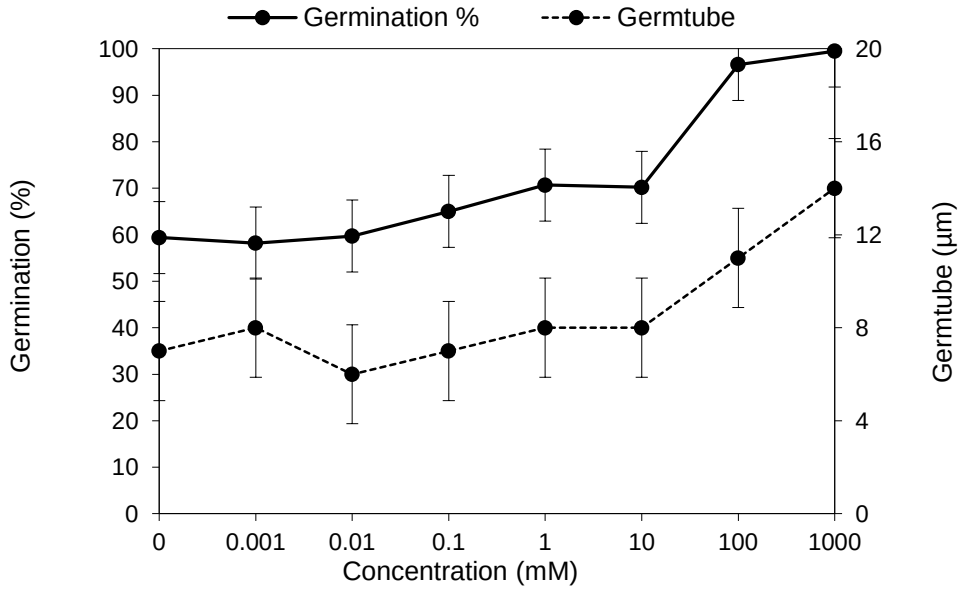

Figure 13. Effect of Glutamic acid on conidial germination (%) **(LSD=7.742)** and germ tube length (µm) **(LSD=2.132)** of *F. mangiferae* grown in SDW after 24h of incubation at 25^{o}C.

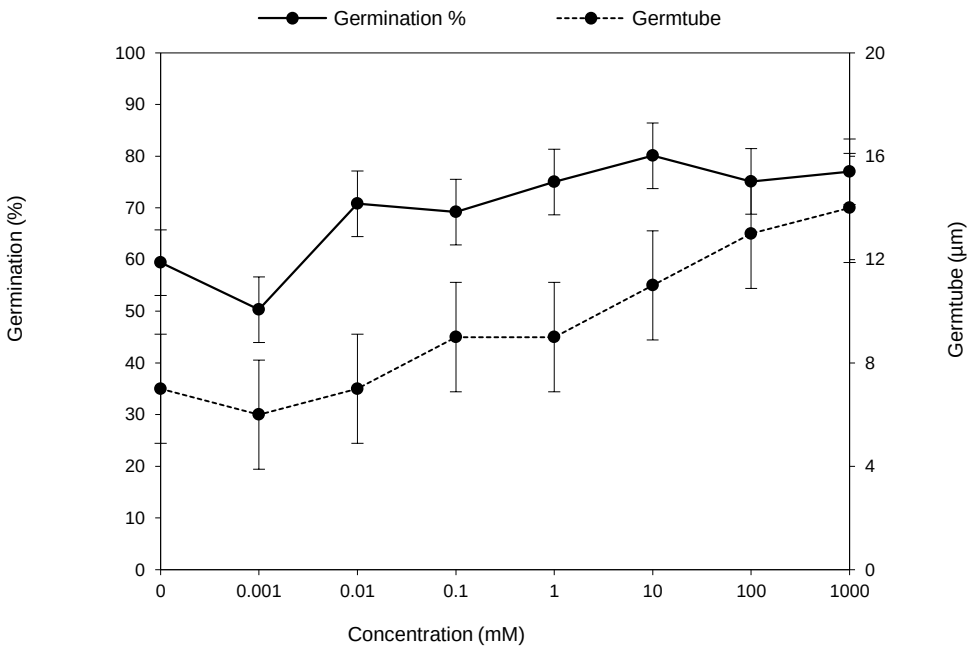

Figure 14. Effect of Proline on conidial germination (%) **(LSD=6.352)** and germ tube length (µm) **(LSD=2.112)** of *F. mangiferae* grown in SDW after 24h of incubation at 25°C.

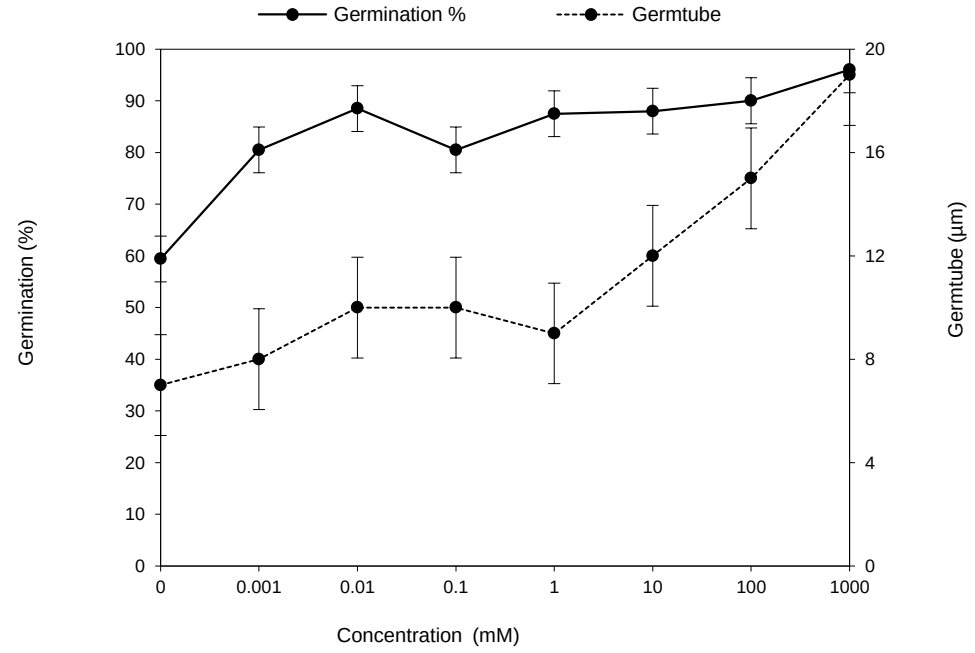

Figure 15. Effect of Alanine on conidial germination (%) **(LSD=4.447)** and germ tube length (µm) **(LSD=1.950)** of *F. mangiferae* grown in SDW after 24h of incubation at 25°C.

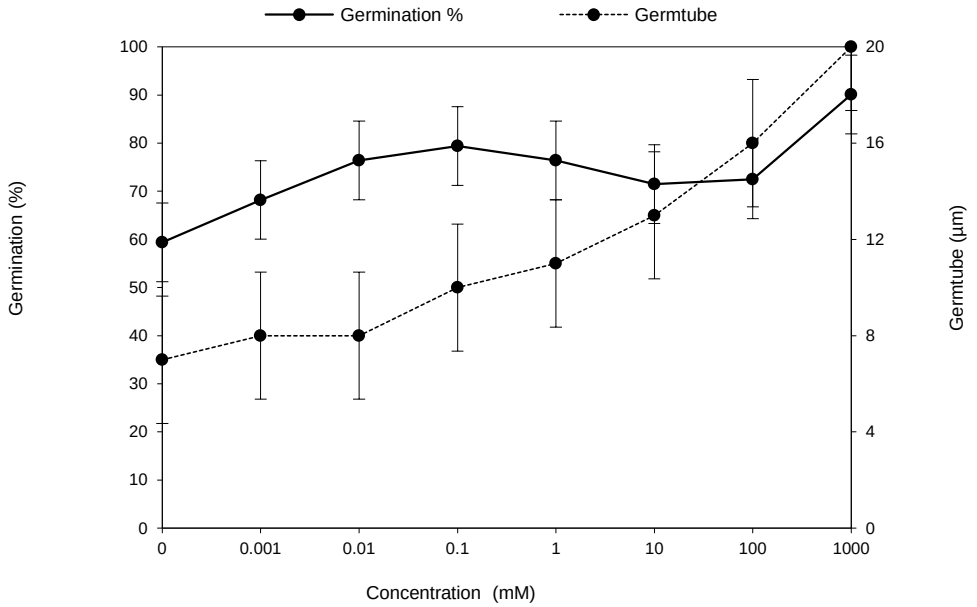

Figure 16. Effect of Aspartic acid on conidial germination (%) **(LSD=8.167)** and germ tube length (µm) **(LSD=2.643)** of *F. mangiferae* grown in SDW after 24h of incubation at 25^{o}C.

Table 3.3. Effect of amino acids on conidial germination (%) of *F. mangiferae* grown in SDW after 24h of incubation at 25^{o}C.

Conc.	**Germination (%)**							
	Glutamic acid		Aspartic acid		Proline		Alanine	
CK.	59.4	cd	59.4	d	59.4	cd	59.4	d
1µM	58.2	cd	68.2	c	50.3	d	80.5	c
10µM	59.7	cd	76.4	bc	71.2	ab	88.5	b
100µM	64.8	cd	79.4	bc	69.2	ab	80.5	c
1mM	70.7	b	76.4	bc	74.7	ab	87.5	b
10mM	70.2	b	71.5	bc	80.2	a	88	b
100mM	96.6	a	72.5	bc	75.3	a	90	b
1M	99.5	a	90.1	a	77.1	a	96	a
LSD	**7.742**		**8.167**		**6.352**		**4.447**	

Means followed by the same letter in the same column are not significantly different.

3.4.6. Inorganic nitrogen forms (NH_4, NO_3)

The effect of NH_4 and NO_3 on germination of *F. mangiferae* conidia and germ tube growth was investigated (Figure 17). NO_3-N influenced germination of conidia and germ tube growth only at the highest concentration tested (100 mM). Lower concentrations were not significantly different from the control treatment . NH_4-N, however, showed no influence on germination rate of conidia nor germ tube growth at all concentration tested.

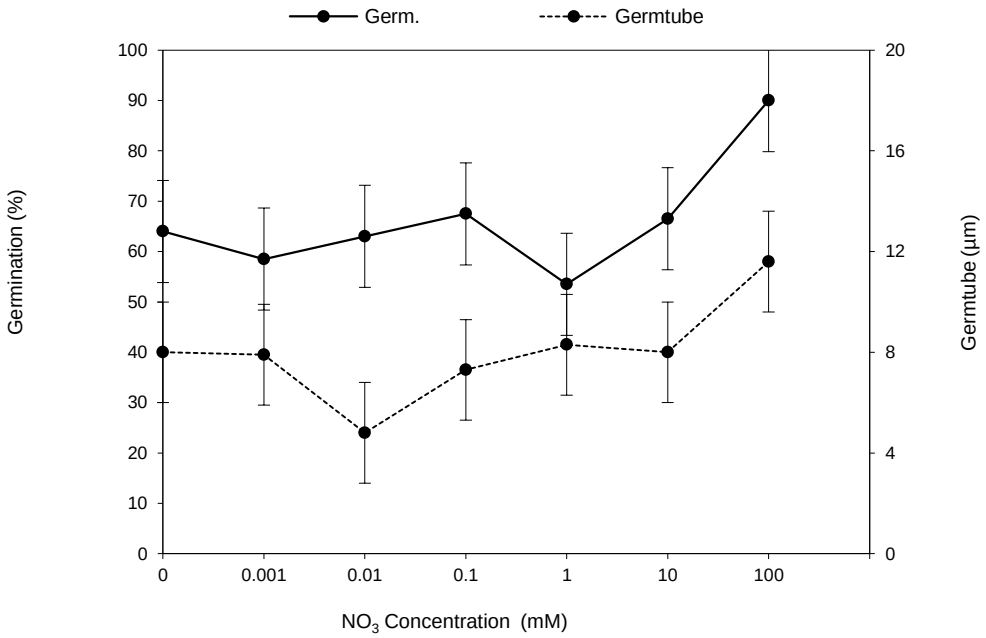

Figure 17. Effect of NO_3 on conidial germination (%) **(LSD=8.044**) and germ tube length (µm) **(LSD=2),** of *F. mangiferae* after 24h of incubation at 25^{o}C.

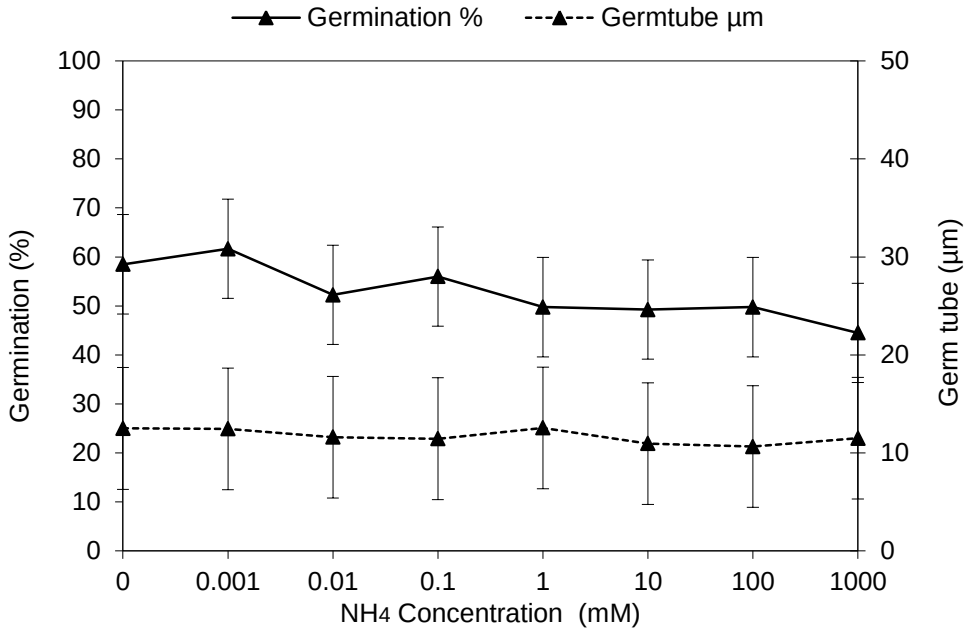

Figure 18. Effect of NH_4 on conidial germination (%) **(LSD=10.137)** and germ tube length (µm) **(LSD=6.216)** of *F. mangiferae* grown in SDW after 24h of incubation at 25^{o}C.

3.4.7. Cations

The effect of the cations Fe^{+2}, Cu^{+2}, Zn^{+2}, Ca^{+2}, Mg^{+2} and k^{+} on germination and germ tube growth of *F. mangiferae* was investigated. Conidial germination and germ tube growth decrease with increasing concentrations of the cations Fe^{+2} (Figure 19), Cu^{+2} (Figure 20) and Zn^{+2} (Figure 21) but in different proportions. In the case of Fe^{+2}, and Zn^{+2}, both parameters declined sharply above the concentration of 100 µM and stopped completely at the concentrations above 10 mM. In the case of Cu^{+2}, all concentrations completely inhibited conidial germination and germ tube growth. Conidial germination and germ tube growth, however, were enhanced by the cations K^{+} (Figure 22), Ca^{+2} (Figure 23) and Mg^{+2} (Figure 24) at the concentrations above 10mM.

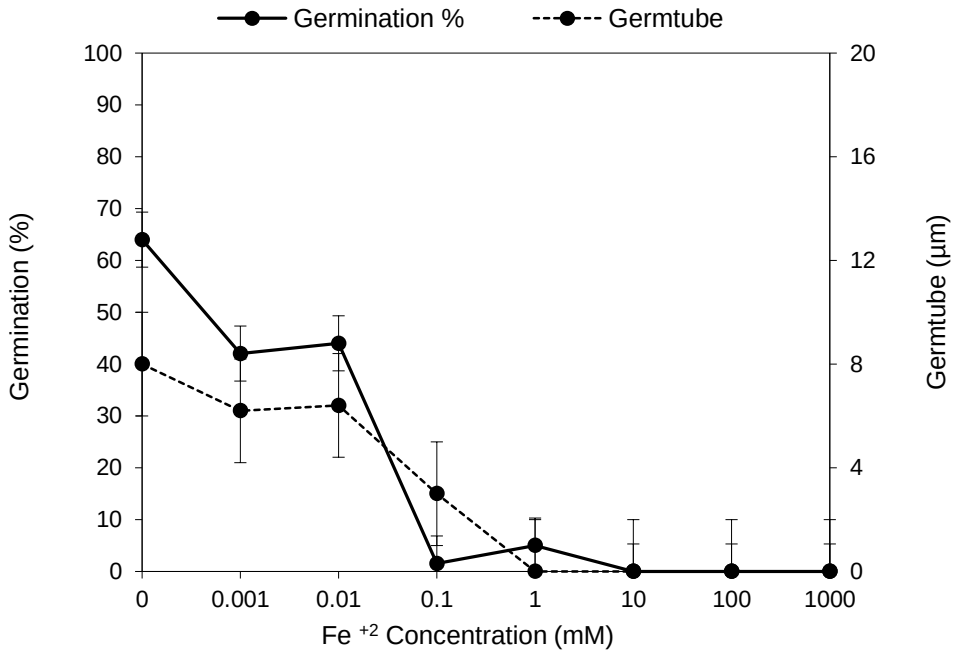

Figure 19. Effect of Fe^{+} on conidial germination (%) **(LSD= 5.309)**, and germ tube length (µm) **(LSD= 4)** of *F. mangiferae* grown in SDW after 24h of incubation at 25°C.

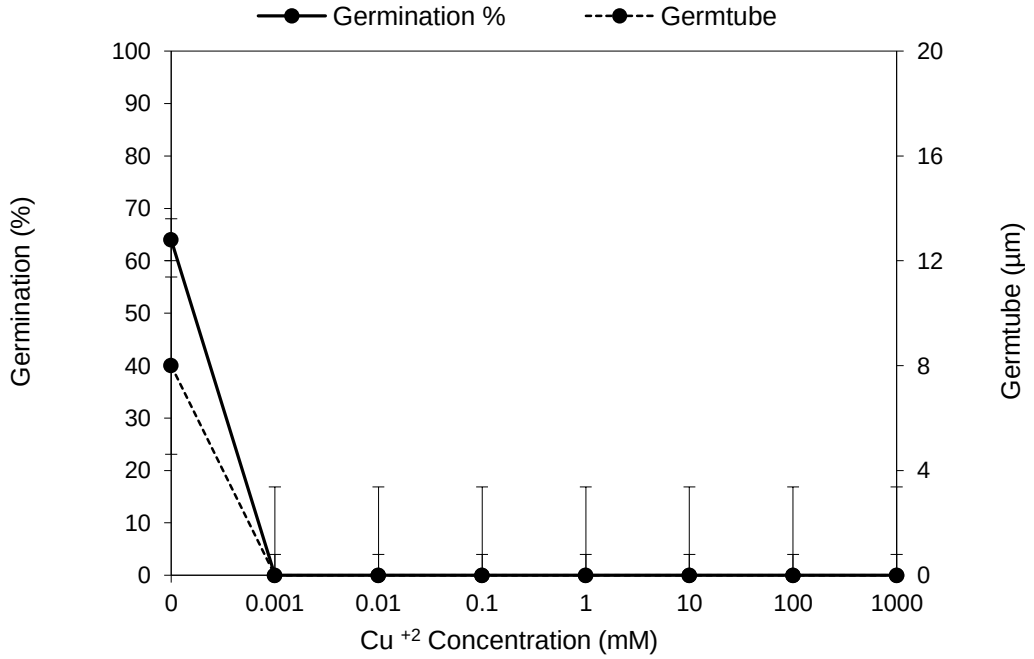

Figure 20. Effect of Cu^{+2} on conidial germination (%) **(LSD= 4)**, and germ tube length (µm) **(LSD= 7.178)** of *F. mangiferae* grown in SDW after 24h of incubation at 25°C.

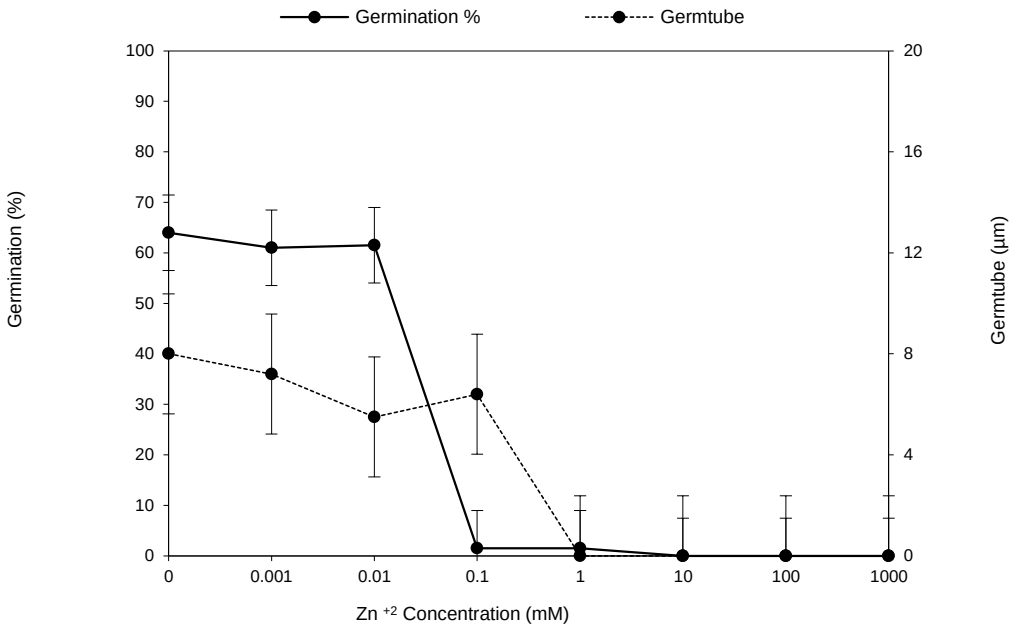

Figure 21. Effect of Zn^{+2} on conidial germination (%) **(LSD= 7.485),** and germ tube length (µm) **(LSD= 3.75)** of *F. mangiferae* grown in SDW after 24h of incubation at 25°C.

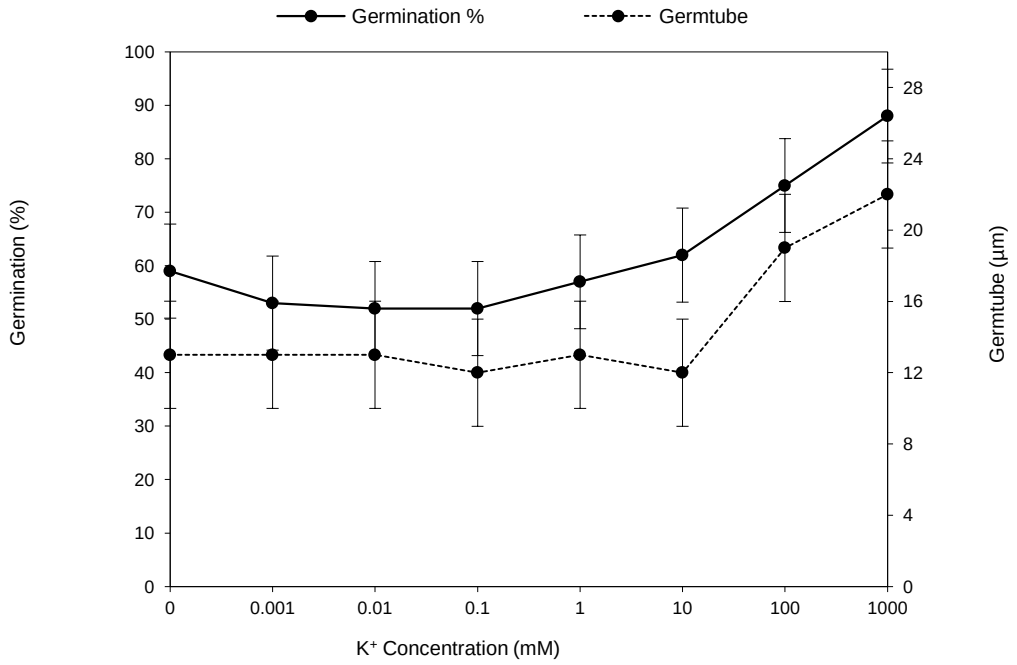

Figure 22. Effect of K^{+} on conidial germination (%) **(LSD= 8.786),** and germ tube length (µm) **(LSD= 6.012)** of *F. mangiferae* grown in SDW after 24h of incubation at 25°C.

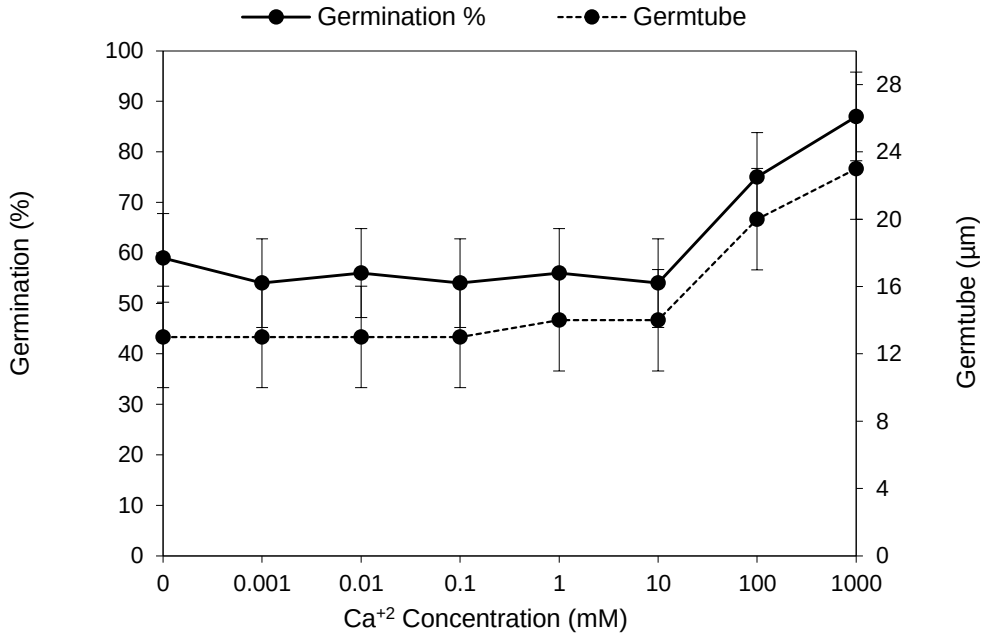

Figure 23. Effect of Ca^{+} on conidial germination (%) **(LSD=7.485)**, and germ tube length (µm) **(LSD= 3.75)** of *F. mangiferae* grown in SDW after 24h of incubation at 25°C.

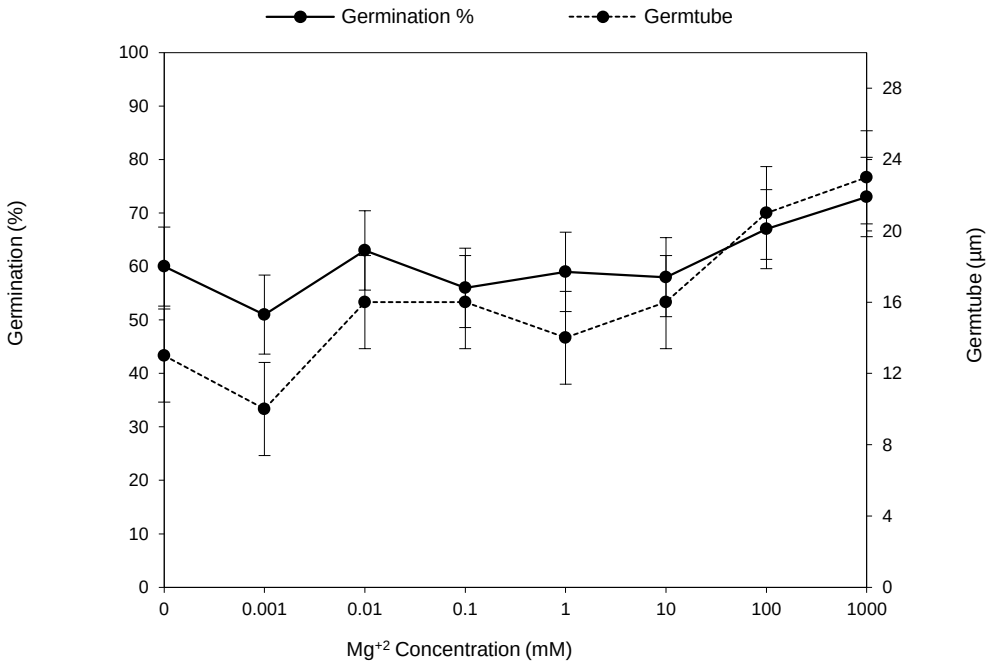

Figure 24. Effect of Mg^{+2} on conidial germination (%) (**LSD= 7.4**), and germ tube length (µm) **(LSD= 4.609)** of *F. mangiferae* grown in SDW after 24h of incubation at 25°C.

3.4.8. pH values

The effect of pH on germination of conidia and germ tube growth of *F. mangiferae* was assessed in SDW and in glucose solution (Figure 25). Conidia germinated and germ tubes were able to grow at pH values of 6-12; no germination or growth observed at pH= 0-5. The highest germination percentages and germ tube growth were observed at pH 7-8, but consistently declined afterward until reaching its minimum at pH 12. Germination of conidia and germ tube growth almost behaved the same under SDW and 100mM glucose in respect to pH values.

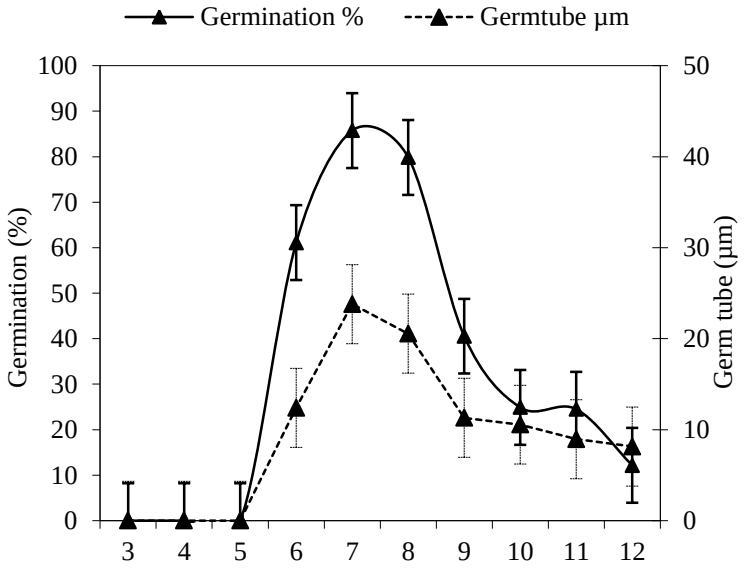

Figure 25. Effect of pH on conidial germination (%) **(LSD=8.22)** and germ tube length (µm) **(LSD=4.34)**, of *F. mangiferae* grown in SDW after 24h of incubation at 25^{o}C

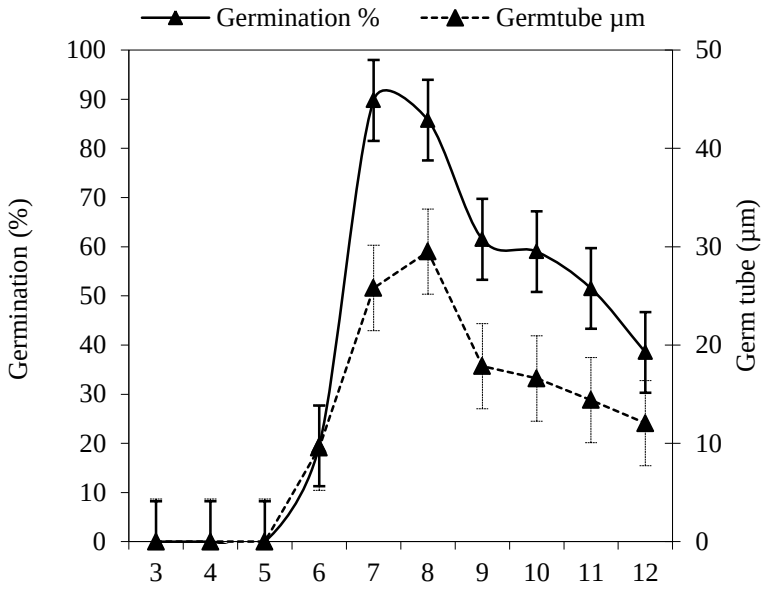

Figure 26. Effect of pH on conidial germination (%) **(LSD=8.22)** and germ tube length (µm**) (LSD=4.34)**, of *F. mangiferae* grown in 100mM glucose after 24h of incubation at 25^{o}C.

3.4.9. Oxidants (hydrogen peroxide and menadione)

The effect of the oxidants hydrogen peroxide (H_2O_2) and menadione ($C_{11}H_{18}O_2$) on germination of conidia and germ tube growth was assessed in SDW (Figure 27 and 28). Germination of conidia and germ tube growth were not affected at low concentrations of hydrogen peroxide (0-1mM). However, at concentration above 1mM, conidia germination and germ tube growth were totally inhibited . Menadione, however, was more toxic and total inhibition of germination and germ tube growth were observed at lower concentrations (0.1- 100 mM).

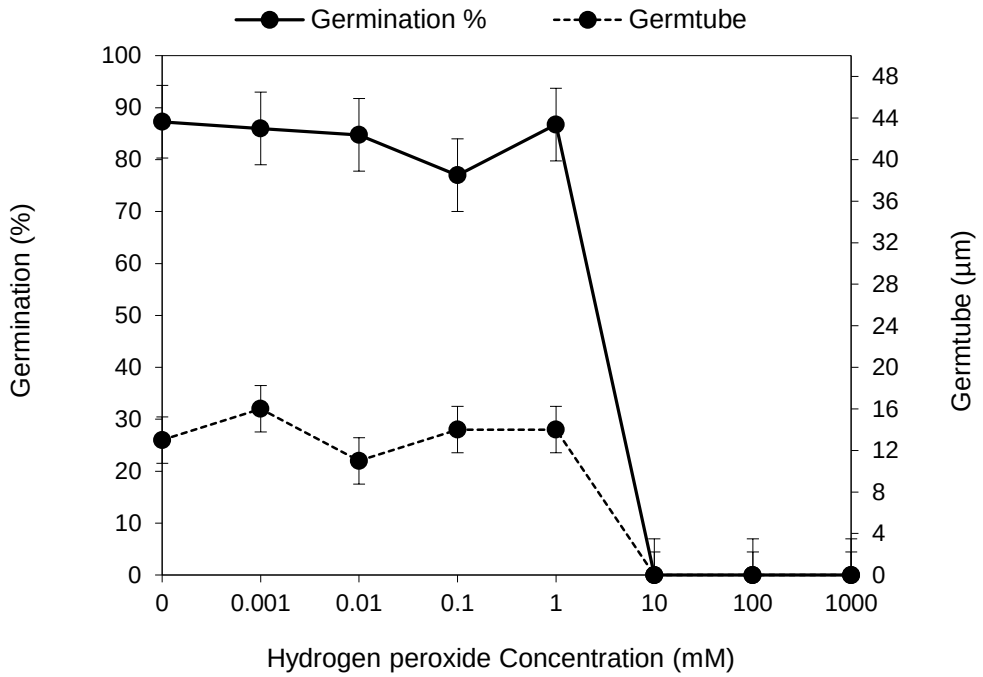

Figure 27. Effect of the oxidant hydrogen peroxide on conidial germination (%) **LSD= 5** and germ tube length (µm) **LSD=3.2** of *F. mangiferae* in SDW after 24h of incubation at 25°C.

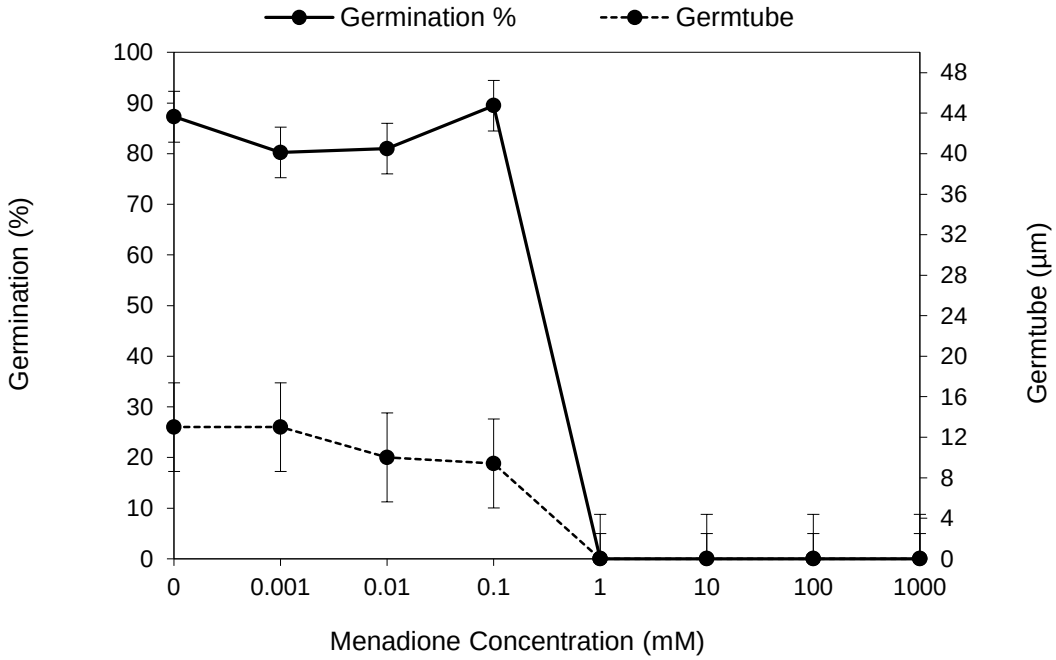

Figure 28. Effect of the oxidant menadione on conidial germination (%) **LSD=(3.233)** and germ tube length (µm) **LSD= (4.388)**, of *F. mangiferae* in SDW after 24h of incubation.

3.5. Management of Mango Malformation

3.5.1. Biological control with *T. harzianum*.

3.5.1 Effect of *T. harzianum* metabolites on mycelium growth rate of *F. mangiferae* ($Q_{2.4}$).

The effect of fungitoxic metabolites produced by *T. harzianum* (Jn14 and Jn58) on mycelium growth rate of *F. mangiferae* ($Q_{2.4}$) was evaluated *in vitro*. Both *T. harzianum* isolates (Jn14 and Jn 58) culture filtrates reduced mycelium growth of *F. mangiferae* significantly compared to the control in the two concentration used (Figure 29). MGR of *F. mangiferae* decreased with increasing concentration of *T. harzianum* culture filtrates.

T. harzianum (Jn14 and Jn58) culture filtrates at the highest concentration (40%), reduced MGR of *F. mangiferae* by (69%) and (58%), respectively, compared to the control with no significant difference in between.

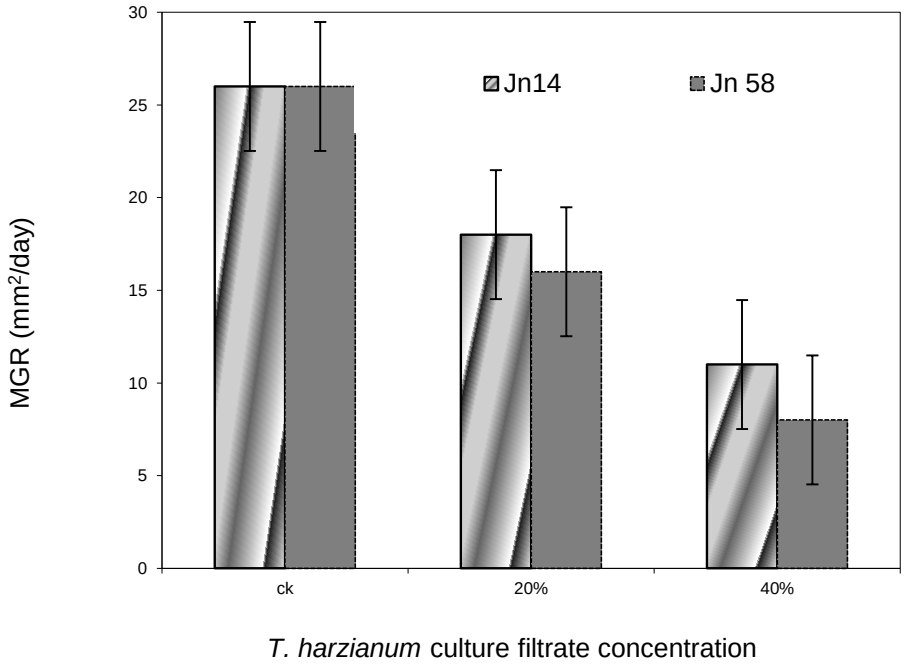

Figure 29. Effect *T. harzianum* fungitoxic metabolites on mycelium growth rate of *F. mangiferae* ($Q_{2.4}$) in PDA at 25ºC (**LSD=3.48**).

3.5.2. Chemical control

3.5.2.1. The effect of the fungicide Aliete (Fosetyl-Al) on mycelia growth rate of *F. mangiferae* ($Q_{2.4}$) and *T. Harzianum* (Jn14 and Jn58).

The effect of the fungicide Aliete (Fosetyl-Al) on mycelia growth rate of *F. mangiferae* ($Q_{2.4}$) and *T. harzianum* (Jn14 and Jn58) was evaluated *in vitro* using several concentrations (Figure 30). Both *T. harzianum* isolates tested were obviously and significantly more tolerant to the fungicide *in vitro* than *F. mangiferae*, with no difference in tolerance in between. *F. mangiferae* mycelial growth was reduced by 53% at the highest concentration tested compared to the control. However, *T. harzianum* mycelial growth was reduced by only 20% compared to the control at the highest concentration of the fungicide Fosetyl-Al tested (800 ppm).

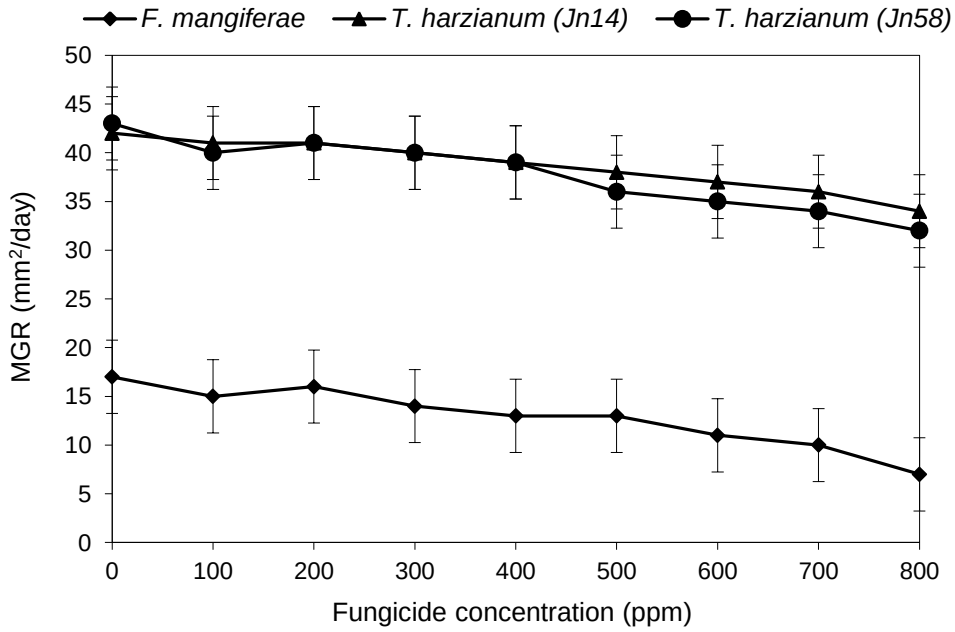

Figure 30. Effect of the fungicide Fosetyl-Al (Aliete) on mycelium growth rate of *F. mangiferae* ($Q_{2.4}$) and *T. harzianum* (Jn14 and Jn58)(**LSD=3.754).**

3.5.2.2. The effect of the fungicide Aliete (Fosetyl-Al) on conidial germination of *F. mangiferae* ($Q_{2.4}$) and *T. harzianum* (Jn14 and Jn58)

The effect of the fungicide Aliete (Fosetyl-Al) on conidial germination of *F. mangiferae* ($Q_{2.4}$) and *T. harzianum* (Jn14 and Jn58) was evaluated *in vitro* using several concentrations (Figure 31). The results showed that conidia of *T. harzianum* (Jn 14) were the most tolerant for the fungicide Fosetyl-Al *in vitro*, and almost at all concentrations tested. *T. harzianum* (Jn58), however, the conidial germination capacity declined dramatically at concentrations above 4 ppm reaching 0% when exposed to the highest concentration of the fungicide. *F. mangiferae* conidia were the least tolerant to the fungicide and the germination capacity declined sharply at all concentrations until it stopped completely at the highest concentration tested (10 ppm).

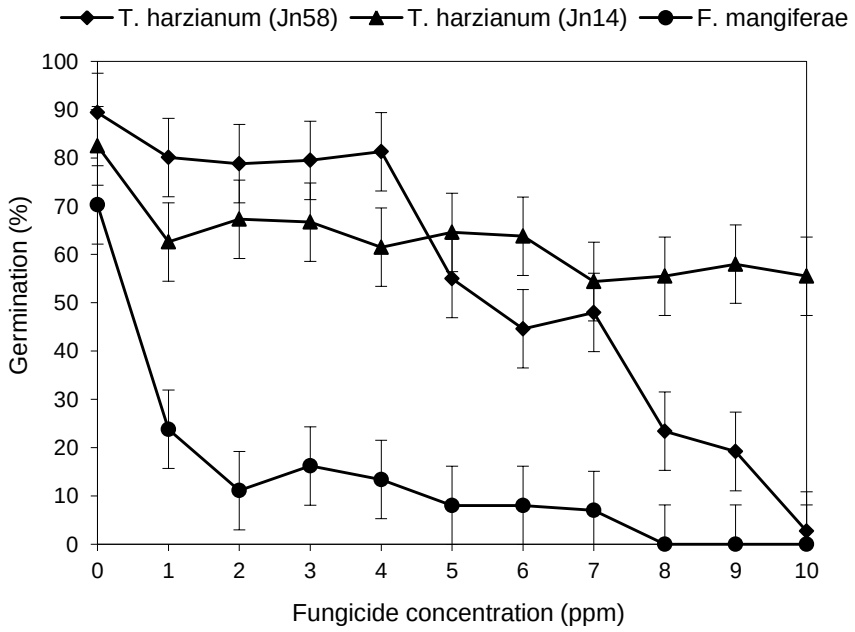

Figure 31. Effect of the fungicide Fosetyl-Al (Aliete) on the germination (%) of *F. mangiferae* and *T. harzianum* at different concentration (0-10 ppm). **LSD= 8.124**

Chapter 4

4. Discussion

The disease survey made in mango orchards in North of the West Bank proved the presence of Mango Malformation in the main mango production areas of Palestine. *F. mangiferae* was isolated from the majority of suspected samples collected from Qalqelia which indicates that the disease entered from Israeli production areas. Its seems that this was the entrance point but the disease may not have yet moved North to Tulkarm production areas since non of the collected samples from Tulkarm had *F. mangiferae.*

F. mangiferae isolated from infected mango samples collected from North of the West Bank was identified based on morphological characters described by (Booth, 1971). The isolates in the present study matched the descriptions and displayed the characteristic described for the species (Wollenweber and Reinking, 1925; Booth, 1971; Kuhlman et al., 1978; Nelson et al., 1983). Suspected plant samples cultured on PDA gave dense colonies with abundant aerial mycelium. Colonies from single-conidium of most of the isolates formed light orange color at first and later turned into dominant light to dark purple. Mixed coloration was visible on the obversed side of the petridish with minor variation. Macroconidia were slender, falcate and borne on monophialides. Three to four-septate macroconidia were always found, while microconidia is slightly sickle shaped to straight with dorsal and ventral surface almost parallel. The size of macroconidia conformed to the reported standards for the species ranging from 3.5-5×45-60µm (Gerlach and Nirenberg, 1982). The microconidia were abundant, fusiform, oval to elliptical, and sometimes spindle shaped (Nelson et al., 1983; Iqbal, 2004). None of the seven *F. mangiferae* isolates produced chlamydospores in cultures. Furthermore, *F. mangiferae* can be

identified by (PCR) based on DNA fingerprinting. Ploetz et al., (2002) showed successful results in generating amplification patterns specific to *F. mangiferae*. Saleem (2004) identified molecularly twenty isolates of *F. mangiferae* from malformed tissues of mango obtained from different areas of Pakistan. The potential of RAPD to identify DNA markers related to intraspecific diversification of the pathogens led to study the genetic diversity within *F. mangiferae* population. KalcWright et al., (1992) classified isolates of *Fusarium* sp. collected during a large scale surey in Victoria, by cultural morphology, pathogenicity and a RAPD assay.

As for the selective media used to isolate *F. mangiferae* from soil, Dodine proved to be an effective fungicide for the isolation. The concentration of 1.5 ppm gave the best selectivity for the benefit of *F.mangiferae*. Numbers of other fungi were low enough for the selective isolation of the pathogen. At higher concentrations (e.g. 2.5 ppm), however, it seems that selectivity declined for the favor of other fungi that showed more tolerance to the fungicide. Concerning soil types, no influence what so ever was obvious in relation to the use of Dodine for the isolation of *F. mangiferae* or other fungi from soil. In previous reports dodine have been successfully used to isolate *F. mangiferae* from two types of soil (Clay and Sand) (Nash and Sayder, 1962). Nash medium amended with 1.5g/l dodine with 20g agar, 15g peptone, 1g KH_2PO_4, 0.5g $MgSO_47H_2O$ and 0.5g Chloromphenecol resulted in good isolation of *F. mangiferae*.

After the successful isolation of *F. mangiferae*, isolates growth rate were evaluated *in vitro*. Isolates growth rates ranged from 12.2cm^2/day to 25.3 mm^2/day. The highest mycelia growth rate (25.3 mm^2/day) was recorded by the isolate ($Q_{2.4}$), which was significantly different from the other isolates growth rates; the lowest mycelium growth rate (12.5 mm^2 /day) was recorded by the isolate ($Q_{2.5}$). This variation in growth rate is very common in *F. mangiferae*

and has been documented by several investigates (Iqbal, 2004; Nelson et al., 1983; Ploetz and Gregory, 1993).

The effect of temperature on mycelium growth rate of *F. mangiferae* was studied for *F. mangiferae* isolate ($Q_{2.4}$). The results showed that mycelium growth rate reached a peak at 25°C, and was completely inhibited at 5 and 35°C. Furthermore, it was observed that light stimulated the germination of *F. mangiferae* isolates. The positive effect of light on spore production was also, observed by Akhtar et al., (1999). In addition, Leslie, (2006) and Rossi, (2002) reported that the optimal temperature for *F. mangiferae* growth was around 25ºC and growth was very slow below 18ºC. Iqbal, (2004) showed that 25-30°C is the optimal temperature range for growth of *F. mangiferae*, and germination inhibited at 8°C. Low temperatures (<5°C) completely inhibited conidial germination and colony growth.

Very little or no information has been published regarding conditions affecting germination and growth of *F. mangiferae* and the infection patterns of this pathogen. Conidia of *F. mangiferae* are likely to be the most prevalent propagules resulting in spread of the pathogen (Gamliel-Atinsky et al., 2009). Therefore, evaluating conditions affecting their germination and growth may contribute to a better understanding of the infection process.

Conidial germination and germ tube growth increased proportionally with time of incubation. The highest germination rate (90%) and germ tube growth (28 µm) occurred after 36 hours of incubation at 25 °C. In this direction, Pujol et al., (1997) reported that *Fusarium* spp. reached high degree of conidial germination after 48 hours of incubation. Nelson et al., (1983) reported that *Fusarium* spp. reached complete conidial germination after 96 hours of incubation. This actually agrees with the findings of this study, and if readings

lasted beyond 36 hours, germination rate would have reached 100% ultimately in terms of time.

Conidial germination rates of *F. mangiferae* ($Q_{2.4}$), however, decreased with increasing spore concentrations without significant differences between SDW and fructose. The highest germination rate and germ tube growth was obtained at the lowest spore concentration tested (10^2 conidia/ml). This agrees with other investigators on the topic. Helbig and Carroll, 1984 found that conidia of *F. oxysporum* exhibit a self-inhibition strategy during germination at high concentrations (10^6 conidia/ml or more). It is assumed that at high concentrations, conidia tend to produce specific germination and/or growth inhibitors regardless of the richness of the substrate. the spore germination of *Fusarium* was influenced by the spore concentration and the viscosity of the germination media.

Concerning temperature, conidial germination and germ tube growth were highest at 25°C. Both growth parameter declined at temperatures below or above 25°C and completely inhibited at temperature below 5°C or above 35°C. Similar results were found by Leslie, (2006); Rossi, (2002) who showed that conidial germination and mycelial growth required temperatures >5°C in order to commence the germination and growth processes. Low temperatures (< 5°C) did not permit conidial germination and colony growth. The temperature requirements for germination is usually in the same range as for growth, but the differences in the optimum for germination and growth maybe existing between or within fungal species (Griffin, 1994). Optimal temperatures for growth and germination vary between *Fusarium* spp. Leslie, (2006); Rossi, (2002). They showed that the optimal temperature for germination and growth of *F.*

mangiferae conidia was 28 and 25°C, respectively. Therefore, this temperature range does not appear to be a limiting factor in seasonal disease development of mango malformation in mango cultivation areas worldwide Huber and Gillespie, (1992). Akhtar et al., (1999) reported that mycelial growth rate was better observed at temperature between 25-30ºC on potato dextrose agar medium.

In an attempt to evaluate which exogenic nutrient can triggers germination and enhance growth of *F. mangiferae* spores, three sugars (glucose, sucrose and fructose) were tested. All three sugars stimulated germination and germ tube growth but in different proportions and according to the concentration used. The mechanism of sugar sensing by *F. mangiferae* conidia is unknown. As diverse sugars and acetate induce germination with similar efficiency, it appears unlikely that nutrient sensing occurs by plasma membrane proteins (Forsberg and Ljungdahl, 2001). One explanation for the particular important activity of fructose in conidial germination could be that this sugar is preferentially taken up by a fructose specific transport system. This is surprising since glucose is usually the most efficient hexose not only as a nutrient, but also as a signalling compound (Doehlemann et al.,2005).

In the same direction, four amino acids were tested in terms of conidial germination and germ tube growth stimulation. All four amino acids increased conidial germination and enhanced germ tube growth in different proportions and respective to the concentrations used. Growth parameters increased with increasing amino acids concentrations. Glutamic acid was able to boost germination to almost 100% at the highest concentration tested (1000 mM). In this direction, it was found that Alanine inhibited germination of *Fusarium* spp.

at concentrations above 600 µg/ml whilst Proline had no significant influence on germination at concentrations below 1000 µg/ml with variations in germ tube lengths. Johnes and Woltz, 1972 showed that Tryptophan and Nicotinic Acid had no influence on conidial germination of *Fusarium* wilt, but Proline weakly enhanced germination compared to Alanine. On the other hand, Asparagine moderately increased conidia germination of *Fusarium* wilt.

Furthermore, the effect of six cations on conidial germination and germ tube growth of *F. mangiferae* was evaluated. Cu^{+2} proved to be very toxic at all concentrations, while Fe^{+2} and Zn^{2+} decreased growth parameters sharply at concentrations above 0.01mM. On the other hand, the cations Ca^{+2}, Mg^{+2} and K^{+} showed no effect at lower concentrations, but increased growth parameters at the concentrations above 10 mM. In this direction, several investigators worked on cations and plant disease development. Helbig and Carroll, 1984 showed that the Fe^{+2} reduced the conidial germination of *Fusarium oxysporum*.

It is very likely that conidia before germination is not affected at low concentrations of cation availability in the growth substrate. However, after germination, germ tube growth becomes more sensitive to a wide range of cation concentrations in the growth media. Cu^{+2} at all concentration completely inhibited conidial germination and germ tube growth. Huber and Schneider, 1982, reported that Cu^{+2} inhibited conidial germination of *F. oxysporum*, while K^{+}, Ca^{+2} and Mg^{+2} enhanced conidial germination and germ tube growth at all concentrations tested.

Concerning nitrogen forms, only the nitrate form of nitrogen enhanced germination and germ tube growth but only at the highest concentrations tested (100 mM). Ammonium nitrogen showed no influence on the growth parameters

of *F. mangiferae*. Huber and Watson (1974), discovered that NH^{+4} increased the germination of *F. oxysporum*, while NO^{3} decreased the germination of *F. oxysporum*. Chang and Chang, (1999) reported that *G. graminis* was better on the medium with NH^{+4}, while others grew better on NO^{-3} as the only nitrogen source. It is worth mentioning that no work has been done what so ever on *F. mangiferae* relations with nitrogen forms before this study.

As for the microclimate pH conidial germination was significantly impaired by extreme pH ranges (below 7 and above 9). Conidia germinated well at pH ranging from 6-8 with the highest germination rate at pH 7. Akhtar et al., (1999) found a similar results that pH 7 is the optimal value for conidial germination of *Fusarium* spp. Vautard and Fevre, (2003) reported that the optimal pH range for *Fusarium oxysporum* on tomato ranged from 6 to 8.

Furthermore, the effect of oxidants hydrogen peroxide (H_2O_2) and menadione ($C_{11}H_{18}O_2$) on germination of conidia and germ tube growth of *F. mangiferae* was evaluated. Hydrogen peroxide (H_2O_2) decreased growth of conidial germination and germ tube of *F. mangiferae* sharply at concentrations above 1 mM. On other hand, menadione ($C_{11}H_{18}O_2$) was more toxic than hydrogen peroxide, where its decreased sharply the conidial germination and germ tube growth above 1mM.

The management of Mango Malformation is not less challenging than understanding the early events of infection and epidemiological aspects of the disease. The biological control study of *F. mangiferae* showed that *T. harazianum* (Jn14 and Jn58) fungitoxic metabolites was able to reduce mycelium growth of *F. mangiferae* significantly compared to the control in the

two concentrations tested. *T. harazianum* (Jn 14 and Jn 58) at the highest concentration (40%), reduced MGR of *F. mangiferae* by 69% and 58% respectively, compared to the control. Tjamos et al. (1992) showed that *T. harzianum* controls *F. oxysporum* by competing for space and nutrients. *Trichoderma* was able to encroach into the inhibition zone of *Fusarium* and extend mycelia towards *Fusarium* followed by heavy sporulation immediately on the colony of *Fusarium* which depicts a step in the mechanism of parasitic activity of *Trichoderma* (Brunner et al., 2005). The mode of action of *T. harzianum* on pathogen includes mycoparasitism, competition and antibiosis (Elad, 1996). Mycoparasites utilize fungal cell-wall-degrading enzymes such as chitinases and glucanases to dissolve their fungal hosts' cell walls and penetrate the cells (Elad, 1995). Competition is effective when the pathogen conidia need exogenous nutrients for germination and germ-tube elongation (Blakeman, 1993).

Concerning chemical control, Fosetyle-Al (Aliete ®) fungicide effect on conidia and mycelium growth of *F. mangiferae* ($Q_{2.4}$) and *T. harzianum* (Jn14 and Jn58) was investigated under several concentrations. Both *T. harzianum* (Jn14 and Jn58) tested were obviously and significantly more tolerant (growth reduced by 20%) to the fungicide *in vitro* than *F. mangiferae* (growth reduced by 53%). According to Bayer facts sheet, Aliete ® acts on spore germination as an inhibitore and stimulates plants defense mechanisms. Wada et al., (1990) tested three chemicals against *F. moniliforme* isolates. The fungus proved sensitive to Benlate at 12.5 ppm. In a similar study, Benlate and Carbendazim proved highly effective against the fungus *F. mangiferae* at 10 ppm. Kumar, (1983) found Carbendazim to be highly effective fungicide *in vitro* against *Fusarium sp*. isolated from malformed mango tissues. Pujol et al., (1997) determined the minimum inhibitory concentration of amphotericin B, micinazole, ketoconazole,

flucytosine and fluconazole for isolates of different *Fusarium* species. Kummar and Beniwal, (1992) and Nene and Thapliyal, (1993), reported that *Fusarium* spp. are sensitive *in vitro* to Benlate, Carbendazim, Copper oxychloride and Trimeltox. Akhtar et al., (1999) determined the *in vitro* sensitivity of *F. mangiferae* to six fungicides viz, Benlate, Antracol, Topsin-M, Dithans M-45, Anvil and Nordox. Benlate and Topsin- fungal growth at lower concentrations.

As a conclusion of this study, it was shown that the epidemiology of this disease was as complex as its management, and from looking at the literature, it is definitely important that investigations are still missing in various epidemiological aspects such as survival of the pathogen in soil, infection venues, and pathogen vectoring if any. In terms of the early events of infection, this study revealed the importance of several physical and chemical factors. Finally, field studies after *in vivo* tests are necessary when it comes to the practical management of the disease.

العوامل المؤثرة على وبائية و إنبات ابواغ فطر *Fusarium mangiferae*, المسبب لمرض تشوه أزهار المانجو

إعداد

فداء أبو شرار

إشراف

الأستاذ الدكتور رضوان بركات

الملخص :

يعتبر مرض تشوه ازهار المانجو والذي يسببه الفيوزاريوم مانجيفيرا *Fusarium mangiferae* من اهم الامراض المحددة للإنتاج في جميع مناطق زراعة المانجو في العالم حيث يؤدي تشوه وتجعد الشماريخ الزهرية قلة في العقد والإثمار.

تهدف هذه الدراسة لمعرفة وجود وانتشار المرض في مناطق زراعة المانجو في شمال الضفة الغربية ودراسة العوامل الكيميائية والفيزيائية التي تؤثر على نمو وتطور المرض وبعض البدائل الميسرة للمكافحة.

واظهر المسح الميداني لمدة عامين انتشار المرض في حقول المانجو في منطقة قلقيلية وأظهرت نتائج الدراسات المخبرية المعمولة على المسبب الفطري لهذا المرض بان درجات الحرارة المثلى لنمو ابواغ الفطر هي 25°م. كما اشارة الدراسات الى ان السكريات (السكروز, الجلوكوز والفركتوز) والأحماض الامينية (جلوتاميك أسيد برولين. النين والاسبارتك اسيد) والكاتيونات (الكالسيوم, المغنيسيوم والبوتاسيوم) والنيترات تؤدي الى زيادة النمو ونسب انبات الابواغ بينما العناصر الصغرى (الزنك, الخارصين والحديد) والمؤكسدات (هيدروجين اوكساید و مينوداين) ادت الى انخفاض في نمو ونسب انبات الابواغ لهذا الفطر.

كما اشارة الدراسات الى ان نواتج ايض فطر *T. harzianum* ادت الى انخفاض نمو الفطر الممرض كما تبين ان فطر *T. harzianum* يتحمل المبيد الفطري Fosetyl-Al بقدر اكبر مما يتحمله الفطر الممرض.

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بسم الله الرحمن الرحيم

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كلية الدراسات العليا

برنامج الموارد الطبيعية وارداتها المستدامة

العوامل المؤثرة على وبائية و إنبات ابواغ فطر الفيوزاريم مانجيفيرا, المسبب لمرض تشوه أزهار المانجو

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