CHARACTERISATION AND TOXICITY STUDIES OF *Fusarium* ISOLATES

NOR AZLIZA BINTI ISMAIL

UNIVERSITI SAINS MALAYSIA

2016

CHARACTERISATION AND TOXICITY STUDIES OF *Fusarium* ISOLATES

by

NOR AZLIZA BINTI ISMAIL

Thesis submitted in fulfillment of the requirements for the degree of

Doctor of Philosophy

MARCH 2016

ACKNOWLEDGMENT

In the name of Allah, the Most Gracious, the Most Merciful.

Thanks to my Creator, Allah SWT for giving me the strength, the good health and the peaceful mind in order to live life, to learn and to work on my research project, hereby completing my study. The process of earning a doctorate is a long, exhaustive road and obviously, it is not completed singlehandedly. Thus, there are a number of remarkable individuals I need to address without whom this dissertation might have not been written.

I would like to acknowledge my supervisor, Professor Dr. Latiffah Zakaria for her time and effort in checking this dissertation, shares her knowledge and expertise from the beginning until the final revision of this dissertation. Her constant feedback and advice were influential and essential throughout the writing process. I sincerely thanked her for countless revisions she did to the betterment of my dissertation. Upon saying that, I would be negligent to not mention my former supervisor, Professor Baharuddin Salleh. The seeds of interest in *Fusarium* were planted 11 years ago by him. Words are not enough to express my deepest gratitude to him, for he was the one who realised my potential, allowed me freedom to explore what I think the best for my work and never once doubted my judgement when I was his student. He was also the one whom I turned to when I was in crisis situations. For me, he truly is the best mentor that is hard to find, difficult to part with and impossible to forget.

I would like to express my heartfelt gratitude to one of the most important people in my life; my dear husband, Muhammad Firdaus Othman for remains willing to engage with the struggle and discomfort of having a life companion like me. He was

always standing by me in most difficult times, constantly offering me a shoulder to cry on and was always ready to lend ears to all my complaints and grumbling. To the world, you may be just one person, but to me, you are my world. I also have to give a special mention to my beloved son, Ziyaad as-Solihin, whom I owed the most. All the sacrifices he had done and all the times he had spent without my presence were irreplaceable. He was with me all the way through my PhD journey long before he was born. Thus, this dissertation was especially dedicated to him. My thankfulness was also extended to my parents and family. Your prayers for me were what sustained me thus far.

My deepest appreciation also goes to my fellow labmates who have helped me stay sane through these years. We absolutely have shared tears and laughs together. My student's life at Laboratory 107 would not be the same if they were not there. There are parts in me wanting to stay but sometimes in life, I have to let go even a good thing like a friendship. Perhaps we do not stay in each other's life but we surely do stay in each other's heart. To all my labmates who are still facing the hardships of earning the light of knowledge, the journey may not be easy, but it is going to worth it. I also warmly thank Mr. Kamaruddin, Mrs. Shafawati Mohamad and all staff at School of Biological Sciences by giving an endless help in numerous ways to finish my labworks. Without their help, my experimental works might have not been accomplished.

Finally, I wanted to express my gratitude to Ministry of Higher Education for providing me a financial support through MyBrain15 (MyPhd) scholarship. I am also indebted to all Malaysians for indirectly funding my education up to this level. It was

indeed one of the biggest motivations knowing that I have been entrusted with this opportunity that I need to push my very best to the finish line.

To all people that I have mentioned and those are not mentioned here, may Allah SWT gives you the best in return. InsyaAllah.

Thank You.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
a_w	Water activity
BEA	Beauvericin
BLAST	Basic Local Alignment Search Tool
dH_2O	Distilled water
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
EtBr	Ethidium Bromide
f. sp.	*forma speciales*
FB_1	Fumonisin B_1
FB_2	Fumonisin B_2
FB_3	Fumonisin B_3
FB_4	Fumonisin B_4
FFSC	*Fusarium fujikuroi* species complex
FLD	Fluorescence Detector
FOC	*F. oxysporum* f. sp. *cubense*
FUM	Fumonisin biosynthetic gene
GA	Gibberelic Acid
HPLC	High Performance Liquid Chromatography
ICNafp	International Code of Nomenclature for algae, fungi and plants
IGS-RFLP	Intergenic Spacer-Restriction Fragment Length Polymorphism
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
MEGA	Molecular Evolutionary Genetic Analysis
MeOH	Methanol
$MgCl_2$	Magnesium chloride
$MgSO_4.7H_2O$	Magnesium sulfate
MON	Moniliformin
MP	Maximum Parsimony
mtSSU	Mitochondrial Small Subunit
$Na_2B_4O_7$	Sodium borate
NaH_2PO_4	Sodium dihydrogen phosphate
NaOCl	Sodium hypochlorite
OPA	*Ortho*-phthalaldehyde
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Broth Agar
PPA	Peptone Pentachloronitrobenzene Agar
rDNA	Ribosomal Deoxyribonucleic Acid
rpm	Revolutions per minute
SDS	Sudden Death Dyndrome
SPE	Solid Phase Extraction

TBE	Tris Borate-EDTA
TEF-1α	Translation Elongation Factor-1α
TLC	Thin Layer Chromatography
UHPLC	Ultra High Performance Liquid Chromatography
VCRU	Vector Control Research Unit
WA	Water Agar
ZEN	Zearalenone
β	Beta

PENCIRIAN DAN KAJIAN TOKSISITI PENCILAN *Fusarium*

ABSTRAK

Spesies di dalam genus *Fusarium* merupakan kulat patogenik kepada pelbagai tumbuhan di seluruh dunia. Beberapa spesies mampu menghasilkan fumonisin B_1 (FB_1) sejenis mikotoksin yang berbahaya kepada manusia dan haiwan. Tujuan kajian ini adalah untuk mengenal pasti dan menciri spesies *Fusarium* yang berassosiasi dengan sembilan perumah di Malaysia, menentukan hubungan filogenetik di antara spesies, menentukan kebolehan spesies ini menghasilkan FB_1 dan toksisiti ekstrak kasar kulat mengandungi FB_1 terhadap anak pokok padi dan larva nyamuk *Aedes*. Di dalam kajian ini, 125 pencilan daripada sembilan perumah dikenalpasti sebagai *F. verticillioides* (12 pencilan), *F. proliferatum* (51 pencilan), *F. fujikuroi* (24 pencilan), *F. andiyazi* (enam pencilan) dan *F. oxysporum* (32 pencilan) menggunakan jujukan TEF-1α. Pohon filogenetik parsimoni maksimum mnggunakan jujukan TEF-1α dan β-tubulin memisahkan kelima-lima spesies kepada lima klad berbeza di mana pencilan daripada spesies yang sama dikelompokkan dalam klad yang sama. Variasi genetik dapat dilihat pada pencilan *F. proliferatum* dan *F. oxysporum* yang membentuk beberapa sub-klad. Gen *FUM1* dikesan di dalam 96 daripada 125 pencilan tetapi hanya 63 pencilan positif FB_1 apabila diuji dengan jalur ujian RIDA®QUICK FUMONISINS, menunjukkan kehadiran gen *FUM1* tidak semestinya berassosiasi dengan penghasilan FB_1. Berdasarkan analisis UHPLC, semua 63 pencilan menghasilkan FB_1 dengan aras kepekatan antara 0.6 µg/g ke 29.2 yang mana dianggap sebagai penghasil FB_1 yang rendah, mencadangkan risiko kontaminasi FB_1 di Malaysia juga rendah. Ekstrak kasar *F. verticillioides* (Q5566O) menunjukkan kesan

perencatan yang signifikan ke atas pemanjangan akar pada kepekatan 25, 50 dan 100 µg/ml dan pemanjangan pucuk pada kepekatan 50 dan 100 µg/ml, menyokong hipotesis bahawa FB_1 bersifat fitotoksik terhadap anak benih padi. Ekstrak ini juga toksik terhadap larva nyamuk dengan kepekatan yang menyebabkan 50% kematian (L_C50) adalah sangat rendah iaitu pada 79.3 µg/ml. Keputusan kajian ini menunjukkan bahawa pencilan *Fusarium* yang berassosiasi dengan pelbagai perumah di Malaysia berupaya menghasilkan FB_1 dan menunjukkan kesan toksisiti ke atas anak benih padi dan larva yang diuji. Walaupun FB_1 dihasilkan pada aras kepekatan yang rendah, pengumpulan mikotoksin boleh terjadi di bawah keadaan yang sesuai untuk pertumbuhan kulat. Keputusan kajian juga mencadangkan potensi kontaminasi FB_1 pada tanaman pertanian di lapangan, menunjukkan keperluan untuk mengawal kontaminasi kulat di peringkat sebelum dan selepas tuai.

CHARACTERISATION AND TOXICITY STUDIES OF *Fusarium* ISOLATES

ABSTRACT

Species in the genus *Fusarium* are pathogenic fungi to a wide variety of plants worldwide. Some species are able to produce fumonisin B_1 (FB_1), a mycotoxin that is harmful to humans and animals. The aims of this present study were to accurately identify and characterise *Fusarium* species associated with nine hosts in Malaysia, to determine the phylogenetic relationship between the species, to determine the ability of these species to produce FB_1 and to test the toxicity of fungal crude extract containing FB_1 on rice seedlings and mosquito larvae. In this study, 125 isolates of *Fusarium* species from nine hosts were identified as *F. verticillioides* (12 isolates), *F. proliferatum* (51 isolates), *F. fujikuroi* (24 isolates), *F. andiyazi* (six isolates) and *F. oxysporum* (32 isolates) using TEF-1α sequences. Maximum parsimony phylogenetic tree using TEF-1α and β-tubulin sequences resolved the species into five different clades of which the isolates from the same species were grouped in the same clade. Considerable genetic variation was observed for *F. proliferatum* and *F. oxysporum* isolates, supported by a number of sub-clades formed. *FUM1* gene was detected in 96 out of 125 isolates but only 63 were positive for FB_1 when tested with RIDA®QUICK FUMONISINS test strip, indicating that the presence of *FUM1* gene was not necessarily associated with FB_1 production. Based on UHPLC analysis, all 63 isolates produced FB_1 with concentration levels ranging from 0.6 µg/g to 29.2 µg/g which were regarded as low FB_1 producers, suggesting that the risk of FB_1 contamination in Malaysia was also low. The crude extract of *F. verticillioides* (Q5566O) showed significant inhibitory effects on the root elongation at concentrations of 25, 50 and 100 µg/ml and the shoot elongation at 50 and 100 µg/ml, supporting the hypothesis that

FB_1 is phytotoxic to plant seedlings. The extract was also toxic to mosquito larvae with concentration resulting in 50% mortality (L_C50) was very low, which was at 79.3 µg/ml. The results of this present study revealed that *Fusarium* isolates associated with various hosts in Malaysia were able to produce FB_1 and demonstrated the toxicity effects on the tested seedlings and larvae. Although low levels of FB_1 were produced, accumulation of the mycotoxin can occur under conditions that favour the fungal growth. The results also suggest a potential for FB_1 contamination on agricultural crops in the field, implying the need to control of the fungal contamination at pre- and post-harvest stages.

CHAPTER 1

GENERAL INTRODUCTION

The genus *Fusarium* comprises a group of plant pathogenic fungi responsible for various types of diseases to agricultural crops worldwide (Leslie & Summerell, 2006). Some species are cosmopolitan and have a wide host range, causing diseases to different hosts and some have a narrow host range. For example, *F. proliferatum* is a pathogen to asparagus, onions, garlic and wheat, while *F. thapsinum* is pathogenic only to sorghum (Leslie & Summerell, 2006). Some *Fusarium* species are human and animal pathogens causing keratitis to contact lens wearer, skin and nails infection and cutaneous mycosis to turtles (Walsh *et al*., 2004; O'Donnell *et al.*, 2010; Santos *et al*., 2015). *Fusarium* is also known as toxigenic fungi, produce harmful metabolites known as mycotoxins. Mycotoxins are low molecular weight natural products produced by filamentous fungi as secondary metabolites that are not essential for their growth (Bennett & Klich, 2003). The functions of mycotoxins are unclear and minimally understood but are believed as some sorts of communication between species or used during competition with other organisms under nutrient-limiting environments (Desjardins, 2006; Khaldi & Wolfe, 2011; Zain, 2011).

Among mycotoxins produced by *Fusarium*, fumonisin B_1 (FB_1) is regarded as the most important, causing numerous toxic effects and diseases to humans and animals (Desjardins, 2006; Brown & Proctor, 2013). Fumonisin B_1 has been associated with cancer, birth defects and increased susceptibility to HIV in humans (Marasas, 2001; Williams *et al*., 2010). In animals, FB_1 causes brain and lung diseases (Marasas *et al*., 1988; Harrison *et al.*, 1990). Further studies of FB_1 effects on numerous

experimental animals have demonstrated that FB_1 is nephrotoxic, hepatotoxic and embryotoxic (Wan Norhasima *et al.*, 2009; Scott, 2012).

Fumonisin B_1 also inhibits the development of various plant seedlings such as tomatoes and maize in a dose-dependent manner, causes damages in cell membranes and also reduces chlorophyll synthesis (Boyette & Abbas, 1992; Lamprecht *et al*., 1994; Williams *et al*., 2007). Fumonisin B_1 was also associated in causing blight disease symptoms, leaf lesions and stunting of the plants (Glenn *et al*., 2008).

Fusarium species are field fungi and the occurrence of mycotoxins like FB_1 can pose agricultural risk in terms of crop production, yield and quality as well as health risk to those consuming contaminated products. In addition to FB_1, other types of important mycotoxins produced by *Fusarium* are tricothecenes, zearalenone (ZEN), moniliformin (MON) and beauvericin (BEA) (Desjardins, 2006).

Many reports have stated that FB_1 is mainly produced by species in the *Fusarium fujikuroi* species complex (FFSC) such as *F. verticillioides*, *F. proliferatum* and *F. nygamai* (Desjardins, 2006). A few strains of *F. oxysporum* have also been reported to produce FB_1 (Proctor *et al*., 2004; Irzykowska *et al*., 2012). Morphological characteristics of species in the FFSC and *F. oxysporum* isolates are highly similar and some characters are overlapping (Leslie & Summerell, 2006). Thus, the identification using morphological characteristics can lead to misidentification (Kvas *et al*., 2009). The correct species names are very important in order to determine the presence and distribution of FB_1-producing *Fusarium* species as well as to estimate the potential risk of FB_1 contamination.

The use of protein-coding genes such as Translation Elongation Factor-1α (TEF-1α) allows precise identification of *Fusarium* species (O'Donnell *et al*., 1998b; Geiser *et al*., 2004; Kvas *et al*., 2009). The gene is highly informative due to its high sequence polymorphism and consistently appears as a single-copy (Geiser *et al*., 2004). Phylogenetic analysis using TEF-1α combined with other genes such as β-tubulin and calmodulin are also widely used to further resolve species identification. By using phylogenetic analysis, relationships between species can also be determined and mycotoxins production can be predicted (O'Donnell *et al*., 2000; Kristensen *et al*., 2005; Watanabe *et al*., 2013).

Fumonisin B_1 is among the most toxic metabolites to humans, animals and plants, but this mycotoxin appears to receive little interest among researchers in Malaysia. Not much information is available on the occurrence and status of FB_1 as a common contaminant in agriculturally important crops. Previous studies have identified several *Fusarium* isolates from vegetables and grasses that can produce FB_1 (Khayoon *et al*., 2010; Nur Ain Izzati & Wan Hasmida, 2011; Nor Azliza *et al*., 2014) but the identification of the isolates was solely based on morphological characteristics which could lead to misidentification as the isolates morphologically identified are members of FFSC.

Fumonisin B_1 has been reported to have inhibitory effects on the growth of some important crops like maize and soybean and might also be involved in pathogenicity (Desjardins & Hohn, 1997). Rice is an important crop in Malaysia and FB_1-producing species, namely *F. proliferatum* and *F. fujikuroi* have been recovered from rice (Nur Ain Izzati *et al*., 2009; Nik Mohd Izham, 2014). There is a possibility that FB_1 also has its toxicity effects on rice plant development.

Animal models such as mice, rabbit and duckling are commonly used in FB_1 toxicity tests to screen and to determine mortality or pathological injuries. But the use of these animals are expensive and involved ethical issues (Krewski *et al*., 2010). Another alternative method for FB_1 toxicity test is the use of brine shrimp larvae. But toxicity studies dealing with brine shrimp larvae sometimes are difficult because the larvae are a bit sensitive and high natural mortality can easily occur during the test. *Aedes aegypti* is a vector of dengue, a viral disease affecting humans mostly found in tropical countries and is among the most resistant vector to insecticides worldwide (Tan *et al*., 2012). In the past two decades, *A. aegypti* were used to determine insecticidal activity of beauvericin (BEA) (Grove & Pople, 1980). Therefore, *A. aegypti* larvae can also be used to assess the toxicity of FB_1. In the present study, the vulnerability of the larvae to FB_1 was observed.

Thus, the objectives of the present study are as follows:

1. to accurately identify *Fusarium* isolates from Fusarium culture collection of Universiti Sains Malaysia

2. to determine the phylogenetic relationships within and between isolates of the identified *Fusarium* species

3. to determine the ability of *Fusarium* species to produce FB_1 *in vitro*

4. to determine the toxicity of *F. verticillioides* extract (Q5566O) containing FB_1 on rice seedlings and mosquito larvae.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy of *Fusarium*

The genus *Fusarium* is a group of asexual (anamorph) filamentous fungi with banana-shaped macroconidia as its primary characters (Leslie & Summerell, 2006). The genus belongs to the phylum Ascomycota in a class Sordariomycetes and order Hypocreales. Beginning in 1935, numerous documented taxonomic schemes were proposed by *Fusarium* researchers all over the world to make species identification easier (Table 2.1). The number of *Fusarium* species varied from nine to 1000 based on the taxonomical systems used, creating a confusion among *Fusarium* researchers.

A few *Fusarium* species have sexual (teleomorph) stage, and thus, the nomenclature is changed to *Gibberella*, *Nectria* or *Haemonectria* (Leslie & Summerell, 2006). However, following the changes by International Code of Nomenclature for algae, fungi and plants (ICNafp) in 2011, this dual nomenclature of anamorph and teleomorph are no longer used (Hibbett & Taylor, 2013; Geiser *et al*., 2013; Aoki *et al*., 2014). After 2013, the use of both anamorph and teleomorph names are not allowed and the pre-existing asexual and sexual names are standardised to only one name (Hawksworth, 2012). Thus, asexual names or anamorph of *Fusarium* is formally used for species with sexual stages, replacing the name *Gibberella*, *Nectria* and *Haemonectria* (Geiser *et al*., 2013).

Table 2.1. Significant evolutions of *Fusarium* taxonomy throughout the year

Year	Event
1809	Link proposed *Fusarium* as a genus, initially known as *Fusisporium*
1935	Wollenweber and Reinking published the first monograph of *Fusarium* species, 'Die Fusarien', containing nearly 1000 species consolidated into 16 sections consisted of 65 species, 55 varieties and 22 forms
1940s	Snyder and Hansen reduced the number of species in 16 sections to only nine species
1950	Raillo proposed a taxonomic system which emphasised on conidial characters such as shapes, lengths and septa
1955	Bilai published a book 'Fusarii' as a continuation of Raillo's work and suggested combining some species in section Liseola with Elegans and Gibbosum with Discolor
1952	Gordon worked on *Fusarium* from various plants and soils and included teleomorph characters to define *Fusarium* species
1971	Booth published a monograph, 'The Genus *Fusarium*' and recognised 44 species. Booth's most significant contribution is the information on conidiophores and conidiogenous cells for identification
1982	Gerlach and Nirenberg published 'The Genus *Fusarium*: A Pictorial Atlas' and recognised 78 morphologically distinct species
1983	Nelson, Toussoun and Marasas published a manual, '*Fusarium* species*:* An Illustrated Manual for Identification'. They recognised 41 species and another 16 insufficiently documented species.
2006	Leslie and Summerell published 'The *Fusarium* Laboratory Manual' providing descriptions of 70 different species with combination of morphological, biological and phylogenetic species concepts as well as a compilation of techniques used in *Fusarium* species identification

2.2 *Fusarium* as plant pathogen

The wide distribution of *Fusarium* species is due to the ability to grow in a wide range of substrates, the effective dispersal mechanism, ability to utilise organic matter and the adaptability in all types of soils for a long time (Nelson, 1981). Thus, it is common for opportunistic species to colonise plant parts and live there as saprophytes, endophytes or pathogens. Diseases caused by *Fusarium* species varied and several diseases have nearly devastated the economy of the plant growers worldwide. Infection by *F. graminearum* and *F. verticillioides* on wheat, maize and barley, for instance, has caused losses of a billion of dollars annually (Wu, 2006; Zain, 2011; Weguloa *et al*., 2014). Sudden death syndrome (SDS) of soybean caused by *F. virguliforme* has also affected soybean production in North and South America (Aoki *et al*., 2003). The disease is ranked as among the top ten diseases that severely reduce soybean yield in the United States. Panama disease (Fusarium wilt) of banana caused by *F. oxysporum* f. sp. *cubense* (FOC) is one of the most destructive plant diseases in the world (Ploetz, 2006). The disease has caused losses in banana plantations and affects the production in all banana-producing regions in Asia, Africa, Australia, and the tropical Americas. To date, there is no effective control measure due to the persistence of the pathogen in soils (Zhang *et al*., 2013).

Fusarium species are polyphagous, infecting different types of plant parts and causing various types of diseases. In cereals, head, grain and seeds are the main parts infected by *Fusarium*. In dicots, *Fusarium* species commonly infect the root, causing vascular wilt by colonising the xylem of which the mycelium causes vessel obstruction and blocking water transportation to the aerial parts of the plant. *Fusarium oxysporum*

is the most common species causing vascular wilt diseases in most dicot plants such as banana, tomato, beans and pea (Leslie & Summerell, 2006).

2.3 *Fusarium* as human and animal pathogen

The role of *Fusarium* as a human pathogen was first emerged when it was isolated from a child with acute leukaemia and from skin lesions of burn patients (Cho *et al*., 1973; Abramowsky *et al*., 1974). *Fusarium* species is the most common pathogens causing fungal keratitis in human (Nucci & Anaissie, 2002, 2007). The incidence of fungal keratitis increases during harvest season among agricultural workers due to the exposure to airborne soils and contaminated plant materials.

Fusarium solani is well-known for its ability to cause keratitis related to contact lens wear (Chang *et al*., 2006; Khor *et al*., 2006). Skin and nail infections caused by opportunistic *F. solani* and *F. oxysporum* have also been reported among immunocompetent and immunocompromised patients worldwide (Romano *et al.*, 1998; Godoy *et al*., 2004). In more severe cases, a patient with acute leukaemia was found to have *F. solani* brain abscess (Garcia *et al*., 2015). It was presumed that the fungus entered the cerebrum *via* the nasal route. Meanwhile, in Europe, *F. verticillioides* is the most prevalent species associated with human infectious diseases (Tortorano *et al*., 2014).

In animals, *F. solani* has been associated with brain disease in dogs and cutaneous mycosis in turtles (Cabańes *et al*., 1997; Vans *et al*., 2004; Santos *et al*., 2015). Mass mortalities of sea turtle embryos due to the infection by *F. solani* has been reported in major nesting areas in Brazilian coasts and influenced the populations number of these endangered reptiles (Sarmiento-Ramírez *et al*., 2014).

2.4 *Fusarium* as mycotoxin producer

Fusarium species can produce a wide range of mycotoxins. Mycotoxins occur mostly in the mycelium but may also be present in the spores. There are more than 100 types of mycotoxins produced by *Fusarium* species and of these, tricothecenes, ZEN and fumonisins are the most important and natural contaminants of agricultural crops worldwide (Desjardins, 2006; Yazar & Omurtag, 2008). Mycotoxin-producing fungi can enter crop materials before harvest, during post-harvest, storage and during processing into food and feed products. *Fusarium* species are field fungi and many toxigenic *Fusarium* are plant pathogens. Therefore, the presence of these pathogens can also cause mycotoxin contamination on crops especially cereal grains. Deoxynivalenol and fumonisins are among mycotoxins produced by *F. graminearum* and *F. verticillioides*, two species that are pathogenic to maize (Desjardins, 2006).

Production of mycotoxins by *Fusarium* species can continue post-harvestly during storage of harvested grains due to carry-over from the field. *Fusarium* species can also grow saprophytically on stored plants (Glenn, 2007). The growth of saprophytic *Fusarium* species colonising the debris and/or in other plant parts can also result in mycotoxin accumulation when stored under improper storage conditions (Bacon *et al*., 2001). These mycotoxins can enter human and animal dietary systems through direct and indirect contaminations (Mangia, 2009). Direct contamination takes place when the food or feed are infected with toxigenic fungi which is subsequently followed by mycotoxin formation. While, indirect contamination occurs due to the mycotoxins that remain in the final products although the fungi is removed or killed during the processing. These mycotoxins originate from raw materials that have

previously been contaminated with mycotoxin-producing fungi in the field or during storage (Mangia, 2009).

Consumption of contaminated feeds can cause diseases to animals, causing feed refusal, abnormalities in reproductive organs and mortality. In humans, the health risks associated with the consumption of mycotoxin-contaminating food products can be as mild as nausea and dizziness to more severe effects such as bleeding, deformities and cancers (Yazar & Omurtag, 2008). Mycotoxin contamination can also cause economic losses of crop and animal production. Contaminated crops result in increased costs for farmers and distributors including extra drying costs, excess storage capacity, losses in transit as well as in markets.

2.5 Fumonisin B_1 (FB_1)

Fumonisins are a group of mycotoxins mainly produced by *Fusarium* species and high levels of fumonisins production have been consistently found among strains of *F. verticillioides* and *F. proliferatum* of the FFSC (Desjardins, 2006). Among fumonisins, FB_1 is the most abundantly produced and highly toxic to humans and animals. Fumonisin B_1 was first isolated by Gelderblom *et al*. (1988) from *F. verticillioides* strain MRC 826 in South Africa. It has been reported that other fumonisin analogues, mainly FB_2 are also produced by other fungal genera, namely *Aspergillus niger* and *Alternaria alternata* (Scott, 2012; Pitt *et al*., 2013).

Fumonisin B_1 naturally contaminate maize and maize-based products worldwide in which the reports are largely gathered from Africa, South America and Europe (Ariño, *et al*., 2007; Njobeh *et al*., 2012; Garrido *et al.*, 2012). Fumonisin B_1 is also found to naturally contaminate plants other than maize such as rice (Kim *et al*.,

1998; Shetty, 2011; Makun *et al*., 2011), asparagus (Seefelder *et al.*, 2002; Liu *et al*., 2005), spices, aromatic and medicinal herbs (Kosoglu *et al*., 2011; Kong *et al*., 2012). Dried fig samples were also found to be contaminated with FB_1 (Karbancıoglu-Guler & Heperkan, 2009). High contamination of FB_1 was also reported in medicinal plants such as leaves of orange tree and black tea (Martins *et al*., 2001), garlic bulbs (Seefelder *et al*., 2002) and soybeans (Aoyama *et al*., 2010). Contamination by FB_1 has also been reported in typical food maize-based products mainly from Mexico and Italy such as corn flakes, tortillas, polenta and broa (de la Campa *et al*., 2004; Lino *et al*., 2007; Muscarella *et al*., 2008).

Several factors can influence FB_1 accumulation in the field including increased temperatures, drought stress, humidity and insect infestation (Miller, 2001; Marín *et al*., 2004; Paterson & Lima, 2010). High temperatures and drought stress have been demonstrated to favour the colonisation of *F. verticillioides* on maize while, insects act as vectors spreading the fungus, or as wounding agents, allowing easier fungal access to plants (Dowd, 1998). Some climatic events such as changes in rainfall patterns are also likely to influence the fumonisin production. Temperature and moisture conditions during storage also affect fungal infection on maize and fumonisin production where water availability for fungal growth plays a key role (Fandohan *et al*., 2003).

Fumonisin B_1 is not mutagenic but is a type of promoter that promotes a later stage of carcinogenesis (Shier *et al*., 2003). Fumonisin B_1 is poorly absorbed from digestive tract and rapidly excreted *via* feces (Shephard *et al.*, 1994; WHO, 2002). However, small amount of FB_1 are retained in liver and kidneys. The consequences of ingestion of FB_1 vary from noticeable diseases with high mortality, decreased

resistance to pathogens and reduced animal productivity (Escrivá *et al*., 2015). The target organs differed according to species and sex of the animals. For instance, in swine, lung, liver and pancreas are primary target organs, whereas in rats and mice, FB_1 affects the liver and kidney. Based on toxicity studies in humans, the main target organs of FB_1 toxicity are the liver and kidneys (Stockmann-Juvala & Savolainen, 2008).

Fumonisin B_1 has also been reported to be phytotoxic to seedlings, callus and leaves of various host plants such as maize, tomato and weeds (Van Asch *et al.*, 1992; Lamprecht *et al*., 1994; Abbas *et al*., 1995) as well as causing necrosis of detached tomato leaves (Lamprecht *et al*., 1994). It has been suggested that FB_1 suppresses plant defense mechanisms (Abbas *et al*., 1995; Baldwin *et al*., 2014), reduces chlorophyll synthesis (Vesonder *et al*., 1992) and causes membrane damage (Abbas *et al*., 1991; 1993). In an experiment by Nelson *et al*. (1993), stem rot of maize was observed only by strains that produced high concentrations of fumonisin *in vitro*. These data provide indirect evidence that FB_1 has toxicity effects on plants. Fumonisin B_1 has also been reported to be associated with virulence of fungal pathogens during disease development (Desjardins *et al*., 1995; Desjardins & Plattner, 2000).

2.5.1 Effects of FB_1

Fumonisin B_1 interrupts the synthesis of sphingolipids and sphingosine products when exposed to this mycotoxin. Sphingolipids consist of a major group of structural materials and lipid signaling molecules in eukaryotic cells (Berkey *et al*., 2012). Sphingolipids can be found in plasma membranes, brain and nerves and involves in cellular events such as cell proliferation, differentiation, motility, growth,

senescence and apoptosis (Lahiri & Futerman, 2007). Fumonisin B_1 is a potent inhibitor of ceramide synthase, an enzyme that catalyses the conversion of sphinganine and sphingosine to ceramide (Wangs *et al.*, 1991; Norred *et al*., 1997). The inhibition of this enzyme lead to accumulation of free sphingoid bases in cells and tissues, thus, disrupts cell functions and signaling pathways such as apoptosis and mitosis (Stockmann-Juvala & Savolainen, 2008). The disruption can further cause imbalance of cell death and replication which potentially contribute to carcinogenesis.

In animals, FB_1 was found to cause equine leukoencephalomalacia, the softening of brain tissues in horses (Kriek *et al*., 1981; Harrison *et al*., 1990; Marasas, 1995). The leukoencephalomalacia disease causes death to horses within a few hours to 1 week after consuming feeds contaminated with FB_1. During surveys in America and Africa, high levels of FB_1 were found in feed samples associated with leukoencephalomalacia (Marasas, 1995). The consumption of FB_1 also causes lung edema in swine known as porcine pulmonary edema (Marasas, 1995). Intravenous injection of pure FB_1 has been demonstrated to cause pulmonary edema in experimental swine (Colvin & Harrison, 1992; Haschek *et al*., 1992). Surveys conducted in areas associated with pulmonary edema outbreaks in America have found high levels of FB_1 in feed samples (Marasas, 1995). It has been suggested that swine consuming naturally contaminated feeds containing approximately 100 µg/g of fumonisins are at risk for pulmonary edema.

In humans, FB_1 has been associated with high incidence of oesophageal cancer. The causal agent (s) of oesophageal cancer is still unknown but the incidence rates are significantly higher in areas with maize as a staple diet (Desjardins, 2006). High incidence of oesophageal cancer was reported in Transkei region, an area with high

maize contamination with *F. verticillioides* (Marasas *et al*., 1981, Rheeder *et al*., 1992), in several rural areas in China (Chu & Li, 1994; Yoshizawa *et al*., 1994) and Golestan, a province in Iran (Alizadeh *et al*., 2012). Although the causes of oesophageal cancer are unknown, fumonisins remain candidates as the causal agent. For this reason, fumonisins, mainly FB_1 are classified as possibly carcinorgenic to humans (group 2B) (IARC, 1993). Epidemiological studies have suggested that FB_1 is associated with neural tube defects among infants in regions where fumonisin-contaminated maize is consumed such as Eastern Cape Province of South Africa, the Northern provinces of China and along the Texas-Mexico border in Northern America (Marasas *et al*., 2004; Missmer *et al*., 2006). Fumonisin B_1 is believed to disrupt the folate receptors on the membrane causing the reduction in folate absorption. This deficiency further causes the incidence of neural tube defects (Steven & Tang, 1997).

Since the discovery of FB_1, many countries have set a regulatory limit in order to minimise the risk of consuming contaminated food and feed products. Maximum limits for human consumption are 1 µg/g in the Europe Union and 2 to 4 µg/g in the USA (WHO, 2002; FAO, 2004). The recommended levels of fumonisin concentration in animal feed is 5 µg/g for horses and other equine species, 10 µg/g for porcine species, 50 µg/g for cattle, and 50 µg/g for poultry (Miller *et al*., 1996). In 2002, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) released a provisional maximum tolerable daily intake of 2 mg/kg body weight for fumonisins. As far as is known, no regulations are established on fumonisin limits in tropical countries including Malaysia (D'Mello & Macdonald, 1997).

2.5.2 Chemical structure of FB_1

Fumonisin B_1 was chemically characterised by Bezuidenhout *et al*. (1988). Fumonisin B_1 is non-fluorescent, water-soluble and polar, consist of a linear 20-carbon backbone with an amine, hydroxyl, methyl and tricarboxylic acid constituents. Fumonisin B_1 is a diester of propane-1, 2, 3-tricarboxylic acid and 2- amino- 12, 16-dimethyl-3, 5, 10, 14, 15- pentahydroxycosane (Figure 2.1).

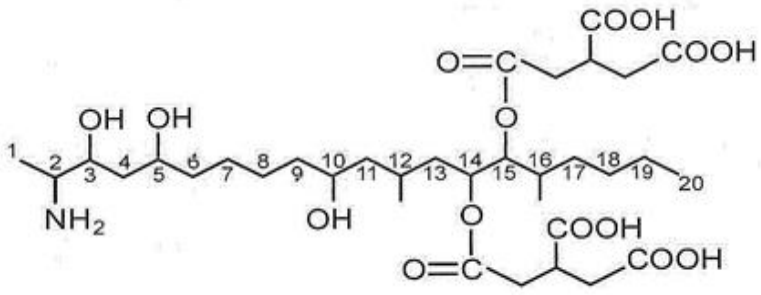

Figure 2.1. Structure of FB_1 (Bezuidenhout *et al*., 1988)

There are different types of fumonisin analogues such as A, C and P series but the B series (B_1, B_2, B_3 and B_4) produce large amount of fumonisins and the most important series compared to the other series (Desjardins, 2006; Ahangarkani *et al*., 2014). In the fumonisin B series, FB_1 is produced at the highest level (~70%) and the most toxic to humans and animals (Marasas *et al*., 2004). Meanwhile, FB_2, FB_3 and FB_4 are commonly produced at much lower levels than FB_1 (10 - 20%) (Nelson *et al*., 1993; Desjardins, 2006).

2.5.3 *FUM* cluster and FB_1 biosynthetic pathway

Most secondary metabolite gene clusters are species specific or genus specific, and little is known on how they are formed during evolution. The ability to synthesise

fumonisins is sporadic across the genus *Fusarium* due to the presence or absence of *FUM* cluster among FB_1-producing species (Figure 2.2).

Figure 2.2. Fumonisin biosynthetic gene cluster in *F. verticillioides*. *FUM20* is an additional small gene and is located between *FUM2* and *FUM13* (Proctor *et al*., 2013)

The *FUM* genes are organised as a cluster of 17 genes including two additional genes, *FUM20* and *FUM21* (Brown *et al*., 2007). *FUM1* gene is the first gene detected in the *FUM* cluster (Proctor *et al*., 1999; Desjardins & Proctor, 2007). Subsequently, additional four genes, namely *FUM6, FUM7, FUM8* and *FUM3* adjacent to *FUM1* were identified (Seo *et al*., 2001), followed by *FUM10* to *FUM19* (Proctor *et al*., 2003). Among these genes, *FUM1, FUM8, FUM6, FUM13* and *FUM3* are among the most important and required in fumonisin biosynthesis. Disruption or deletion of these genes causes to the loss or 99% reduction in the ability to produce fumonisins (Proctor *et al*., 1999; Butchko *et al*., 2003; Desjardins & Proctor, 2007).

The detection of *FUM* genes using PCR has facilitated fumonisin analysis as these genes are directly involved in its biosynthesis (Dawidziuk *et al*., 2014). The PCR assay using specific primers can also discriminate between fumonisin-producing and nonproducing isolates, thus providing a rapid sorting method in the laboratory (Dissanayake *et al*., 2009). Researchers also utilise genes from the *FUM* cluster as an additional marker for phylogenetic and taxonomic studies of the fumonisin-producing *Fusarium* species and found a good correlation between *FUM* gene expression and FB_1 production (Gonzάlez-Jaèn *et al*., 2004; Baird *et al*., 2008; Stępień *et al*., 2011a).

FUM1 is an initiator gene in fumonisin biosynthesis. This gene is involved in the formation of fumonisin backbone, thus, its presence is primarily required compared to other additional genes (Proctor *et al*., 1999; 2004). Previous studies suggested that the detection of this gene is an early indication of fumonisins production (Sánchez-Rangel *et al*., 2005; Sreenivasa *et al*., 2007; Cruz *et al*., 2013). Detection of *FUM1* gene is now routinely applied in laboratory studies before any application of qualitative and/or quantitative methods to analyse FB_1 production.

2.5.4 *Fusarium* species associated with FB_1

Most FB_1-producing *Fusarium* species are members of the FFSC and occasionally *F. oxysporum* (Desjardins, 2006; Aoki *et al*., 2014). *FUM* genes have been detected in *F. verticillioides*, *F. proliferatum*, *F. nygamai*, *F. fujikuroi* and *F. globosum*, and in strain FRC O-1890 identified as *F. oxysporum* from Korea (Proctor *et al*., 2004). All these species except *F. globosum* have been isolated from various hosts in Malaysia particularly from diseased plant parts such as root and stem of rice, asparagus, banana and oil palm (Al-Amodi, 2007; Nur Ain Izzati, 2007; Hafizi *et al*., 2013).

Fusarium verticillioides is among the pathogen of maize ear rot, which is prevalent in temperate and tropical areas such as in the USA, Italy, Slovakia and Indonesia (Srobarova *et al*., 2002; Logrieco *et al*., 2002; Darnetty *et al*., 2008). Besides being reported as the causal agent of maize ear rot, *F. verticillioides* is also consistently reported to produce high levels of FB_1 (Desjardins, 2006). The occurrence of FB_1 in maize-growing areas is high where *F. verticillioides* predominates and the severity of infection by *F. verticillioides* is a good indicator of FB_1 accumulation in maize ears

(Pascale *et al.*, 2002). Previous studies have also revealed that *F. verticillioides* producing FB_1 was also associated with other grain cereals including rice, wheat, sorghum and millet (Silva *et al*., 2004; Alizadeh *et al*., 2012; Busman *et al*., 2012). It has also been recovered from banana, pea and pine nuts (Jiménez *et al*., 1997; Mirete, 2003; Waśkiewicz *et al*., 2013).

Fusarium proliferatum has a broad host range compared to other members in the FFSC (Leslie & Summerell, 2006; Proctor *et al*., 2010). This species is pathogenic to various plants such as asparagus, onion and palm (Armengol *et al*., 2005; Al-Amodi, 2005; Dissanayake *et al*., 2009). *Fusarium proliferatum* can also grow without apparent symptoms in a few hosts including orchids, maize and wheat (Tsavkelova *et al*., 2008; Krnjaja *et al*., 2012; Palaciosa *et al*., 2015). High levels of FB_1 produced by *F. proliferatum* isolated from rice have been reported in several countries such as Nepal and Korea (Desjardins *et al*., 1997; Park *et al*., 2005). The production of FB_1 by *F. proliferatum* associated with asparagus has also been reported (Logrieco *et al*., 1998; Liu *et al*., 2007; Waśkiewicz *et al*., 2010).

Fusarium fujikuroi is the causal agent of bakanae disease with symptoms of abnormal elongation, slender stems and yellowish leaves caused by large quantities of Gibberelic Acid (GA) produced by this species (Sun & Snyder, 1981). *Fusarium fujikuroi* has a narrow host range with rice as the primary host but association with other plant hosts such as pineapple and water grass has also been reported (Carter *et al*., 2008; Waśkiewicz & Stępień, 2012). *Fusarium fujikuroi* is also reported to be FB_1 producer although the production is much lower and the producing isolates were fewer (Desjardins, 2006; Waśkiewicz & Stępień, 2012).

Fusarium oxysporum is a soil-borne fungus and has been isolated from both agricultural and non-agricultural hosts (Weber *et al*., 2006; Nur Ain Izzati *et al*., 2009; Summerell *et al*., 2011). This species usually causing vascular wilt disease in many vegetable and ornamental crops, banana and palms as well as causes crown and root rot diseases (Gunn & Summerell, 2002; Ploetz *et al*., 2003; Zakaria *et al.*, 2012). *Fusarium oxysporum* has been reported to be an FB_1 producer but the production is very low and rare among isolates (Waśkiewicz *et al.*, 2009; 2010; Irzykowska *et al.*, 2012). *Fusarium oxysporum* appear to be of minor importance in FB_1 production probably it is not associated with maize or other major cereal grains such as rice and wheat. Previous studies have shown that *F. oxysporum* produced C series of fumonisins (Seo *et al*., 1996; 1999).

Several *Fusarium* species such as *F. dlaminii*, *F. napiforme*, *F. subglutinans* and *F. thapsinum* have also been reported to produce fumonisins (Leslie *et al*., 1996; Rheeder *et al*., 2002; Desjardins, 2006). However, the *FUM* genes have not yet been detected in these species (Proctor *et al*., 2004).

2.5.5 Identification of FB_1-producing *Fusarium* species

Three different methods are commonly used to identify FB_1-producing *Fusarium* species, namely morphological, biological and molecular identification.

2.5.5 (a) Morphological identification

Morphological identification involves observing the differences and similarities of the fungal characteristics in suitable growing media. For *Fusarium*, the morphological characteristics are defined by the shape and size of macro- and microconidia, the presence or absence of chlamydospores and types of conidiogenous

cells, either monophialides or polyphialides (Nelson *et al*., 1983; Leslie & Summerell, 2006).

For *Fusarium* species, defining a species using morphological characteristics can be challenging as for some species there are overlapping characters, changes in morphology can occur due to mutation and for isolates of the same species, cultural variation can also occur (Leslie & Summerell, 2001; Kvas *et al*., 2009). This method is often laborious and usually requires a broad knowledge of classical taxonomy (Capote *et al*., 2012).

The use of morphological identification is not recommended to identify FB_1-producing species especially members of the FFSC as there is a possibility of misidentification because most species are morphologically similar with few or none distinctive characters to separate them. Cultural variations between isolates of the same species have also been reported (Leslie *et al*., 2001; Summerell & Leslie, 2011). Morphological characterisation is often used to sort out the isolates into smaller groups or morphotypes/morphospecies before conducting molecular identification.

2.5.5 (b) Biological identification

Biological identification is based on the compatibility of two different individuals from the same species to interbreed with each other to produce viable progenies (Mayr, 1963). For biological identification of *Fusarium*, the isolates that can interbreed are grouped in a known mating population (Leslie, 1995).

Biological identification can only be applied for species that has sexual stage or teleomorph where species that can cross-fertile are considered as the same species and grouped in the same mating population (Leslie, 1995). Most *Fusarium* species are

heterothallic (self-sterile) that require a suitable partner carrying opposite mating type alleles to complete the sexual cycle (Leslie *et al*., 2001). Some species such as *F. solani* and *F. graminearum* are self-fertile, termed as homothallic, that are able to sexually reproduce without a partner (Nelson, 1996; Leslie & Summerell, 2006).

Biological identification cannot be used on homothallic species as well as on species with asexual stage only. However, it can be used to identify several species in the FFSC (Leslie *et al*., 2004).

2.5.5 (c) Molecular identification

Molecular identification and phylogenetic analysis are other methods routinely used for identification of *Fusarium* species. For *Fusarium*, protein-coding genes such as TEF-1α, β-tubulin, calmodulin and histone H3 are commonly used as these genes are intron-rich and highly conserved, undergoing minor changes during species divergence. Therefore, these genes are suitable to be used as molecular markers especially to analyse closely related species (Zeller *et al*., 2003; Geiser *et al*., 2004).

Among these genes, TEF-1α and β-tubulin are the most widely applied markers for molecular identification and phylogenetic analysis of *Fusarium* species. Nowadays, the use of the TEF-1α for the identification of species is very common due to its ability to resolve most of the *Fusarium* species (Leyva-Madrigal *et al*., 2014). Thus, the gene is regarded as a barcoding marker for identification of *Fusarium* species. Higher numbers of introns and high level of sequence polymorphism among closely related species compared to other protein-coding genes are among factors that make TEF-1α a chosen molecular marker (Geiser *et al*., 2004). Variations in DNA

sequences of TEF-1α also provide a clear separation within and among isolates of *Fusarium* species, hence, species can accurately be identified (Taylor *et al.*, 2000).

TEF-1α gene was first used as a molecular marker to infer the relationships among Lepidopteran species (Cho *et al*., 1995). In *Fusarium*, it was first used to determine genetic relationships of *F. oxysporum* isolates that caused banana wilt disease (O'Donnell *et al*., 1998a). Since then, TEF-1α has been continuously used as a molecular marker for *Fusarium* species identification and phylogenetic analysis especially species in the FFSC (Sampietro *et al*., 2010; Mohamed Nor *et al*., 2013; Mohammadi *et al.*, 2015). TEF-1α and phylogenetic analysis of species in FFSC recovered from maize, sorghum and wheat identified three species, namely *F. subglutinans*, *F. andiyazi* and *F. thapsinum*, contrasted with morphological identification in which these species were identified as *F. verticillioides*, *F. proliferatum*, *F. thapsinum* and *F. subglutinans* (Sampietro *et al*., 2010).

Phylogenetic analysis using TEF-1α has also been used to infer relationships of *F. oxysporum* isolates (Jiménez-Gasco *et al*., 2002; Webb *et al*., 2012; Li-sha *et al*., 2013). Phylogenetic analysis of *F. oxysporum* from chickpea has clearly separated the pathogenic *F. oxysporum* from non-pathogenic due to identical sequences of TEF-1α shared by pathogenic isolates (Jiménez-Gasco *et al*., 2002). Phylogenetic analysis of *Fusarium oxysporum cubense* (*FOC*) race 4 using TEF-1α from Cavendish in mainland China has shown that the isolates were closely related with *FOC* race 4 isolates from Southeast Asia and Taiwan, which indicated that they may have descended from a common ancestor (Li-sha *et al*., 2013).

β-tubulin gene is also intron-rich protein coding gene used in systematic studies of *Fusarium* species. For *Fusarium* species, primers designed by O'Donnell & Cigelnik (1997) are commonly used to infer phylogenetic relationships. Based on a study by O'Donnell & Cigelnik (1997), β-tubulin provided greater resolution and higher bootstrap support compared to mtSSU and nuclear 28S of rDNA. Although *Fusarium* species identification using sequences of β-tubulin is less accurate, it is still phylogenetically useful especially in combined gene analysis. β-tubulin gene has been used in combination with other genes and region such as mtSSU and ITS by the assumption that phylogenetic accuracy and resolution can be improved when larger number of characters is used (O'Donnell *et al*., 2000). For phylogenetic analysis of Fusarium species, β-tubulin is commonly combined with TEF-1α as both genes have shown to provide more robust and high resolution tree branching pattern (Geiser *et al*., 2005; Lima *et al*., 2009; Walsh *et al*., 2010). For instance, in a study of Geiser *et al*. (2005), the monophyly of *Fusarium* isolates from coffee wilt was not well supported using TEF-1α but the isolates were shown to be monophyletic in combined sequences of TEF-1α and β-tubulin. In a study of Lima *et al*. (2009), the support for the clade representing new *Fusarium* lineage from Brazilian mango were only supported by less than 50% when only β-tubulin was used. However, the support for the clade increased to 94% when β-tubulin was used in combination with TEF-1α. Thus, in some cases phylogenetic analysis requires additional gene sequences to establish the phylogenetic relationships within the same species.

A database, Fusarium-ID created for *Fusarium* identification is available online at http://isolate.fusariumdb.org/index.php containing 5560 sequences from well-characterised species and voucher specimens are also available which improve

the accuracy of sequence data. There is also a quality control regarding correct identification in which all the isolates in the database have been morphologically characterised and verified. Fusarium-ID can also be used in combination with sequences from GenBank for confirmation of species (Geiser *et al*., 2004).

2.5.6 Analytical methods for FB_1 detection

2.5.6 (a) Immunochromatographic methods

Rapid screening methods that can be used at sampling sites are becoming more important to immediately detect toxin contamination. The first step of analytical survey is a simple presence/absence detection test which is sufficient as qualitative analysis. Two methods, immunoassays and lateral flow test strips are commonly used in detection test at sampling sites and are now commercially available for fumonisins screening of agricultural and food commodities (Wang *et al*., 2006; Shephard *et al*., 2011; Scott, 2012). The immunochromatographic strip tests are rapid and user-friendly test formats that do not require either instrument or additional chemicals (Goryacheva *et al*., 2007). The results are based on visual evaluation and usually provide qualitative results as positive or negative based on colour intensity. A quantitative lateral flow devices to measure fumonisin contamination in maize was later developed by Anfossi *et al*. (2010) and a novel format of lateral flow dual immunoassay for simultaneous quantitative detection of multimycotoxins in foods has also been developed (Wang *et al*., 2013).

2.5.6 (b) Chromatographic methods

Chromatographic methods are commonly used for quantitative determination of FB_1 which include Thin Layer Chromatography (TLC), High Performance Liquid