AETIOLOGY AND TOXIGENICITY OF *Fusarium* SPECIES, THE CAUSAL AGENT OF POTATO TUBER DRY ROT

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

20x	20 times
40x	40 times
ACN	Acetonitrile
APMV	Andean Potato Mottle Virus
APZV	Andean Potato Latent Virus
c	Control
FCs	C Fumonisins
CLA	Carnation Leaf Agar
cm	Centimetre
CPRI	Central Potato Research Institute
CF/FAB/MS	Continuous Flow/Fast Atom Bombardment/Mass Spectrometry
conidia/ml	Conidia per millilitre
°C	Degree Celsius
°N	Degree North
°S	Degree South
CH_2Cl_2	Dichloromethane
ddH_2O	Double distilled water
dsp	disease-specific genes
EU	European Union
FDA	Food and Drug Association
$FUMB_1$	Fumonisin B_1
GMB	Grain Marketing Board
kg cm^{-2}	Kilogram per centimetre square
g	Gram

HDPE	High Density Polyethylene
h	Hour
MAP	Mitogen-Activated Protein
µg	Microgram
µg/g	Microgram per gram
M	Molar
MeOH	Methanol
mg/g	Milligram per gram
mg/kg	Milligram per kilogram
ml	Millilitre
mm	Millimetre
min	Minute
MON	Moniliformin
nm	Nanometre
OPA	*o*-phtaldialdehyde
KH_2PO_4	Potassium dihydrogen phosphate
pat	Pathogenicity genes
KOH	Potassium hydroxide
PAMV	Potato Aucuba Mosaic Virus
PCNB	Pentachloronitrobenzene
PDA	Potato Dextrose Agar
PLRV	Potato Leafroll Virus
PMTV	Potato Mop Top Virus
ppm	Part per million
PSTVd	Potato Spindle Tubers Viroid
PVA	Potato Virus A

PVM	Potato Virus M
PVS	Potato Virus S
PVV	Potato Virus V
PVX	Potato Virus X
PVY	Potato Virus Y
PVYN	Potato Virus N
PVY0	Potato Viruso
PYDV	Potato Yellow Dwarf Virus
%	Percentage
NaoCl	Sodium hypochlorite
NaH_2PO_4	Sodium dihydrogen phosphate
R^2	R-squared
spp.	Species
$C_{16}H_{35}N.H_2SO_4$	Tetrabutylammonium hydrogen sulfate
TRV	Tobacco Rattle Virus
TSV	Tobacco Streak Virus
US$	US dollar
UV	Ultraviolet
W	Watt
WA	Water Agar

ETIOLOGI DAN KETOKSIGENAN *Fusarium* SPECIES, EJEN PENYEBAB BUSUK KERING UBI KENTANG

ABSTRAK

Busuk kering Fusarium ialah penyakit yang dilaporkan di seluruh dunia yang berupaya menjangkiti ubi kentang. Sehingga kini, pelbagai kajian telah dilaksanakan untuk mengurangkan kerugian yang disebabkan oleh penyakit ini. Objektif kajian ini adalah untuk mengesahkan etiologi spesies *Fusarium* yang dipencilkan daripada ubi kentang yang dijangkiti busuk kering dan ketoksigenan pencilan terpilih. Satu siri persampelan telah dilakukan di tiga negeri di utara Semenanjung Malaysia iaitu Pulau Pinang, Kedah dan Perlis. Sejumlah 56 sampel ubi kentang dengan gejala khas busuk kering telah dikutip. Selepas pemencilan di atas medium semi-selektif, pepton pentakhloronitrobenzena agar (PPA), 117 pencilan *Fusarium* yang diperolehi ditulenkan melalui spora tunggal dan dikenalpasti secara morfologi kepada tujuh spesies iaitu *F. oxysporum* (54 pencilan; 46.2%), *F. solani* (52 pencilan; 44.4%), *F. proliferatum* (3 pencilan; 2.6%), *F. semitectum* (3 pencilan; 2.6%), *F. subglutinans* (2 pencilan; 1.7%), *F. equiseti* (2 pencilan; 1.7%) dan *F. graminearum* (1 pencilan; 0.8%). Sejumlah 70 pencilan *Fusarium* telah dipilih untuk ujian kepatogenan selama 21 hari. Daripada ujian tersebut, tiga spesies *Fusarium*, iaitu *F. oxysporum*, *F. solani* dan *F. graminearum* menyebabkan jangkitan ke atas ubi kentang yang dilukakan. Ubi kentang yang dijangkiti dengan spesies lain dan dengan kertas turas yang disteril (kawalan) tidak menunjukkan sebarang gejala. Daripada 30 pencilan *F. oxysporum*, 29 pencilan memperolehi nilai indeks 4, yang menunjukkan jangkitan parah berupa busuk kering dan busuk basah, manakala satu pencilan tidak menunjukkan sebarang gejala dengan nilai indeks 1. Daripada 29 pencilan *F. solani*, 28 pencilan adalah

patogen dengan 16 pencilan menunjukkan gejala yang parah dengan nilai indeks 4, 10 pencilan menunjukkan gejala utama busuk kering dengan nilai indeks 3, dua pencilan menunjukkan gejala sederhana busuk kering dengan nilai indeks 2 dan satu pencilan dengan nilai indeks 1 iaitu tiada gejala. Tambahan lagi, satu pencilan *F. graminearum* menunjukkan nilai indeks 4 dengan gejala yang parah. Analisis profil $FUMB_1$ dan MON telah dijalankan ke atas 25 pencilan *F. oxysporum* dan 25 pencilan *F. solani* dengan menginokulasi beras disteril dan dieramkan selama 28 hari di dalam gelap sebelum pengekstrakan dan penapisan. Kedua-dua mikotoksin tersebut dianalisis menggunakan Ultra Performance Liquid Chromatography (UPLC; Water® Associates, USA). Dua puluh pencilan *F. oxysporum* berupaya menghasilkan $FUMB_1$ dalam julat 0.33 µg/g ke 4.23 µg/g. Tujuh pencilan *F. oxysporum* (K7090P, K7304P, R7308P, R7417P, P7381P, P7424P, P7429P) disyaki melebihi had maksima yang ditetapkan untuk jagung (~4.00 µg/g) oleh Food and Drug Association (FDA) dan European Union (EU). Tiada pencilan *F. solani* berupaya menghasilkan $FUMB_1$. Dua puluh empat pencilan *F. oxysporum* juga berupaya menghasilkan MON dalam julat 0.72 µg/g ke 11.23 µg/g. Sebanyak tiga pencilan *F. solani* (K7130P, R7145P and R7341P) menghasilkan MON dalam dalam julat 0.45 µg/g ke 1.34 µg/g. Kajian ini berjaya mencapai objektif-objektif yang telah ditetapkan iaitu mengesahkan etiologi spesis *Fusarium* daripada ubi kentang yang dijangkiti busuk kering dan ketoksigenan pencilan terpilih. Kajian ini diharap mendatangkan manfaat kepada pihak yang terlibat dalam industri makanan dan orang ramai serta memberikan kesedaran tentang kewujudan penyakit ini serta keburukan mikotoksin ke atas tubuh badan manusia apabila ubi kentang yang tercemar digunakan di dalam masakan. Kajian ini adalah yang pertama berkaitan etiologi dan ketoksigenan busuk kering ubi kentang di Malaysia.

AETIOLOGY AND TOXIGENICITY OF *Fusarium* SPECIES, THE CAUSAL AGENT OF POTATO TUBER DRY ROT

ABSTRACT

Fusarium dry rot is a worldwide reported disease infecting potato tubers. Thus, many studies have been conducted with a common aim to minimize losses incurred by the disease. The objectives of this study were to confirm the aetiology of *Fusarium* spp. isolated from potato dry rot and toxigenicity of selected isolates. A series of samplings were conducted in three states in northern Peninsular Malaysia namely Pulau Pinang, Kedah, and Perlis. A total of 56 samples of potato tubers with typical dry rot symptoms were collected. After the isolation on a semi-selective media, peptone pentachloronitrobenzene (PPA), 117 isolates of *Fusarium* spp. recovered were single-spored and identified morphologically into seven species, namely *F. oxysporum* (54 isolates; 46.2%), *F. solani* (52 isolates; 44.4%), *F. proliferatum* (3 isolates; 2.6%), *F. semitectum* (3 isolates; 2.6%), *F. subglutinans* (2 isolates; 1.7%), *F. equiseti* (2 isolates; 1.7%) and *F. graminearum* (1 isolate, 0.8%). Seventy isolates of *Fusarium* species were chosen for pathogenicity test for 21 days. From the test, three species of *Fusarium* i.e., *F. oxysporum*, *F. solani* and *F. graminearum* caused dry rot on artificially wounded potato tubers. Potato tubers inoculated with other species and those with sterile filter paper (control) did not show any disease symptoms. From 30 isolates of *F. oxysporum*, 29 isolates showed Scoring index of 4, which indicated severe infection of dry rot and soft rot and one with no symptom which indicated with Scoring index 1. From 29 isolates of *F. solani*, 28 isolates were pathogenic with 16 isolates showed severe infection with Scoring index 4, 10 isolates showed major dry rot symptoms with Scoring index 3,

two isolates showed minor dry rot symptom with Scoring index 2 and one isolate showed no visible symptom with Scoring index 1. In addition, one isolate of *F. graminearum* showed Scoring index of 4 with severe infection. Analysis of $FUMB_1$ and MON profiles was conducted on 25 isolates of *F. oxysporum* and 25 isolates of *F. solani* by inoculating sterilized rice and incubated for 28 days in the dark prior to extraction and filtration. Both $FUMB_1$ and MON were analysed using Ultra Performance Liquid Chromatography (UPLC; Water® Associates, USA). Twenty isolates of *F. oxysporum* were able to produce $FUMB_1$ ranged from 0.33 µg/g to 4.23 µg/g. Seven isolates of *F. oxysporum* (K7090P, K7304P, R7308P, R7417P, P7381P, P7424P, P7429P) were suspected to exceed the maximum limit practice for maize (~4.00 µg/g) by Food and Drug Association (FDA) and European Union (EU). None of the *F. solani* isolates was able to produce $FUMB_1$. Twenty-four isolates of *F. oxysporum* also were able to produce MON ranged from 0.72 µg/g to 11.23 µg/g. Moreover, three isolates of *F. solani* (K7130P, R7145P, R7341P) were able to produce MON ranged from 0.49 µg/g to 1.34 µg/g. This study achieved all the objectives which were to confirm the aetiology of *Fusarium* spp. isolated from potato dry rot and toxigenicity of selected isolates This research is hoped to be beneficial to the authority involved in food industry and also to the throngs besides alert them on the existence of the disease and detrimental effects of mycotoxins when the contaminated potato tuber is used for cooking. This is the first report on aetiology and toxigenicity of Fusarium dry rot on potatoes in Malaysia.

CHAPTER 1

GENERAL INTRODUCTION

Fusarium dry rot is one of worldwide threats of potato tubers. Infection of dry rot usually commences in the field and progress extensively during storage due to favourable environmental conditions such as high humidity and temperature. One of the produce susceptible to Fusarium dry rot is potato. Some of the *Fusarium* species linked to this threat is *F. sambucinum*, *F. solani*, *F. oxysporum* and *F. avenaceum* (Hanson *et al*., 1996; Choiseul *et al*., 2007; Wustman, 2007). The concern on potato infection is paramount important. Although potato tuber is not planted in Malaysia, the use of this produce is enormous. Potato tuber is processed into fast food and frequently consumed by Malaysians. The fast development especially the fast food industry is one of the proves of increasing potato demands in Malaysia.

The pathogenic patterns of *Fusarium* species are very much influenced by the climates and regions although the hosts are the same. In Iran and Great Britain for instance, *F. sambucinum* was reported as the most aggressive *Fusarium* spp. against potato tubers (Esfahani, 2005; Peters *et al*., 2008b) whereas a study carried out in northern Italy showed that potato tubers were highly infected by *F. solani* and *F. oxysporum* f. sp. *tuberosi* (Manici and Cerato, 1994). In addition, the same species originated from two different regions can be varied specifically, in term of macroconidia sizes. Thus, identification is a critical stage in naming a culture isolated from any samples (Leslie and Summerell, 2006).

Fourteen *Fusarium* spp. were recorded to infect and cause dry rot on the potato tubers. *F. coeruleum, F. sulphureum, F. sambucinum,* and *F. avenaceum, F. oxysporum, F. solani, F. eumartii, F. coeruleum, F. equiseti* and *F. redolens* are among the dry rot agents and be the important cause of spoilage of potato tubers in storage (Peters *et al*., 2008b; Wustman, 2007). The species committed to Fusarium dry rot varies among the regions as many factors may involve in the species pathogenic patterns. A species which is highly pathogenic on potato tuber may also be classified as weakly pathogenic in another area. In Malaysia, no data on *Fusarium* causing dry rot on potato tuber. Therefore, the study was conducted to identify pathogenic *Fusarium* spp. on potato tuber.

Fusarium spp. is able to produce various mycotoxins including fumonisin B_1 ($FUMB_1$) and moniliformin (MON). Mycotoxin is the secondary metabolite produced by *Fusarium* spp. which may cause detrimental effects due to its indirect consumption such as pulmonary oedema in swine. Section Liseola particularly is always related with the ability to produce high concentration of $FUMB_1$. This toxin is frequently reported to be produced by *F. verticillioides*, *F. proliferatum* and *F. subglutinans*. The species are highly pathogenic on maize and associated with maize ear rot (Pascale *et al*., 2002). The member of section *Elegans*, *F. oxysporum* is also able to produce $FUMB_1$ but the report on the production is very few due to low concentration of $FUMB_1$ produced (Nur Ain Izzati and Wan Hasmida, 2011). No record was found on the production of $FUMB_1$ by *F. solani*. The toxin is optimally produced in media that has moderate water activity and is nitrogen limited. The production doubled roughly every 48 hours as long the mycelial dry weight increased (Miller, 2001). MON is highly produced by *F. moniliforme* which is now separated

to a few species belonged to section *Liseola*. Besides, *F. oxysporum* is also able to produce high concentration of MON compare to $FUMB_1$ (Nur Ain Izzati and Wan Hasmida, 2011). Moreover, some isolates of *F. solani* are able to produce MON but in a minute quantity (Chelkowski *et al*., 1990; Nor Azliza, 2008).

In Malaysia, this study is crucial to alert the related authorities and the consumers on the potato tuber safety for consumption due to mycotoxins contamination. Since Fusarium dry rot is a worldwide disease, it has the potential to vastly infect the potato tubers during storage in Malaysia. The identification of *Fusarium* spp. commit to potato dry rot is also important for advance researches.

Thus, the objectives of this study were:

1. To study the aetiology of *Fusarium* isolates from potato dry rot, and
2. To detect the production of $FUMB_1$ and MON by selected isolates of *F. oxysporum* and *F. solani* causing dry rot disease.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Potato Crop

Potato originated in the highlands of Peru, in the region around Lake Titicaca. Potatoes as well as maize allowed the development of civilisations of Huari and Inca. As these civilisations flourished, the potatoes were adapted to meet different environmental conditions, and the produce became domesticated. During Spanish conquest, a Spanish soldier, Pedro de Cieza de Leon was the first European to record the existence of the potato, in 1538, in the Upper Cauca valley which is later known as Colombia (Choiseul, 2008).

In the Netherlands, in 1846 to 1847, the adoption of potato cultivation was practiced since the produce harvested was higher per unit of land compare to wheat and rye. Besides, the produce was assumed to be protected from harm above ground infections. The produce also saved from being destroyed by the soldiers sent by absentee landlords to collect overdue land rents. Since most of the farmers were poor and own no land, thus the potato tubers assisted them a lot in their daily survival. The farmers stored the tubers in shallow ditches in the ground for the winter as they lacked warehouse facilities (Agrios, 2005; Lindeboom *et al*., 2010).

In Ireland, for the first time, at the end of 16th century, Sir Walter Raleigh introduced potatoes at his estate in Youghal Co. Cork. However it is as likely that potatoes were introduced to Ireland from Spain as part of normal trade. In the second half of 19th century, among the prominent varieties were Rocks, Skerry Blue,

Flounder and Champion. Rocks was the dominant varieties planted with almost 40% of total potato plantation area as recorded in 1880. However, Rocks was susceptible to blight during blight outbreak in 1879. Later, variety Champion was popular due to its resistance against fungus and was vastly planted from 1884 to 1892. From 1900 onwards, Champion declined its popularity due to a reduction in its resistance to blight and replaced by Kerr's Pink (Choiseul, 2008).

More potato varieties were discovered and later potato breeding was practiced to gain the valuable potatoes. The modern potato breeding began in England when Knight (1807) made the first recorded hybridizations between varieties by artificial pollination. Besides of Ireland, during the second half of the 19th century, the breeding programmes were intensive in Europe and North America when exchanges of germplasm occurred and many new cultivars were produced by farmers and breeders. North America most popular potato cultivar, Russet Burbank was released in 1914 descended from Rough Purple Chili through three generations of open pollination (Ortiz, 2001). The World Catalogue of Potato Varieties listed more than 4, 500 varieties from 102 countries covering all the potato grown all over the world in 2009 (Pieterse and Hils, 2009).

Modern potato breeding in India and China commenced in 1930s but rapid expansion was reported since 1948 and 1978 respectively (Gaur and Pandey, 2009; Jin *et al*., 2004). Potato breeding programmes in China focused on high yield and diseases resistance. The programmes were later concerned on producing tubers for processing to supply the increasing demand for production of crisps and French fries. Chinese cultivars supply most of the fresh market however North American cultivars currently dominate the processing acreage. Shepody and Kennebec are the major

cultivars used for production of French fries, while cultivars for crisp production include Atlantic and Snowden (Janksy *et al*., 2009).

2.2 Nutritional Value of Potato

Potato contains approximately 78% water, 22% dry matter and less than 1% fat. From the percentage of dry matter, almost 82% is highly digestible carbohydrates. The potato also contains some dietary fibre (up to 3.3%), minute volume of various simple sugars and at least 12 essential vitamins and minerals (Suttle, 2008; Burlingame *et al*., 2009; Bradshaw and Bonierbale, 2010).

The antioxidant compounds are also naturally present which are vital for human health for example carotenoids. The carotenoids in potato are lutein, zexanthin, violaxanthin and xantophylls. Carotenoids are approximately 50 to 100 µg per 100 g of fresh weight in white-fleshed varieties. Deeply yellow to orange-fleshed potato cultivars are enriched with almost 2,000 µg per 100 g of potato tuber fresh weight. Pihlanto *et al*. (2008) concluded that potato and by-products from the potato industry comprise a source of bioactive compounds and antioxidant activities.

The phenolic compounds presumably induce the observed ACE-inhibitory and radical-scavenging potencies of the by-products namely the pulp, liquid and peel fractions. The results of this study indicated that potato proteins were the promising source for the production of bioactive compounds as materials for developing functional foods with a positive impact on cardiovascular health. Predominant phenolic compounds is chlorogenic acid; constitutes 80% of total phenolic acids (Pihlanto *et al*., 2008).

Although potato has a very small portion of protein (0.6 to 1.2% of the fresh weight), their value is almost equal to most cereals such as rice and wheat. A small portion of significant potato starch is resistant to digestion by enzymes in the stomach and small intestine; thus reaching the large intestine for nutrients adsorption. This potato starch is considered to have similar physiological effects and health benefits as fibre. It offers protection against colon cancer, enhances glucose tolerance and insulin sensitivity, lower plasma cholesterol and triglyceride concentrations, increases satiety and assumed to be able to reduce fat storage (Bradshaw and Bonierbale, 2010).

2.3 Potatoes in Food Industry in Malaysia

Since Malaysia is located in area with high temperature and high humidity, potato is not planted in Malaysia. However, the consumption is typically high. Based on the research conducted by Warr *et al.* (2008), potato intake per person by Malaysian was noted to increase based on the data collected in 1990 and 2005. Thus, in 2011, the consumption was estimated to be higher with the rapid development of fast food industry in Malaysia (Table 2.1).

Table 2.1 Comparison of Malaysia's food consumption per person between 1990 and 2005 (Warr *et al*., 2008)

Daily calorie intake	**1990 calories**	**2005 calories**
Vegetables products	2303	2552
Animal products	337	383
Total	**2639**	**2935**
Consumption per person	**kg**	**kg**
Cereal	160.3	171.6
Wheat	32.6	57.6
Rice	118.2	99.9
Maize	6.1	9.3
Starchy roots	26.1	22.8
Cassava	20.9	13.6
Potato	3.2	6.4
Sweet potato	0.3	0.5

Malaysia imported potatoes mainly from China and India due to their good quality and lower prices. Particularly in China, the export value of potatoes to Malaysia increased from US$0.6 million in 1995 to US$21.6 million in 2006. This data proved that the demand and consumption increased and estimated to be higher in 2011 (Warr *et al*., 2008) (Table 2.2). In 2009, almost 22% of total world potato production is recorded in China thus placing this country as the world leading potato producer with an increase in land utilization for potato crops. This data showed an increasing demand of potatoes from the world market including Malaysia (Jansky *et al*., 2009).

Table 2.2 Comparison of Malaysia's potato imports from Australia and China between 1995 and 2006 (Warr *et al*., 2008)

Potato		
	1995	**2006**
	US$m	
Australia	2.2	2.9
China	0.6	21.6

2.4 Diseases of Potato

Potato tubers are prone to infections by oomycetes, fungi, antinomycetes, bacteria and viruses during storage. The favourable conditions during storage such as high humidity and high temperature may assist the pathogens to grow, disseminate and eventually caused a losses (Maldonado *et al*., 1998).

2.4.1 Fungal Diseases

Fusarium dry rot is caused by several *Fusarium* spp. particularly *F. solani*, *F. oxysporum*, *F. sambucinum* and *F. graminearum* (Arora and Khurana, 2004). The spores are able to remain long in the soil. The spores may stick to the periderm of the potato tubers but unable to penetrate through if the entry is not available. These pathogens penetrate the tubers through wounds and bruises formed during harvest, handling, transportation and storage and finally develop extensively when the temperature and humidity are favourable. The symptom is generally visible about a month after storage as small brown lesions on surface of affected tubers (Wharton *et al*., 2007).

The skin of potato tuber becomes wrinkled and shrivels. The internal necrotic tissue usually will dry out thus sometimes a hole is formed and clearly observed on

the surface of the potato tuber (Figure 2.1). When the potato tuber is cut into half, rotted cavities lined with mycelia and spores of various colours from yellow to pink can be seen. Later, the diseased potato tubers get mummified. Under high relative humidity, infected potato tuber with Fusarium dry rot provides an entry to the secondary organisms such as *Erwinia* spp. *Erwinia* spp. is the causal agent of bacterial soft rot. Exudates containing bacteria come out from the infected tubers resulting in soft rot. The infected tubers usually become totally macerated, sunken and produce a strong uneasy smell. Subsequently, the infection will spread among the potato tubers and finally causes a great loss (Wustman, 2007; Bradshaw and Bonierbale, 2010).

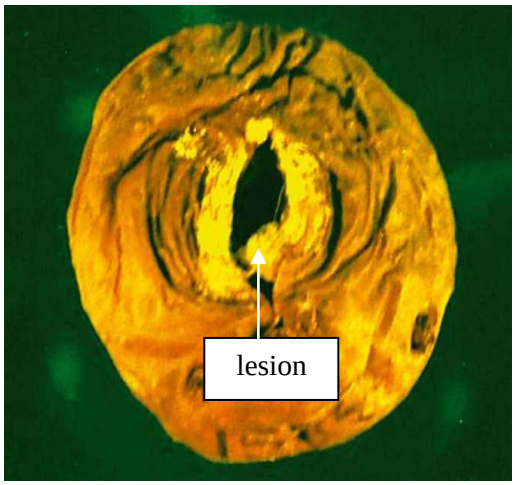

Figure 2.1: Lesion observed on potato tuber due to Fusarium dry rot caused by *Fusarium* spp. (Arora and Khurana, 2004)

Early blight is usually caused by *Alternaria solani*. The lesions are dark, sunken, circular to irregular in shape, shallow and separated from healthy tissue by purplish-brown dry cork layer (Arora and Khurana, 2004) (Figure 2.2).

Figure 2.2: Symptom observed on the potato tuber due to early blight caused by *Alternaria solani* (Arora and Khurana, 2004)

Wart of potato is caused by *Synchytrium endobioticum*. The wart tissues are soft and spongy. Tubers may turn completely warty which desiccate or decay at harvest (Arora and Khurana, 2004; Bradshaw and Bonierbale, 2010) (Figure 2.3).

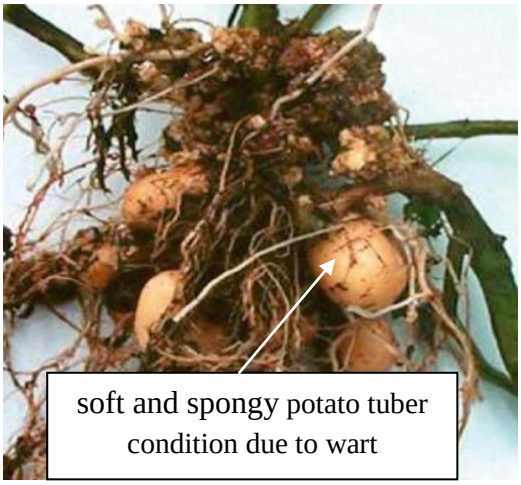

Figure 2.3: Soft and spongy potato tuber due to wart caused by *Synchytrium endobioticum* (Arora and Khurana, 2004)

Black scurf of potato is caused by *Rhizoctonia solani*. The most common symptom observed on the potato tubers is black irregular lumpy encrustations of fungal sclerotia which stick to the surface of the tubers. It may result with tubers cracking, malformation and pitting (El-Kot, 2008) (Figure 2.4).

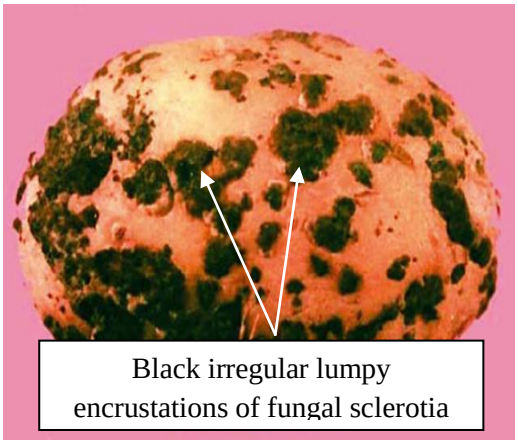

Figure 2.4: Potato tuber condition due to black scurf caused by *Rhizoctonia solani* (Arora and Khurana, 2004)

Charcoal rot of potato is caused by *Macrophomina phaseolina*. Among the symptoms on tubers are a dark light grey, soft and water-soaked lesion developed on the surface of the tuber. Cavity within the lesion becomes filled with black mycelium and sclerotia of the pathogen (Snowdon, 1990) (Figure 2.5).

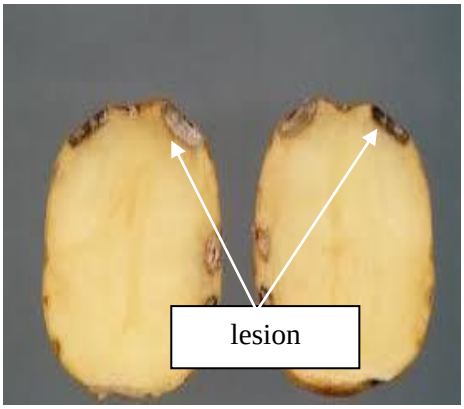

Figure 2.5: Lesion observed on potato tuber due to charcoal rot caused by *Macrophomina phaseolina* (Snowdon, 1990)

2.5 Potato Storage

Potato tubers can be stored up to 10 months under appropriate storage conditions. During storage period, as a living organism, potato will go through the active metabolism. Respiration and transpiration will continuously occur thus contributing the weight loss during storage. Besides, sprouting, changes in the chemical composition, damage by extreme temperature whether too low or too high and spread of the diseases may also contribute to the weight loss during storage. Thus, an appropriate storage conditions play a role to minimize the weight loss and to extend its quality during consumption (Wustman and Struik, 2007).

The respiration in healthy potato tuber shows a minimum rate at 5°C and if the temperature increases, the respiration rate also will increase. Besides, the transpiration also will increase with the increase of the temperature. However, if the temperature drops, especially below the freezing point, it will cause chilling injury on the potato tubers. In addition, sprouting can contribute weight loss by increasing both respiration and transpiration of the potato tubers (Wustman and Struik, 2007).

Although *Fusarium* is a field fungi, the infection on potato tubers usually occurs during harvesting due to crack, bruises or skinning when the produce are dug from underground and also during handling process. When the pathway established on the periderm due to these reasons, it eases the *Fusarium* to penetrate and causes infection especially during storage when the conditions are favorable. Thus, a swift wound healing and suberization is paramount important to treat the injuries and hamper the infection by *Fusarium* and also by any other microorganisms (Shetty *et al*., 1998; Yahia *et al*., 2004).

In addition, the late blight pathogen of potato tubers, *Phytophthora infestans* can infect unwounded tubers thus produced an entry for *Fusarium* spp. to commence the infection. When the relative humidity is high, bacterial soft rot which is caused by *Erwinia* spp. will also play its part. The combination of these pathogens will make the potato tubers condition to become worst. The potato tubers have to be well-stored to avoid favorable conditions for any fungi conidial to develop and cause infection (Sicilia *et al*., 2002).

Thus, in Malaysia, our concern is on the storage management. A thorough inspection on the potato tubers upon receiving the produce is also highly recommended since potato tubers are living organisms which are subjected to losses during the period after the tuber has been separated from the plant. Storability is vital and it is depends on a few factors including weather conditions, storage temperature, sprouting and the presence of diseases and pests in the store. These factors can be manipulated and control by us since Malaysia only imports the produce and it is beyond our capability to involve in pre-harvest and harvesting management (Wustman, 2007).

2.6 History of *Fusarium* Systematics

Wollenweber and Reinking (1935) published their work, 'Die Fusarien' on *Fusarium* taxonomy that has become the standard reference on this subject. Most taxonomic systems of *Fusarium* published since 1935 were based on this publication. Wollenweber and Reinking began with approximately 1000 species of *Fusarium* and organized them into 16 sections which were divided into 65 species, 55 varieties and 22 forms. The characters used by Wollenweber and Reinking to separate the sections

were the presence or absence of microconidia, the shape of microconidia, the presence or absence of chlamydospores, the location of chlamydospores whether intercalary or terminal, the shape of macroconidia and the basal foot cell on the microconidia. Sections were separated into species, varieties and forms based on the length and width of macroconidia including the septation (Nelson, 1991).

Wollenweber and Reinking (1935) studied isolates grown on beerwort agar, carrot decoction agar, oatmeal agar, rice mash, alfalfa stems, barley heads and potato dextrose agar. Emphasize was given on the differences of the growing cultures instead of their similarities which produced a complex and complicated structure of species determination. Many of the characters used by Wollenweber and Reinking to separate the species, varieties and forms were not stable when inoculated on different types of agar and different environmental conditions. Cultural variation and mutation might have been overlooked by both Wollenweber and Reinking since their cultures were not originated from single conidia. In addition, some cultures were named based on two representatives and it was not practical in species determination (Nelson, 1991).

Figure 2.6 shows the relationship of several taxonomic systems of *Fusarium* originated from Wollenweber and Reinking. *Fusarium* taxonomists can be divided into lumpers and splitters, which explained the philosophy employed by taxonomists in determining *Fusarium* species but were not necessarily a reflection of the number of species recognized. From Figure 2.6, Wollenweber and Gerlach are splitters with additional splitters on the left and lumpers on the right. Gordon, Booth and Nelson, Toussoun and Marasas are listed in the middle because they had followed a more moderate philosophy or had combined the results of others with their own finding to erect a taxonomic system.

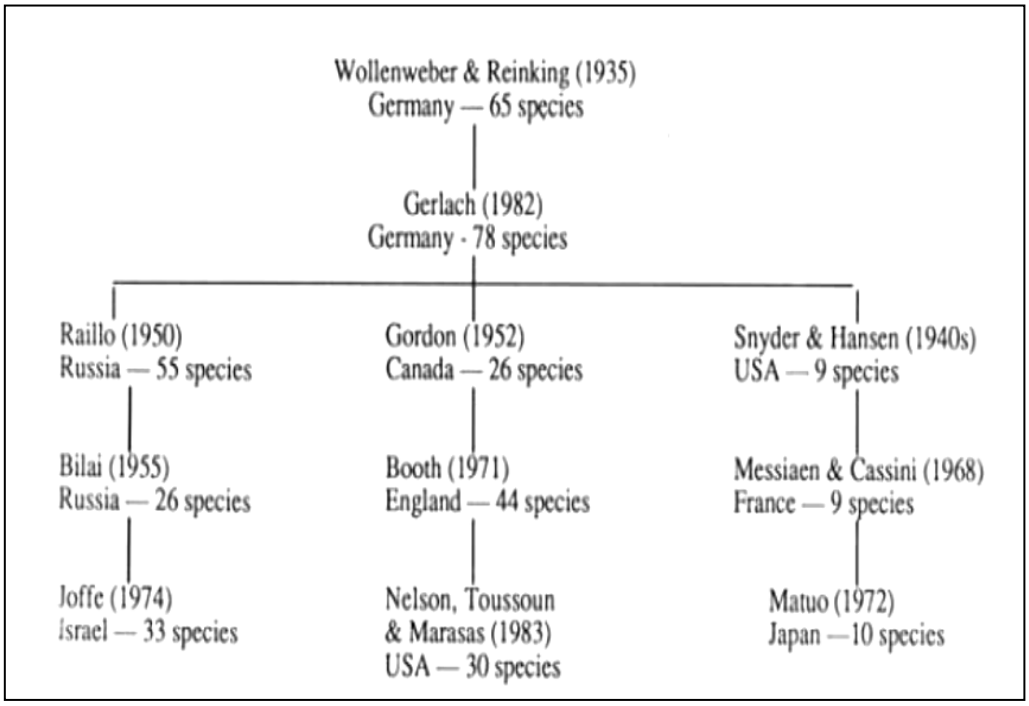

Figure 2.6: Principal taxonomic systems for *Fusarium* species showing the relationship of several taxonomic systems pioneered by Wollenweber and Reinking (Nelson, 1991)

Snyder and Hansen (1940; 1941; 1945) were considered as the ultimate lumpers. In Berkeley, Snyder began an extensive study on biology and taxonomy of *Fusarium* species in cooperation with Hansen and published their results in three major papers. They reduced all of *Fusarium* species from Wollenweber and Reinking's 16 sections into only nine species. Their system was based primarily on the morphology of the macroconidia and proposed the lumping of several sections into one species that was not generally accepted. The lumping of Wollenweber and Reinking's sections of Arthrosporiella, Discolor, Gibbosum, and Roseum into merely one species *F. roseum* has caused a great deal of confusion and controversy. Moreover, Snyder and Hansen (1945) proposed the adoption of the nonbotanical name, cultivar, for certain intraspecies populations differing in conidial morphology. On the basis of continued study, it has been concluded that the concept of a single

species, *F. roseum* as proposed by Snyder and Hansen (1945) cannot be justified and should be abandoned.

Raillo (1935; 1950) studied morphological characters useful in taxonomy of *Fusarium* and concluded that the shape of the apical cell was important in species determination. The incurvature of conidia, length of the apical cell, number of septa and width of conidia were the characters used in separating subspecies and varieties. The cultural characters such as pigmentations, presence of sclerotia and mode of spore formation were useful in separating forms only. Raillo (1935) studied the variability in *Fusarium* by initiating cultures from single conidia and found that the form of the apical cell and the incurvature of conidia remained constant in cultures developed from single conidia. The number of septa was also constant in isolates within a single-conidial culture. However, the mode of spore formation (pionnotes, pseudo-pionnotes and sporodochia) varied within a single-conidial culture.

Based on the continual study and usage as proposed by Synder and Hansen, the designation f. sp. *cerealis* to denote pathogenicity to cereals is not valid as shown by Tammen (1958) and the suggestion to use f. sp. *cerealis* simply to designate pathogenesis is confusing and unnecessary.

Gordon worked with *Fusarium* isolated from cereal seed, various host plants and soil from both temperate and tropical geographical areas (Gordon, 1944; 1952; 1954a; 1954b; 1956a, 1956b; 1959; 1960). Gordon taxonomy system was similar to Wollenweber and Reinking (1935) and also to Synder and Hansen (1940; 1941; 1945). Certain sections particularly Lateritium, Liseola, Elegans and Martiella were modified as proposed by Synder and Hansen (1940; 1941; 1945). Thus, Gordons'

taxonomic system was a compromise between Wollenweber and Reinking and also Synder and Hansen.

Bilai (1955; 1970) recognized the importance of cultural variation or mutation in *Fusarium* isolates. Bilai (1955) did a critical analysis of several characters used in identification by studying variability of individual isolates and establishing the range of variation for some species. In addition, she also studied morphogenesis in single-conidial isolates and the effects of temperature, moisture, length of growth period and composition of the medium, as well as the method of germination and aging of conidia. Based on the observation, Bilai (1970) revised the taxonomy of the genus to include only nine sections, 26 species and 29 varieties, as well as the species in the section Liseola with section Elegans and section Gibbosum with section Discolor.

Messiaen and Cassini (1968) followed Synder and Hansen's system, but they used botanical varieties instead of cultivars at the subspecies level in *F. roseum*. They provided descriptions for each variety and a key was provided for the entire system.

Booth (1971) modified Gordons' taxonomic system and expanded the information on perfect states. A major contribution by Booth was information on conidiophores and conidiogenous cells which are useful in the taxonomy of *Fusarium* species. Booth (1971) also showed the value of polyphialides and monophialides in separating the sections and species. The length and shape of microconidiophores were also shown to be a reliable character in separating *F. oxysporum*, *F. solani* and *F. moniliforme*.

Matuo (1972) also followed Synder and Hansens' system and provided a key to the entire system. Matuo and Kobayashi (1960) reported that *Hypocrea splendens* produced a conidial state that that named *F. splendens*. However, further work showed that this was most likely a *Nectria* hyperparasite. Matuo was also in favour of lumping *F. lateritium* and *F. roseum*, but this concept had received very little support (Matuo and Kobayashi, 1960).

Joffe (1974) examined a large number of isolates of *Fusarium* from soil, wilting or decaying plants and seeds. His philosophy and approach to *Fusarium* taxonomy is similar to Wollenweber and Reinking (1935). Joffe (1974) taxonomic system appeared to be a restatement of Wollenweber and Reinking's sections and species, which included 13 sections, 33 species and 14 varieties.

The techniques used in studying *Fusarium* and establishing new species placed Gerlach with the splitters (Gerlach, 1981). This was the evident from 78 species that appeared in his atlas published with Nirenberg (Gerlach and Nirenberg, 1982), a well-illustrated work that used excellent photographs and line drawings. Gerlach and Nirenberg (1982) grew *Fusarium* cultures on the eight different media used by Wollenweber and Reinking (1935). Their work was more focus on the differences rather than similarities, and the result slight cultural difference might had been the basis for description of a new species or variety. New species were established based on a single culture and in some cases, on a single mutant culture which led to a complex taxonomic system that was difficult to be applied.

Nelson *et al*. (1983) combined several taxonomic systems and their own work to develop a compromise taxonomic system of *Fusarium*. The combination included were description of *F. oxysporum* and *F. solani* by Synder and Hansen (1940; 1941;

1945), and information of conidiophores as described by Booth (1971). They retained of Wollenweber and Reinking (1935) description on toxigenic species as well as some other sections. However, the number of species was reduced and varieties and forms were combined with an appropriate species name. The manual by Nelson *et al*. (1983) was illustrated with photographs of macroconidia, microconidia, conidiophores and chlamydospores which made the species description relatively easier. The manual was cross-referenced to other taxonomic systems such as Wollenweber and Reinking (1935), Synder and Hansen (1940; 1941; 1945), Messiaen and Cassini (1968), Booth (1971), Joffe (1974), and Gerlach and Nirenberg (1982).

Leslie and Summerell (2006) combined several descriptions of *Fusarium* species with their own work on *Fusarium* morphology. The data were collected and compiled into a manual, 'The *Fusarium* Laboratory Manual'. The manual contains images and brief description for species identification.

2.7 *Fusarium* morphology

Macroconidia is one of the most important character used for identification of *Fusarium* species. The shapes of the macroconidia varies and the diagnostic features are the degree of curvature, relative length and general form. The length and septation of the macrocondia differ between species when observed on Carnation Leaf (CL) compare to the observation on the agar. The shape is classified into three; needlelike, dorsinventral curvature along the spore and curved dorsal compare to ventral (Figure 2.7). The apical cells are usually categorized into four general forms namely blunt, paplillate, hooked and tapering (Figure 2.8). The basal cell has four

general forms, foot-shaped, elongated foot shape, distinctly notched and barely notched (Figure 2.9) (Booth, 1971; Nelson *et al*., 1983; Leslie and Summerell, 2006).

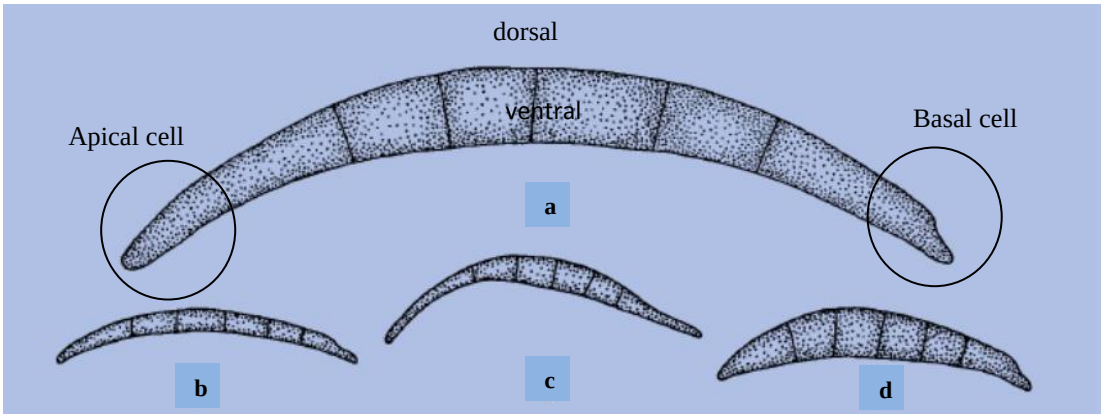

Figure 2.7: Macroconidia of *Fusarium* (a) general shape of macroconidia with apical and basal cells (b) needle-like (c) dorsi-ventral curvature (d) curved dorsal compare to ventral (Leslie and Summerell, 2006)

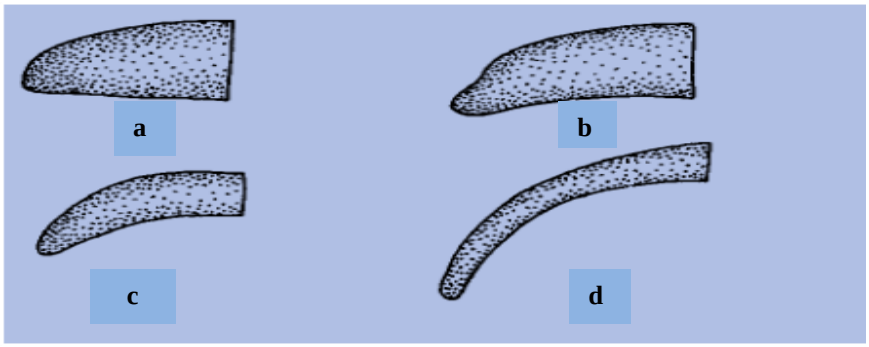

Figure 2.8: Apical cell of macroconidia (a) blunt (b) papillate (c) hooked (d) tapering (Leslie and Summerell, 2006)

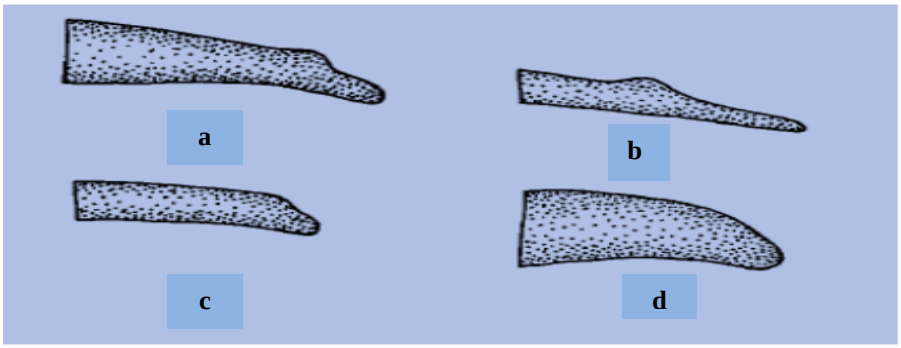

Figure 2.9: Basal cell of macroconidia (a) foot-shaped (b) elongated foot shaped (c) distinctly notched (d) barely notched (Leslie and Summerell, 2006)

Microconidia which formed from the conidiogenous cell is also an important characters of *Fusarium* species identification, however microconidia is absent in some species. The size and septation are the main features of microconidia, in which the septation is usually 0 or 1, and in some species, there are two septa. Microconidial shapes usually observed for identification are oval, reniform (kidney shape), obovoid (almost oval) with a truncate shaped, pyriform (pear-shaped), napiform (turnip-like) and globose (spherical) (Figure 2.10) (Booth, 1971; Nelson *et al*., 1983; Leslie and Summerell, 2006).

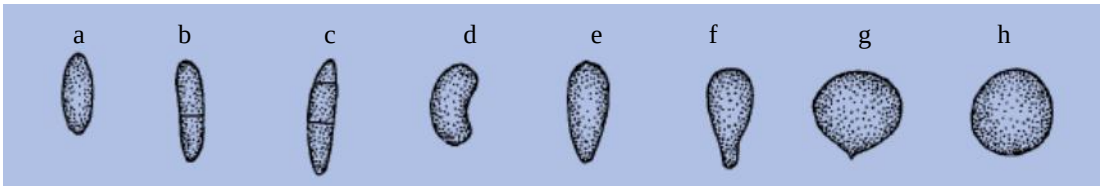

Figure 2.10: Various shapes of microconidia produced by conidiogenous cell found in *Fusarium* species (Leslie and Summerell, 2006)

The conidiogenous cells are divided into monophialides and polyphialides. Monophilaides have a single opening whereas polyphialides have multiple openings per cell. Species producing polyphialides may produce more monophialides compare to polyphialides thus, ample time is needed to detect the presence of polyphialides. Besides, the length of the phialides may vary from very short to very long. Microconidia are commonly arranged on the phialides in faise head or in chains (Figure 2.11) (Booth, 1971; Nelson *et al*., 1983; Leslie and Summerell, 2006).

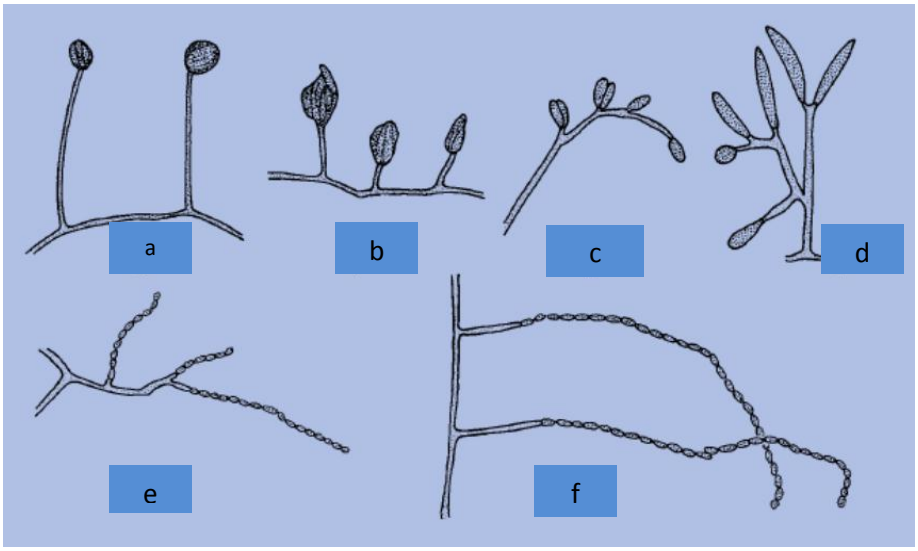

Figure 2.11: Conidiogenous cells commonly found in *Fusarium* spp. (a,b) monophialides with false heads (c,d) polyphialides with false heads (e, f) chains (Leslie and Summerell, 2006)

Chlamydospores are also an important criteria in *Fusarium* species descriptions, which may form singly, doubly, in clumps and in chains (Figure 2.12). The formation of chlamydospores usually take almost six weeks and feasibly observed on soil nutrient agar. Chlamydospores may be found in the aerial mycelia or embedded in the agar. Pseudochlamydospores and swollen hyphae may mistakenly identified as chlamydospores, however chlamydospore has a rough wall and high volume density whereas pseudochlamydospores and swollen hyphae have smooth wall with low volume density (Booth, 1971; Nelson *et al*., 1983; Leslie and Summerell, 2006).

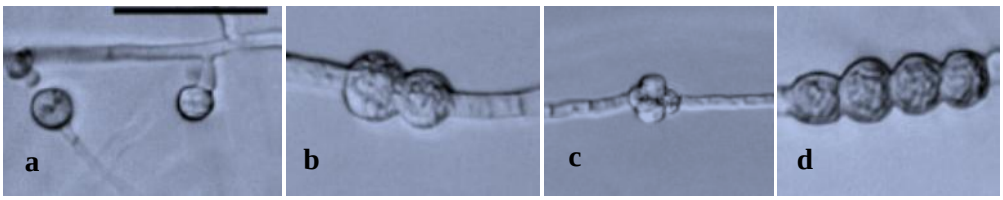

Figure 2.12: Various **c**hlamydospores formation of *Fusarium* (a) single (b) in-pairs (c) clump (d) chain (Leslie and Summerell, 2006)

Secondary characteristics such as pigmentation is also very much crucial for species descriptions. Cultures grown on Potato Dextrose Agar (PDA) showed various pigmentation among the species of *Fusarium*. The pigmentation usually observed after a week of incubation. Growth rate is also measured from a single spore incubated at 25°C and 30°C. The colony diamater is measured after three days of incubation (Leslie and Summerell, 2006).

2.8 Mycotoxins

Mycotoxins are secondary metabolite produced by fungi. Three genera commonly associated with the mycotoxins are *Aspergillus*, *Penicillium* and *Fusarium*. Mycotoxins have a detrimental effects on animals and humans. The most potent mycotoxins frequently reported are aflatoxin, achratoxin, deoxynivalenol, zearalenone and fumonisin which are commonly associated with many mycotoxins contamination worldwide (Marasas *et al*., 2008; Visconti and Perrone, 2008).

The most challenging aspect in mycotoxin issues in developing countries is the development of sustainable prevention and control strategies since the nature also plays its part in mycotxins productions. The food intake contaminated with mycotoxins will cause detrimental effects when highly accumulated in humans or animal body. Thus, food safety is a worldwide issue which need all the countries to gather and enacted legislation on mycotxins (Flannigan, 1991; Piñeiro, 2008).

2.8.1 Moniliformin (MON)

Moniliformin (MON) is a naturally occurring sodium or potassium salt of 1-hydroxycyclobut-1-ene-3,4-dione (Figure 2.13). MON was discovered by Cole *et al*. (1973) produced by a species called *F. moniliforme* Sheldon NRRL 5860 (=ATCC 26263) isolated from maize kernel in the United States. The isolate lost its ability to produce moniliformin during the course of a study to determine the structure and it was subsequently found that most isolates of *F. moniliforme* were either produce only small amounts of MON or did not produce MON (Marasas, 1988).

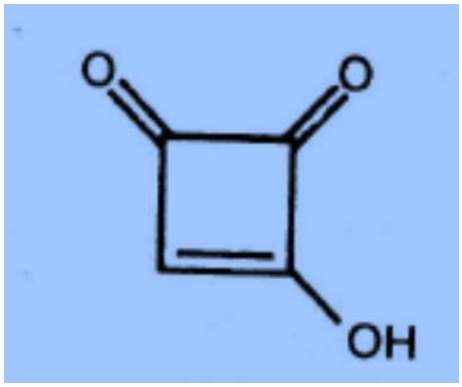

Figure 2.13: Chemical structure of moniliformin (MON) (Flannigan, 1991)

MON was initially isolated and chemically characterized by Springer *et al*. (1974) from *Gibberella fujikuroi* which was isolated from millet (*Pennisetum typhoides*) in Nigeria. In addition to *G. fujikuroi*, MON also had been found to be produced by *F. moniliforme* var. *subglutinans* and *F. sacchari* var. *subglutinans* which are common corn pathogen (Kriek *et al*., 1977; Steyn *et al*., 1978). In a study conducted in Illinois, United States, MON was recovered about 600 mg/kg from corn grit medium. The case study showed that the 50% lethal dose for chicken embryos was 2.8 µg per egg. For one day old chicks dosed with MON by crop intubation and