EFFECT OF SOIL PHYSICAL AND BIOLOGICAL PROPERTIES AND SOIL FLOODING TREATMENT ON DISEASE INCIDENCE OF *Fusarium* WILT OF TOBACCO

MOHD NORHAFEEZ BIN JUSOH

UNIVERSITI SAINS MALAYSIA

2017

EFFECT OF SOIL PHYSICAL AND BIOLOGICAL PROPERTIES AND SOIL FLOODING TREATMENT ON DISEASE INCIDENCE OF *Fusarium* WILT OF TOBACCO

by

MOHD NORHAFEEZ BIN JUSOH

Thesis submitted in fulfillment of the requirements

for the degree of

Master in Science

April 2017

ACKNOWLEDGEMENT

In the name of Allah, Most Gracious, Most Merciful. All praise and glory to Almighty Allah (S.W.T) who gave me courage and patience to perform this work. Peace and blessing of Allah be upon last Prophet Muhammad (Peace Be upon Him).

It is my pleasure to express my sincere and deepest gratitude to my supervisor, Associate Professor Dr. Hideyuki Dhakirullah Nagao and co-supervisor, Professor Dr. Wan Ruslan Ismail for his patience, motivation, enthusiasm and immense knowledge. Above all and the most needed, he provided me unflinching encouragement and support in various ways.

I am grateful for the friendship and support of my friends in the Plant Pathology Laboratory, especially Mohd Nazzary, Jalilah, Muhammad Adli, Nur Zubaidah and Siti Juhana who have listened to me laugh, complain and rejoice in these three years. It is a pleasure to pay tribute also to staffs in the Department of Plant Pathology, School of Biological Sciences especially En Abdullah and En Kamarudin for their help and technical assistance.

Last but not least, I wish to thank my family especially to my beloved wife, Suziana BT Tuan Ab Halim for standing by me in all my endeavors with love and patience, and for making many sacrifices without which I could not have pursued my studies. I wish to record my very special sincere appreciation and thank to my parents, Jusoh bin Ismail and Nuraini binti Johari, and my siblings, who give me the

warm encouragement and have been with me all the times to spur my spirits. Without their supports and caring, it is hard for me to get motivated and inspire to finish my study. Their prayers and moral support will be always in my heart. Thank you everyone. Thank you so much.

TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure

LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of variance
cfu	Colony forming unit
CLA	Carnation leaf-piece agar
DI	Disease incidence
f. sp.	Formae speciales
Fe2(SO4)3	Iron(III) sulfate
Fe-EDTA	Ferum-ethylenediaminetetraacetate
H3PO4	Phosphoric acid
K_2HPO_4	Dipotassium phosphate
KCl	Potassium chloride
KH2PO4	Monopotassium phosphate
MgSO4•7H2O	Magnesium sulfate heptahydrate
Na2B4O7•10H2O	Sodium tetraborate
NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
PCNB	Pentachloronitrobenzene
PDA	Potato dextrose agar
PSA	Potato sucrose agar
PVC	Polyvinyl chloride
var.	variety
WA	Water agar

KESAN CIRI-CIRI FIZIKAL DAN BIOLOGIKAL TANAH DAN RAWATAN TANAH BANJIR TERHADAP INSIDEN PENYAKIT LAYU *Fusarium* PADA TEMBAKAU

ABSTRAK

Penyakit layu *Fusarium* pada tembakau adalah disebabkan oleh *Fusarium oxysporum*. Spesies ini dikenali sebagai patogen tumbuhan sebaran tanah. Oleh itu, kajian ini dijalankan untuk melihat insiden penyakit layu *Fusariu*m pada tembakau berdasarkan tekstur dan ciri-ciri tanah dan kesan rawatan banjir bagi populasi *F. oxysporum*. Dalam kajian ini, beberapa kaedah telah dilakukan antaranya pengecaman spesies *F. oxysporum*, analisis tekstur sampel tanah dan analisis ciri-ciri fizikal dan biologi tanah serta kesan rawatan banjir terhadap populasi *F. oxysporum* di dalam tanah. Pencilan *F. oxysporum* yang diperoleh daripada 22 jenis tanah telah dicamkan secara morfologi berdasarkan pemerhatian ciri-ciri mikroskopik dan makroskopik. Daripada 22 sampel tanah yang telah dianalisa, terdapat 18 sampel tanah mempunyai tekstur tanah jenis berpasir dan empat sampel tanah mempunyai tekstur jenis tanah lom berpasir. Kedua-dua tekstur tanah didapati tiada kolerasi signifikan dengan insiden penyakit. Tetapi tekstur tanah jenis berpasir memperlihatkan korelasi signifikan ($P<0.05$) dengan populasi bakteria. Analisis fizikal tanah iaitu pH, kelembapan, ketumpatan pukal, porositi dan ketumpatan partikel didapati kesemuanya tidak mempunyai kolerasi signifikan ($P<0.05$) dengan insiden penyakit. Tetapi pH tanah didapati menunjukkan korelasi signifikan ($P<0.05$) dengan populasi *F. oxysporum*. Bagi analisis biologi tanah merangkumi populasi fungi, *F. oxysporum*, actinomycetes dan bakteria. Keputusan menunjukkan populasi fungi dan actinomycetes tidak mempunyai kolerasi signifikan ($P<0.05$) dengan insiden penyakit

manakala populasi *F. oxysporum* dan bakteria memperlihatkan korelasi sinifikan ($P<0.05$) dengan insiden penyakit. Kajian rawatan banjir menunjukkan populasi *F. oxysporum* f. sp. *niveum* berkurang dengan korelasi signifikan ($P<0.05$) setelah rawatan banjir dilakukan. Kesimpulannya, tekstur tanah, ciri fizikal tanah dan populasi fungi serta actinomycetes tidak mempunyai kolerasi signifikan dengan insiden penyakit layu *Fusarium* pada tembakau. Manakala, populasi *F. oxysporum* dan bakteria menunjukkan korelasi signifikan dengan insiden penyakit layu *Fusarium* pada tembakau. Kaedah rawatan banjir dapat mengurangkan populasi *F. oxysporum* f. sp. *niveum* di dalam tanah. Maklumat yang diperoleh daripada kajian ini boleh digunakan untuk formulasi kaedah kawalan penyakit layu *Fusarium* atau penyakit lain melalui sebaran tanah. Selain itu, ia boleh juga diguna dalam kajian tanah supresif untuk mengurangkan insiden penyakit pada tanaman pertanian bagi mengelak penyakit menjadi teruk.

EFFECT OF SOIL PHYSICAL AND BIOLOGICAL PROPERTIES AND SOIL FLOODING TREATMENT ON DISEASE INCIDENCE OF *Fusarium* WILT OF TOBACCO

ABSTRACT

The *Fusarium* wilt of tobacco is caused by *Fusarium oxysporum*. This species is known as soilborne plant pathogen. Therefore, this study was conducted to survey the disease incidence of *Fusarium* wilt of tobacco based on physical and biological soil characteristics and the effect of flooding treatment on the population of *F. oxysporum* in soils. In this study, several methods have been conducted such as the identification of *F. oxysporum* species, analysis on soil texture, soil physical and biological characteristics and also soil flooding experiment on *F. oxysporum* population in the soil. The isolates of *F. oxysporum* collected from 22 soil samples has been identified based on the microscopic and macroscopic characteristics. From the 22 soil samples analyzed, a total of 18 soil samples were sandy and four soil samples were loamy sand. Both soil textures showed no significant correlation to the disease incidence. The soil physical analysis of pH, moisture, bulk density, porosity, particle density showed no significant correlation ($P<0.05$) on disease incidence. However, soil pH showed a significant correlation ($P<0.05$) to the population of *F. oxysporum*. For soil biological properties, the population of fungi, *F. oxysporum*, actinomycetes and bacteria were analyzed. The results showed fungi and actinomycetes population does not have significant correlation ($P<0.05$) on disease incidence while *F. oxysporum* and bacteria population showed a significant correlation ($P<0.05$). The soil flooding treatment showed that the population of *F. oxysporum* were significantly reduced ($P<0.05$) after the flooding treatment was conducted. As for the conclusion,

soil texture, soil physical, fungi and actinomycetes populations showed no significant correlation on disease incidence of *Fusarium* wilt of tobacco. Besides, the population of *F. oxysporum* and bacteria showed a significant correlation on the disease incidence of *Fusarium* wilt of tobacco. The soil flooding treatment showed that the population of *F. oxysporum* was reduced in the soil after flooded. The results obtain from this study can be used to formulate a disease control methods on *Fusarium* wilt disease or other soil borne disease. Besides, this result also can be used in the study of suppressive soil to reduce the disease incidence on crops to prevent serious disease from happen.

CHAPTER 1 INTRODUCTION

The genus *Fusarium* has been known as the genus that contains many plant-pathogenic fungi and can cause diseases to plants, human and also domesticated animals (Goldschmied *et al*., 1993; Krcmery *et al*., 1997; Boonpasart *et al*., 2002). It has been known that at least one *Fusarium*-associated disease in many diseased plants (Leslie and Summerell, 2006). One of the most widely dispersed *Fusarium* species is *Fusarium oxysporum* and it can be recovered from most soils including arctic (Kommedahl *et al*., 1988), tropical or desert (Joffe and Palti, 1977; Mandeel *et al*., 1995) and cultivated or not cultivated soils (McMullen and Stack, 1983, 1984). Moreover, in the *Fusarium* genus, *F. oxysporum* is the most economically important species given its numerous hosts and the level of loss that can result when it infects a plant (Leslie and Summerell, 2006). Plant pathogenic *F. oxysporum* species often can cause vascular wilt diseases (Nelson *et al*., 1981; Beckman, 1987; Summerell and Rugg, 1992), damping-off problems (Nelson *et al*., 1981) and crown and root rots (Jarvis and Shoemaker, 1978). The vascular wilt diseases of plant occur when the xylem vessels of the infected plant are blocked due to the formation of gels in the host plant's cell wall (van der Molen *et al*., 1986: Shi *et al*., 1992).

According to Leslie and Summerell (2006), *F. oxysporum* species can be identified using the morphological features of the fungus including the short phialides formed on the hyphae, the production of microconidia in false heads, the shape of the macroconidia and microconidia and the production of chlamydospores. The macroconidia of *F. oxysporum* can be observed with short to medium in length, falcate to almost straight, thin walled and usually 3-septate and short apical cell with slightly

hooked in some isolates. For microconidia, the shape may be oval, elliptical or reniform with usually 0-septate and are formed abundantly in false heads on short monophialides. When the fungus is cultured on cultured medium, the colony morphology appeared to be varies widely. The color of the colony pigmentation observed can be range in color from white to pale violet and the mycelia may be floccose, sparse and abundant (Leslie and Summerell, 2006).

Specific strains of *F. oxysporum* have been known to infect only a small number of host plants that are differentiated on the basis of pathogenicity known as formae specialis. These strains have very similar or identical morphological characteristic and it cannot be differentiated from non-pathogenic or saprophytic strains (Leslie and Summerell, 2006). In tobacco, the vascular wilt disease cause by *Fusarium* was first reported in 1921 by Johnson, and this disease has been reported in most part of the world including America, Canada, Netherland, India, Japan, Philippine, Russia and other country (Lucas, 1975). In Malaysia, Nik Masdek (1982) and Yusup (1985) reported that *F. oxysporum* as the main cause for vascular wilt disease in tobacco. The disease will show symptoms such as the vascular tissue turning to brownish or black coloration. In the early stage of infection, the leaves will turn into yellowish in color and half part of the plant become wilted and dried (Azmi, 1991).

Fusarium oxysporum is known to be dispersed by soil hence it is called as soilborne pathogen (Garibaldi *et al*., 2004). As this species inhabit the soil to propagate, it can be difficult to remove this species entirely from the soil. Years ago, many treatments have been experimented to control the spread of *F. oxysporum* within the soil by using chemical and resistant cultivars but did not yield promising results. In 1892, Atkinson recognized the type of soil that can limit the disease incidence of *Fusarium* wilt, known as suppressive soils. It has been reported that in *Fusarium* wilt

suppressive soil, the suppression effects are generated by the activity of several bacteria and fungi such as *Alcaligenes* sp. (Yuen and Schroth, 1986), *Bacillus*, *Trichoderma* (Sivan and Chet, 1989), *Pseudomonas* spp. (Kloepper *et al.,* 1980; Scher and Baker, 1982; Lemanceau and Alabouvette, 1993), Actinomycetes (Amir and Amir, 1989) and nonpathogenic *F. oxysporum* (Rouxel *et al.,* 1979; Alabouvette *et al.,* 1984; Alabouvette, 1986; Larkin *et al.,* 1996; Larkin and Fravel, 1998, 1999). Besides the biotic action in the suppressive soil, suppression effect also can be caused by soil abiotic properties such as soil pH, organic matter content and clay content (Stotzky and Martin, 1963; Amir and Alabouvette, 1993; Hoper *et al.*, 1995).

Other than the suppressive soil effect on *Fusarium* wilt disease cause by *F. oxysporum*, flooding technique has been studied in order to control the disease infection derived from soil pathogen. Flooding has been use to reduce the soil fungal population (Stover *et al.*, 1953; Stover, 1954) and being used to control diseases such as tobacco black shank in Sumatra, *Sclerotinia* on celery in Florida and *Fusarium* wilt of banana in Central America. In the 50s and 60s, researcher has been investigating on the effect of soil flooding experiment in *Fusarium* wilt of banana caused by *F. oxysporum* f. sp. *cubense* and the result showed that the wilt incidence can be reduced (Stover *et al.,* 1953, 1961; Stover, 1954, 1955, 1962a, b).

In Malaysia, the tobacco plantation takes place in east coast of peninsular Malaysia, mainly in Kelantan region and Terengganu region. Form the field survey analysis, it was indicated that *Fusarium* wilt disease caused by *F. oxysporum* was one of the main disease that infect the tobacco plant in the plantation region. This disease can lead to economic loss to tobacco farming industry, where the quality of the infected tobacco plant cause by *Fusarium* wilt disease would be poor.

As the *F. oxysporum* is the soilborne pathogen, research need to be made on the study on soil characteristic effect on the incidence of *Fusarium* wilt to know whether the population of *F. oxysporum* in the soil can be influenced by soil characteristics or not. Soil physical and biological properties need to be analyzed to find the factors that correspond to the disease incidence of *Fusarium* wilt. The study of correlation between soil characteristic properties with *Fusarium* wilt disease incidence is important because it can improve the disease management and the result can be used to plan a suitable control method in the future.

The objectives of this research are as below:

1- To conduct a field survey and soil sampling on *Fusarium* wilt disease incidence and morphological identification on *F. oxysporum*
2- To analyze and correlate the soil physical and biological properties with incidence of *Fusarium* wilt disease
3- To conduct a study on soil flooding treatment on *F. oxysporum* f. sp. *niveum* population density before and after flooding treatment

CHAPTER 2 LITERATURE REVIEW

2.1 *FUSARIUM* WILT

2.1.1 *Fusarium* taxonomy and classification

Link (1809) was first to described the genus *Fusarium* based on the presence of banana- or canoe-shaped conidia. Earlier studies of *Fusarium* reported more than 1000 species of *Fusarium* that cause plant diseases described based on the diagnosis, identification and enumeration of the taxa. However, in many cases most of the *Fusarium* species were poorly defined and the type of specimens were no longer available. Numerous species were identified using several types of culture media and various characters that led to species diagnosis became very complicated. Wollenweber and Reinking (1935) later simplified Link complicated system.

A simplified taxonomy system was introduced by Wollenweber and Reinking (1935) which species with similarities were grouped into the same section based on the shape of the foot or basal cells of macroconidia; presence and the shape of the microconidia; occurrence and location of the chlamydospores. Sixteen sections were described using this system namely Arachnites, Arthrosporiella, Discolor, Elegens, Eupionnotes, Gibbosom, Lateritium, Liseola, Macroconi, Martiella, Pseudomicrocera, Roseum, Spicarioides, Sporotrichiella, Submicrocera and Ventricosum which included 65 species, 55 varieties and 22 forms. However, in Wollenweber and Reinking (1935) taxonomic system, the culture used was not originated from single conidia and the cultural media and incubation periods were not standardized. Their species concept remained based on the differences between isolates, rather than on similarities of the isolates.

In the 1940s and 1950s, Snyder and Hansen (1945) demonstrated the identification of *Fusarium* species using cultures derived from a single spore that reduced the number of species within the genus to nine from Wollenweber and Reinking's species descriptions. Snyder and Hansen recognized nine species which were: *F. episphaeria*, *F. lateritium*, *F. moniliforme*, *F. nivale*, *F. oxysporum*, *F. rigidiuscula*, *F. roseum*, *F. solani* and *F. tricinctum* which was based on morphological features of the macroconidia and variability of the species. The Snyder and Hansen species concept was easy to apply and virtually every isolate could be identified to species with relative ease and this species concept also popular among diagnosticians. *Fusarium oxysporum* and *F. solani* are the two wildly used species described by Snyder and Hansen (1945) but have little doubt that these taxa contain more than a single species as defined with more current species concepts (Baayen *et al*., 2000; O' Donnell, 2000; Suga, *et al*., 2000).

Another *Fusarium* taxonomist, Raillo, proposed a classification system in the mid-1930s which based on cultures derived from single conidia but the work was only published in 1950 (Leslie and Summerell, 2006). In Raillo studies, the morphological characteristics of the apical cell was used as the main character for species delimitation and the incurvature of conidia, length of the apical cell, number of septa and width of conidia were used to separate sub-species and varieties while colony pigmentation, presence of sclerotia and mode of spore formation were used to separate special form (Leslie and Summerell, 2006). Raillo (1935) observed the apical cell, incurvature of conidia and number of septa were constant while length and width of conidia, number of sclerotia, and the mode of spore formation varied in isolates within a single conidial culture.

In 1950s, Bilai (1955) also studied the cultural variation or mutation of *Fusarium* species and also focused on the effects of temperature, moisture, length of growth period and composition of the medium. As a result, Bilai introduced nine sections, 26 species, and 29 varieties were introduced and section Liseola was combined with section Elegans and section Gibbosum with section Discolor. However, the taxonomic system by Bilai (1955) was not widely accepted and difficult to understand.

Booth (1971) made a significant development of *Fusarium* taxonomy with the publication of a monograph, The Genus *Fusarium*. The monograph included keys to the sections and species of *Fusarium* adapted from taxonomic system of Wollenweber and Reinking's system. The use of morphology of the conidiogenous cells, especially those producing the microconidia had been introduced by Booth as a species-level diagnostic character. It is essential to use conidiogenous cell morphology for distinguishing some of the species in sections Liseola and Sporotrichiella (Leslie and Summerell, 2006).

A monograph entitled *Fusarium* Species: Their Biology and Toxicology was published by Joffe (1986) based on a large number of *Fusarium* isolates from soils, wilting or decaying plants and seeds. The approach was regarded as 'modern systems' and was identical to the system proposed by Wollenweber and Reinking (1935), Gerlach (1981) and Gerlach and Nirenberg (1982). It appears that Joffe so-called 'modern system' was a simplified system of Wollenweber and Reinking's taxonomic system. Based on Joffe (1986) classification, 13 sections, 33 species and 14 varieties were depicted.

A pictorial atlas of *Fusarium* species with clear photographs and line drawings was published by Gerlach and Nirenberg (1982) to enhance the original

drawings by Wollenweber and Reinking. The taxonomic system was based on differences rather than similarities and a few new species were described from a single culture and in some cases from a single mutant culture. From the Gerlach and Nirenberg (1982) taxonomic system, 78 species were described and arranged into sections despite the taxonomic system considered to be very complex.

Nelson *et al.* (1983) pointed out that for the identification of all *Fusarium* species, there was no single taxonomic system that was completely satisfactory. Thus, several systems available at that time were combined by Nelson *et al.* (1983) with results of their own studies to develop a compromised identification system of *Fusarium* species. As a result, the species varieties and forms were combined with appropriate species name and the number of species was reduced. They also mentioned that many of the varieties and forms may have been cultural variants or mutants cultures. A manual contains photographs of macroconidia, microconidia, conidiophores and chlamydospores produced on carnation leaf-piece agar (CLA) as the medium produced uniform microscopic characters was published by Nelson *et al.* (1983).

A manual based on 40, 000 cultures of *Fusarium* was published by Burgess *et al.* (1994). Taxonomic system that emphasized less on the use of sections for species identification because the boundaries to differentiate species are difficult especially when introducing new species was developed by Burgess *et al.* (1994).

A manual which contain a compilation of species descriptions by several researchers was produced by Leslie and Summerell (2006). In the manual, the morphological, biological and phylogenetic species concepts were used and included as a guide for *Fusarium* species identification.

The genus *Fusarium* is described as imperfect fungi in which the asexual spores are produced on or within aerial hyphae and has unknown or lack of sexual structures and reproduction cycle (Agrios, 2005). The latest classification of the genus *Fusarium* is as follows (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=5506).

Superkingdom: Eukaryota
Kingdom: Fungi
Subkingdom: Dikarya
Phylum: Ascomycota
Subphylum: Pezizomycotina
Class: Sordariomycetes
Subclass: Hypocreomycetidae
Order: Hypocreales
Genus: *Fusarium*

2.1.2 *Fusarium* wilt disease

The *Fusarium* wilt disease is one of the diseases caused by *Fusarium* spp. which occur at the vascular part of the host plant and most of the disease is caused by formae specialis of *Fusarium oxysporum*. As the disease occurred on the infected host plant, the vascular section became brownish, discolored and resulting in wilting. *Fusarium* wilt disease has been known to cause major damage on agricultural and horticultural crop plants around the globe. Important crops that have been attacked by this disease including banana, cotton, cabbage, flax, melon, tomato, oil palm, watermelon, muskmelon, aster, carnation, tulip, sweet potato, pea, gladiolus and lettuce.

The first case of *Fusarium* wilt disease identified was occurred in cotton plant (Atkinson, 1892) caused by *F. oxysporum* f. sp. *vasinfectum* and inflicts serious loss

of crop yields in United States and India. In present days, this disease continues to affect many main cotton production regions in the world mainly in West Africa, Turkey and Australia. In China, this disease had becoming the most important disease for cotton plant (Kelman and Cook, 1977). In *Fusarium* wilt of cotton, certain factors such as pathogen's virulence, cultivar's susceptibility, climate conditions, soil types and fertility and nematodes interactions tends to manipulate the total yield losses in cotton plantation field (Atkinson, 1892; Subramaniam, 1950; Al-Shukri, 1969; Garber *et al*., 1979; Armstrong and Armstrong, 1981; Wang *et al.,* 1999).

In past days, researcher had suggested various explanations in defining the method and mechanism on how the *Fusarium* spp. established in vascular system of host plants and later causing the wilt disease. Mechanical plugging of the vascular system had been proposed by Smith (1899) in which responsible for the wilting of cotton, cowpea, and watermelon plants infected with *Fusarium* sp. Then, fungal toxins were suggested instead of plugging that cause the symptoms that developed from vascular infections (Bisby, 1919; Brandes, 1919; Haskell, 1919). Later, reports still being published and investigated regarding to the pathogenesis and new suggestion had being made, suggesting that either injury of cells due to fungal toxins or vascular dysfunction and water stress are the grand cause of the symptoms developed. The spread of *F. oxysporum* may have been due to several factors such as incorporating tobacco stalks and remains back into the soil after harvesting and the spread of the chlamydospores between fields through movement of fields equipment (Wichuk et al., 2011).

2.1.3 *Fusarium* wilt of tobacco

Fusarium wilt of tobacco (*Nicotiana tabacum* L.), is a widespread disease caused by *Fusarium oxysporum* and occurs in many countries and results in severe damage in localized areas (Lucas, 1975). In United States, the *Fusarium* wilt of tobacco disease was first reported in Maryland by Johnson (1921) in 1916. Later, the same disease was first reported in Connecticut in 1943 by Anderson (1944) in a research plot that had previously been planted to sweet potato. By 1980's and 1990's, the *Fusarium* wilt of tobacco affect approximately 20% of tobacco production fields in Connecticut and Massachusetts, causing severe disease and the removal of heavily infested fields from tobacco production (LaMondia, 2015). The *Fusarium* species that cause the wilt disease have been designated as *F. oxysporum* f. sp. nicotianae, *F. oxysporum* f. sp. *batatas* and *F. oxysporum* f. sp. *vasinfectum* based on host range and ability to cause disease on multiple hosts such as sweet potato and cotton as well as tobacco (Armstrong and Armstrong, 1968; Smith and Shaw, 1943). Four races of *F. oxysporum* pathogenic to tobacco plant has been distinguished by Armstrong and Armstrong (1968). *Fusarium* wilt of tobacco showed typical symptoms of *Fusarium* wilt such as yellowing, drying and death of leaves, often vertically distributed on one side of the plant or even one side of the lead midvein. The vascular tissue showed chocolate-brown discoloration (Lucas, 1975; Shew and Lucas, 1991). The exterior of the green tobacco stalk becomes discolored as the discoloration become visible after a period. Finally, the entire tobacco plant will become dry, necrotic and die. The *Fusarium* wilt of tobacco is most severe in sandy loam soils and under warm conditions (Lucas, 1975).

In *Fusarium* wilt management of tobacco, many different tactics may be attempted to control this disease such as plant resistance, sanitation, rotation,

nutrition, nematode management and fumigation or biofumigation but, not all tactics are equally effective. For *F. oysporum* f. sp. *nicotianae* in soils, there are no completely effective chemical controls, although soil fumigation may result in moderate reductions in disease severity (LaMondia, 2015). Methyl bromide or chloropicrin is the most consistently effective fumigants against *F. oxysporum* (Bennet et al., 2011), but the use of methyl bromide is cease and both methyl bromide and chloropicrin resulted in poor wrapper leaf quality and burn (Taylor, 1987). The use of metam sodium in soil fumigation to reduce *Fusarium* wilt of cotton caused by *F. oxysporum* f. sp. *vasinfectum* was not generally effective and results were not different from non-treated controls (Bennet *et al.,* 2011). The chlamydospores of *Fusarium* may be resistant to fumigation, especially when the spore resides in crop debris (LaMondia, 2015). Lucas (1975) reported that the development and widespread use of wilt-resistant tobacco cultivars are the most effective control of *Fusarium* wilt worldwide. In Connecticut, tobacco cultivars with resistance to *Fusarium* wilt pathogen were released (LaMondia, 2012; LaMondia and Taylor, 1991, 1992; Lucas, 1975) but wilt symptoms still developed under high inoculum potential, suggesting that the resistant cultivars are not immune to *Fusarium* infection.

2.1.4 Mechanism in disease infection

In understanding the pathogenesis of *Fusarium* wilt disease, the penetration, establishment and colonization of pathogens within the water conducting vascular system need to be investigated and studied. According to Federation of British Plant Pathologist (1973), pathogenesis term has been defined as “the sequence of processes in *disease* development from the initial contact between a *pathogen* and its *host* to

completion of the *syndrome*''. In *Fusarium* wilt mechanism, *Fusarium* established and colonized the vascular part of its host by moving from initial contact point via roots penetration directly or through wounds opening and later develops the wilt symptom. Chlorosis, one-sided and total wilting, vascular discoloration and plant death are the typical wilt symptoms.

In the root of banana, melon, China aster, radish and cabbage, the pathogen invades the root cap and move intercellularly through the elongation zone (Brandes, 1919; Smith and Walker, 1930; Ullstrup, 1937; Reid, 1958). Cut or wounding of host plant also tends to favors wilt pathogen to enter xylem tissue directly as in cases with sweet potato tubers (McClure, 1949). The wilt pathogen tends to multiple and spread throughout the xylem vessel elements after it penetration into the vascular system (Tisdale, 1917; Brandes, 1919; Ullstrup, 1937; Chambers and Corden, 1963; Pennypacker and Nelson, 1972).

In the xylem parenchyma cells surroundings, the pathogens penetrate it by moving through the half-bordered pit pairs between the xylem parenchyma cells and the xylem vessel elements (Reid, 1958; Peterson and Pound, 1960; Pennypacker and Nelson, 1972; Philips and Stipes, 1976). In the xylem vessel elements, the pathogen colonizes and produces mass and rapid formation of conidia. In banana and tomato plant, extensive sporulation in the xylem vessel elements has been reported (Beckman *et al.,* 1961; Trujillo, 1963; Elgersma *et al.,* 1972). Within the vascular system, the perforated plates of xylem vessel elements becomes a barrier to the pathogen spores as they are found to move freely along the vascular stream (Beckman *et al.,* 1961; Trujillo, 1963). At the perforation plates, the trapped spores germinated and the germ tube penetrates the perforation plate and producing hyphae beyond the plates which subsequently producing conidiophores and conidia

(Beckman *et al.*, 1961, 1962). The pathogens colonize the host rapidly by this trapping, penetration and sporulation strategy. However, in carnation (Pennypacker and Nelson, 1972), muskmelon (Reid, 1958) and flax (Tisdale, 1917) plant, the blocking of conidia at the perforation plate was not found probably due to the anatomical configurations of the perforation plates of these plants.

The xylem vessel elements also produce gels which immobilized the conidia from travelling along the vascular system besides trapping the spores by the perforation plates (Beckman *et al.*, 1961). However, the gels functions are strict because it can only contain the pathogens for a short time (Beckman, 1966). In the process of host colonization and establishment, the *Fusarium* wilt pathogens have been reported to produce enzymes (Gothoskar *et al.*, 1953, 1955; Deese and Stahmann, 1962a, b) which can be found in carnation and tomato (Pierson *et al.*, 1955; Pennypacker and Nelson, 1972).

Meanwhile, the invasion of *Fusarium* wilt pathogen has been responded by host plant to contain the pathogens by variety of ways such as the formation of gels, gums, and tyloses in the xylem vessel elements. In the vessel elements, the conidia are trapped by the gels and gums formed by the host plant (Beckman *et al.*, 1961) but are not too effective as conidia can overcome the vascular occlusion because of the gels formed are short-lived and unable to produce tyloses (Beckman, 1966). Carnation, sweet potato, mimosa, watermelon and cotton (McClure, 1949; Bugbee, 1970; Nishimura, 1971; Pennypacker and Nelson, 1972; Phillips and Stipes, 1976) have been reported to produced gums and gels and no reports as for the occurrence in tomato (Pierson *et al.*, 1955; Chambers and Cordens, 1963). In banana, it has been reported that the formation of tyloses have effect on in localizing *F. oxysporum* f. sp.

cubense (Beckman *et al.,* 1961) but fail to contain *F. oxysporum* f. sp. *batatas* in sweet potato (McClure, 1949).

Host plant frequently produces hypertrophied and hyperplastic cells in response towards infection and in the cortex of banana roots invaded by *F. oxysporum* f. sp. *cubense,* such cells formation has been found by Wardlaw (1930). In tomato and carnation, similar hyperplastic activity has been reported in the infected xylem parenchyma of vascular tissue (Chambers and Cordens, 1963; Pennypacker and Nelson, 1972).

2.2 *FUSARIUM OXYSPORUM*

2.2.1 *Fusarium oxysporum* taxonomy

Fusarium oxysporum can be recovered from most soils – Arctic (Kommedahl *et al.,* 1988), tropical or desert (Joffe and Palti, 1977; Mandeel *et al.,* 1995), and cultivated or not (McMullen and Stack, 1983 and 1984) and is the most widely dispersed of the *Fusarium* species. It also can be recovered from marine algae (Granchinho *et al.,* 2002) and dispersed by insects (Gillespie and Menzies, 1993). *Fusarium oxysporum* is known by its numerous hosts and the level of loss that can be result when it infects a plant and is the most economically important species in the *Fusarium* genus (Leslie and Summerell, 2006). Many agriculture-important crops have been attacked by *Fusarium* spp. and causes diseases such as vascular wilt, cankers, dieback, cortical rots, head blights, leaf spots, root rots and fruit rots. One of the *Fusarium* spp. that causes important disease is *Fusarium oxysporum*, which attack susceptible host plant to cause vascular wilt disease. The vascular wilt disease can cause destructive effect if not controlled and combined with the ability of the

pathogen to adapt and survive for a prolonged period in soils, this disease met difficulties to be controlled. Agriculture-important crops such as tomato, cabbage, flax, banana, pea, sweet potato, lentil, tobacco, muskmelon, watermelon, cotton and ornamental crops such as carnation, chrysanthemum, tulip, daffodil, stock, aster, gladiolus and trees such as mimosa, date palm, and oil palm has been reported to be attacked by formae specialis of *F. oxysporum*.

In *Fusarium oxysporum* taxonomic system, Wollenweber and Reinking (1935) include *F. oxysporum* in section Elegans which described to three subsections which are Orthocera, Constrictum and Oxysporum. Macroconidia formations on sporodochia and macroconidia width are the basis criteria to separate the subsections. Wollenweber and Reinking further separated each subsection according to species, varieties and forms. However, despite their contribution, this system not easy to apply because the identification keys proposed are difficult to be used and the system only emphasis on spore measurements and characteristics. Through the Section Elegans from Wollenweber and Reinking's system, a simplified taxonomic system was described by Snyder and Hansen (1940) using single spore technique and identical condition to grow cultures. They found that the spores' characteristics such as length, width and septation varied greatly. As a result, a species concept has been developed by Snyder and Hansen (1940) which all members of the Section Elegans form one natural species, *F. oxysporum*. Basic morphologic features in the Section Elegans previously described by Wollenweber and Reinking system (1935) have been incorporated in this new species concept.

Fusarium wilt of cotton (*Gossypium* spp.) was the first vascular wilt disease described which is caused by *F. oxysporum* f. sp. *vasinfectum* (Atkinson, 1892). Many cotton plantation regions over the world has affected by *Fusarium* wilt of

cotton including USA, Egypt, Tanzania, West Africa, China, Turkey, Australia and India. In the studies on *Fusarium* wilt of cotton, certain variables have noted to contribute on losses caused by this disease. Such variables are host resistance, general plant health, inoculums potential, environmental factors, presence of nematodes and use of chemical fertilizers. In cotton plant, the symptom can be appeared from seedling emergence to maturity of the cotton plant.

2.2.2 Life cycle of *F. oxysporum*

Chlamydospores formation allowed most formae specialis of *F. oxysporum* remain dormant in decaying host tissue until stimulated to germinate as showed in crops such as banana (Stover, 1962a, 1970), aster (Baker, 1953) and flax (Kommedahl *et al*., 1970). Stover (1962a, 1970) described that the germination of chlamydospores can be stimulates by host or nonhost plant roots, or contact with pieces of fresh noncolonized plant debris. Conidia, new chlamydospores and hyphae may be formed after the chlamydospores germinated (Stover, 1970). The pathogens enter the host plant either through wounds or directly. In sweet potato, vascular wounding by freshly cut stems, roots, or fresh leaf scars serves as penetration sites for *F. oxysporum* f. sp. *batatas* (Wr.) Snyd. & Hans (McClure, 1949). Johnson (1921) reported that in tobacco (*Nicotiana tabacum* L.), initial infection occurs largely dependent on wounding as it can greatly increase the occurrence of a successful infection. *F. oxysporum* f. sp. *batatas* is also known to infect tobacco plant when the roots are cut or damaged by transplanting, but remained healthy after inoculated without wounding (McClure, 1949). Wounding is shown to be main penetration sites in the study of pathogens infection of banana (*Musa sapientum* L.) caused by *F. oxysporum* f. sp. *cubense* (E. F. Sm.) Snyd. & Hans (Sequeira *et al.*,

1958). Stover (1962b) reported that infection does not occur on healthy main roots without wounds or injuries.

Other mode of infection is direct penetrations which not require a wound for infection and involves many formae speciales of *F. oxysporum*. Smith and Walker (1930) reported that penetration occur intercellularly in the apical meristemtic regions of the roots, the zone of elongation and on occasion through root hairs like in the case of *Fusarium* wilt of cabbage (*Brassica oleracea* L., var. *capitata* L.) caused by *F. oxysporum* f. sp. *conglutinans* (Wr.) Snyd. & Hans., race 1 (Pound and Fowler, 1953; Armstrong and Armstrong, 1966).

2.2.3 Formae specialis and pathogenic races

Fusarium oxysporum is host-specific pathogen which cause vascular wilt diseases and only infect a small number of host plants. This specific strain is subdivided based on pathogenicity known as formae specialis. Some examples of formae specialis and host plants are *F. oxysporum* f. sp. *cubense* – banana, *F. oxysporum* f. sp. *batatas* – sweet potato, *F. oxysporum* f. sp. *niveum* – watermelon and *F. oxysporum* f. sp. *lycopersici* – tomato. Compared with non-pathogenic or saprophytic *F. oxysporum* strains, the formae specialis strains cannot be differentiated morphologically and has similar or identical morphological characteristic. Booth (1971) reported over 100 formae speciales and races of *F. oxysporum* have been described. The prospects for disease control knowing the nature of the diversity comprised within forma specialis can be achieved using genetic resistance (Gordon and Martyn, 1997). Besides formae specialis, *F. oxysporum* strains are grouped in ‘races’, which correspond to cultivar-level

specificity. According to Armstrong and Armstrong (1981), races are defined by their differential interaction with host genotypes, that the cultivars known to carry one or more major gene for resistance (Simons *et al.,* 1996). Races can be determined by the single genes in host plant (e.g., races 1 and 2 of *F. oxysporum* f. sp. *lycopersici* and the corresponding I and I-2 genes in tomato (Stall and Walter, 1965; Jones and Woltz, 1981). Other definition on races is selectivity of isolates to distinct plant species as showed by Armstrong and Armstrong (1981) on isolates infecting crucifer.

2.3 DISEASE CONTROL AND MANAGEMENT

2.3.1 *Fusarium* wilt disease control

Control strategies have been applied and researched to focus on long term effect in controlling this soilborne pathogen. The *F. oxysporum* is difficult to be controlled because of probably it will never disappeared and is almost impossible to eradicate from soil (Alabouvette, 1999). The disease control management has been applied using chemical, practical and biological elements to contain this host specific pathogen. Example of past and recent control strategies on *Fusarium* wilt diseases are soil fumigation, fungicide, soil solarization, resistant cultivar, monoculture, crop rotation and biological control agents.

2.3.2 Chemical control

Chemical control strategies are applied to control *Fusarium* wilt pathogen and other soilborne pathogen using chemical means, to kill and inhibit the spores from geminated. Examples of chemical-base control are soil fumigation and fungicide treatment. In the past times, soil fumigation has been applied to control *Fusarium* wilt of banana cause by *F. oxysporum* f. sp. *cubense*. In Jamaica, attempted has been made to fumigate banana soils without success (Rishberth and Naylor, 1957). Early phase detection of *Fusarium* wilt is said to be effective in preventing the outbreaks of the disease. Fumigation treatment using methyl bromide has been used in Africa and was able to stop the outbreaks of early-detected *F. oxysporum* f. sp. *cubense*. However, the disease only can be contained for approximately 2 years, and the *Fusarium* wilt disease spread even more rapidly than before after the treatment (Herbert and Marx, 1990). The uses of soil fumigants are not very effective in the long term and the uses of chemicals are not sustainable in near future. Furthermore, the use of methyl bromide as soil fumigant has been banned throughout the world.

Fungicides have been used to control the spread of *Fusarium* wilt disease and some of the treatment can contribute to success against the disease. This treatment is applied by many farmers to maximize their agriculture yields by eradicating the pathogen spores and provide unfavorable soil condition to the pathogens' growth and germination. Commonly used fungicides are belonging to the benzimidazole group such as benomyl, carbendazim, thiabenzole and thiophanate. In *Fusarium* wilt of tomato, the disease can be control by applying benomyl as a drench or a spray on young tomato plants in the greenhouse (Thanassoulopoulos *et al.*, 1970) and as a soil drench on muskmelon plants (Maraite and Meyer, 1971). In *Fusarium* wilt of sweet