MORPHOLOGICAL IDENTIFICATION, MOLECULAR CHARACTERIZATION AND PATHOGENICITY OF *FUSARIUM* SPECIES ON CHILLI PEPPER IN TSUNAMI-AFFECTED AREAS, ACEH PROVINCE, INDONESIA

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I hope this thesis has great benefits for the knowledge generally in agriculture and especially in plant pathology, the pathogenic *Fusarium* species causing wilt and necrotic lesion disease symptoms on chilli pepper.

TABLE OF CONTENTS

CHAPTER 3 – MATERIALS AND METHODS 32

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µg/ml	Microgram per Mililiter
CLA	Carnation Leaf Agar
dai	Day after Inoculation
dNTPs	Deoxynucleotide Triphosphates
DSI	Disease Severity Index
G	Granule
g/l	Gram per Liter
kg/ha	Kilogram per Hectare
$MgCl_2$	Magnesium chloride
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
NADP	Nicotinamide adenine dinucleotide phospate
p	p value (<0.05)
PCNB	Pentachloronitrobenzene
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
PEP	Phosphoenolpyruvate
PPA	Peptone Pentachloronitrobenzene Agar
RAPD	Random Amplified Polymorphic DNA
rpm	Revolutions per minute
RuBP	Ribulose biphosphate
SL	Soluble
ssp.	Subspecies
u	Unit

PENGECAMAN MORFOLOGI, PENCIRIAN MOLEKULAR DAN KEPATOGENAN SPESIES *FUSARIUM* PADA CILI DI KAWASAN TERJEJAS TSUNAMI, PROPINSI ACEH, INDONESIA

ABSTRAK

Cili (*Capsicum annuum)* adalah tanaman sayur-sayuran bernilai tinggi di Aceh dan bahagian lain di Indonesia dan Malaysia. Buahnya digunakan secara meluas sebagai sayur-sayuran, rempah-ratus, dan ubat-ubatan di seluruh dunia. Terdapat beberapa penyakit penting dari segi ekonomi yang disebabkan oleh spesies *Fusarium* telah dilaporkan pada tanaman ini, iaitu lecur anak benih, layu, reput akar, reput batang, dan reput buah. Di dalam kawasan terjejas tsunami di Propinsi Aceh, dua daripada gejala penyakit tersebut sering kali diperhatikan, iaitu layu dan luka nekrosis yang dijangka disebabkan oleh spesies *Fusarium*. Dalam kajian ini, pencilan *Fusarium* yang berasosiasi dengan cili di kawasan terjejas tsunami (Aceh) dan kawasan bukan terjejas tsunami di Indonesia dan Semenanjung Malaysia (sebagai perbandingan) telah dipencil, dicam, dicirikan dan diuji kepatogenannya. Pengecaman ke peringkat spesies telah dijalankan berdasarkan ciri morfologi pada kultur yang ditumbuhkan pada PDA dan CLA, dan seterusnya dicirikan lagi melalui analisis RAPD. Sejumlah 503 pencilan *Fusarium* diperolehi dari puing tanah dan tanaman cili berpenyakit dari semua kawasan pensampelan telah dicamkan menjadi lapan spesies, *F. semitectum*, *F. solani*, *F. proliferatum*, *F. oxysporum*, *F. dimerum*, *F. equiseti*, *F. subglutinans,* dan *F. sterilihyphosum*. Perbezaan frekuensi taburan dan kepelbagaian spesies *Fusarium* yang jelas telah diperhatikan antara kawasan terjejas tsunami dan bukan terjejas tsunami. Walau bagaimanapun, untuk ciri morfologi dalam spesies, telah ditunjukkan bahawa ciri-ciri spesies adalah sama di kawasan terjejas dan bukan terjejas tsunami. Keputusan analisis kluster UPGMA dengan jelas menyokong adanya perbezaan strain *Fusarium* kepada lapan spesies

yang berbeza. Wakil strain setiap spesies telah diuji kepatogenannya pada anak benih cili yang sihat. Keputusan ujian kepatogenan yang dilakukan dengan menggunakan kaedah pindaan tanah dan ampaian spora menunjukkan bahawa *F. semitectum, F. solani, F. proliferatum, F. oxysporum, F. dimerum, F. subglutinans,* dan *F. equiseti* dari kawasan terjejas tsunami dan bukan terjejas tsunami adalah bersifat patogen secara signifikan ($P<0.05$) terhadap cili, kecuali *F. sterilihyphosum*. Gejala layu dan luka nekrosis yang disebabkan oleh strain patogenik yang diperhatikan adalah sama seperti yang terdapat di lapangan. Spesies *Fusarium* yang paling patogenik menjangkiti tanaman cili adalah *F. oxysporum, F. solani, F. proliferatum,* dan *F. subglutinans.* Kajian ini adalah laporan pertama spesies *Fusarium* yang berasosiasi dengan cili di Indonesia, iaitu *F. semitectum, F. proliferatum, F. dimerum, F. subglutinans, F. equiseti* dan *F. sterilihyphosum,* sedangkan di Semenanjung Malaysia adalah *F. oxysporum, F. proliferatum, F. dimerum, F. equiseti* dan *F. sterilihyphosum.* Juga disimpulkan bahawa tsunami tidak memberi kesan terhadap morfologi, kepatogenan dan kepelbagaian genetik spesies *Fusarium* yang menyebabkan layu dan luka nekrosis pada tanaman cili.

MORPHOLOGICAL IDENTIFICATION, MOLECULAR CHARACTERIZATION AND PATHOGENICITY OF *FUSARIUM* SPECIES ON CHILLI PEPPER IN TSUNAMI-AFFECTED AREAS, ACEH PROVINCE, INDONESIA

ABSTRACT

Chilli pepper (*Capsicum annuum*) is a high value vegetable crop in Aceh and other parts of Indonesia and Malaysia. The fruits are widely used as vegetables, spices, and medicines worldwide. There are several economically important diseases caused by *Fusarium* species have been reported on this crop, such as damping-off, wilt, root rot, stem rot, and fruit rot. In the tsunami-affected fields of Aceh Province, two of the disease symptoms were commonly observed, namely wilts and necrotic lesions that were suspected to be caused by *Fusarium* species. In this study, *Fusarium* isolates associated with chilli pepper in tsunami-affected areas (Aceh) and non-tsunami affected areas in Indonesia and Peninsular Malaysia (as a comparison) were isolated, identified, characterized and tested for pathogenicity. Identification into species level was conducted based on morphological characters on cultures grown on PDA (Potato Dextrose Agar) and CLA (Carnation Leaf Agar), and further characterized by RAPD analysis. A total of 503 isolates of *Fusarium* obtained from soil-debris and diseased chilli pepper plants from all sampling areas were identified into eight species, *F. semitectum*, *F. solani*, *F. proliferatum*, *F. oxysporum*, *F. dimerum*, *F. equiseti*, *F. subglutinans*, and *F. sterilihyphosum*. The difference frequency of distribution and diversity of *Fusarium* species were clearly observed between tsunami-affected and non-tsunami affected areas. However, for morphological characters within species, it was shown that the species characters were similar in both tsunami-affected and non-tsunami affected areas. Results of the UPGMA cluster analysis clearly supported the differentiation of *Fusarium* strains into distinct eight species. Representative strains of each species were tested for their pathogenicity on healthy chilli pepper seedlings. Results of pathogenicity tests by using soil amendment and spore suspension methods revealed that *F. semitectum, F. solani, F. proliferatum, F. oxysporum, F. dimerum, F. subglutinans,* and *F. equiseti* from tsunami-affected and non-tsunami affected areas were significantly ($P<0.05$) pathogenic to chilli pepper, except *F. sterilihyphosum.* Wilt and necrotic lesion symptoms caused by the pathogenic strains were similar to those observed in the field. The most pathogenic *Fusarium* species infecting the chilli pepper plants were *F. oxysporum, F. solani, F. proliferatum,* and *F. subglutinans.* This is the first report of *Fusarium* species associated with chilli pepper in Indonesia, namely *F. semitectum, F. proliferatum, F. dimerum, F. subglutinans, F. equiseti* and *F. sterilihyphosum,* while in Peninsular Malaysia were *F. oxysporum, F. proliferatum, F. dimerum, F. equiseti* and *F. sterilihyphosum*. Also, it is concluded that tsunami has not affect the morphology, pathogenicity and genetic diversity of *Fusarium* species causing wilt and necrotic lesion symptoms on chilli pepper plants.

CHAPTER 1

INTRODUCTION

In Aceh, most Acehnese's cuisines use chilli pepper among other spices, and therefore this crop is widely cultivated. Database from the Republic of Indonesia Ministry of Agriculture in 2012 (http://aplikasi.deptan.go.id/bdsp/ newkom-e.asp; 25th April 2012) showed that the plantation areas of chilli peppers in Aceh province before tsunami in 2004 were 9,681 ha with a production of 43,536 ton or 4% of total national production but after tsunami in 2009, the areas have markedly reduced to 7,266 ha with a production 34,820 ton, or merely 3.2%. The significant reduction in total chilli pepper production was due to the reduced plantation areas affected by tsunami and other factors such as pests and diseases.

Among fungal diseases infecting chilli pepper is *Fusarium,* causing damping-off, root rot, wilt diseases, and fruit rot. Damping-off and root rot diseases have been reported on chilli pepper in New Mexico (Goldberg, 1995); Bengkulu, Indonesia (Winarsih and Syafrudin, 2001); Assiut, Egypt (Abo-Elnaga and Ahmed, 2007); and Poland (Jamiołkowska, 2008), while Fusarium wilt affected chilli pepper in Egypt (Attia *et al.*, 2003), and Thailand (Wongpia and Lomthaisong, 2010). The latest report of fruit rot on chilli pepper was from Trinidad, West Indies (Rampersad and Teelucksingh, 2011). Nishikawa *et al.* (2006) and Tewksbury *et al.* (2008) found that *Fusarium* associated with diseases of chilli pepper were categorized as seed borne pathogens. Damping-off and wilt diseases are the main problems on chilli pepper cultivated in Aceh as well as in other areas in Indonesia and Malaysia. However, the *Fusarium* species associated with both diseases are not well documented, moreover in these two countries.

For *Fusarium* species identification, three different basic species concepts are commonly applied, namely morphological, biological and phylogenetic species concept. Morphological species concept of *Fusarium* is still widely used by numerous workers as some species can be identified by using morphological characters. Morphological species concept is based on observable morphological characters, such as conidia sizes and shapes, presence or absence of microconidia, macroconidia, presence of chlamydospores, growth rates, colony morphology and pigmentations, odours, and other observable characteristics (Leslie and Summerell, 2006). Moreover, publication manuals by Gerlach and Nirenberg (1982) and Nelson *et al.* (1983) are still widely used for morphological identification.

Identification by using morphological characteristics is not always easy as some *Fusarium* species shared similar characters. Moreover, morphological characters are easily changed depending on environmental conditions. Therefore, molecular characteristics are used to assist in identification and characterization (Yergeau *et al.*, 2005; McPherson and Møller, 2006; Allison, 2007). Among the molecular methods, random amplified polymorphic DNA (RAPD) which is a polymerase chain reaction (PCR) based technique is commonly used for characterization and to observe genetic diversity of *Fusarium* species (Abd-Elsalam *et al.*, 2004; Sabir, 2006; Lin *et al.*, 2009). RAPD is DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence (William *et al.*, 1990).

Although there are reports of pathogenic *Fusarium* species on chilli pepper, there is limited information on the occurrence of *Fusarium* on chilli pepper in Indonesia, particularly in Aceh and also Malaysia. Thus, the main objectives of the present study were:

1. To isolate and identify *Fusarium* spp. from diseased chilli peppers in tsunami-affected areas, in Aceh and to compare those from non-tsunami affected areas in Peninsular Malaysia and Indonesia.
2. To determine pathogenicity of the *Fusarium* spp. on chilli pepper to fulfill the Koch's postulates.
3. To characterize the genetic relationship of the *Fusarium* strains by using random amplified polymorphic DNA (RAPD).

CHAPTER 2

LITERATURE REVIEW

2.1 Chilli Pepper Plant

Based on the oldest archaeological evidence in the Tehuacán Valley of south-central Mexico, chilli pepper has been used as human diet for at least 7,200 years before the plant was domesticated. Birds were believed to be responsible in spreading the seeds of wild chilli peppers to other parts of America (DeWitt and Bosland, 2009). Eventually, the genus *Capsicum* has been known since pre-Columbian civilization and was domesticated among other crops in their agricultural activities. Five species of chilli peppers, namely *Capsicum annuum, C. baccatum, C. chinense, C. frutescens*, and *C. pubescens* were domesticated by the native people in different regions in North, Central and South America (Eshbaugh, 1993; Bosland, 1996; Bosland and Votava, 2012). Molecular study conducted by Walsh and Hoot (2001) showed the distribution patterns of genus *Capsicum* from dry region of the Andes uplands (Peru and Bolivia) subsequently migrated to the north or east, and into the tropical regions of South America.

Five hundred years ago, Christopher Columbus with his expedition to Americas discovered chilli pepper in the Caribbean and brought them to Europe (DeWitt and Bosland, 2009). In the 16th century, the distribution of chilli pepper spread to Africa when Portuguese colonized Brazil, and chilli pepper was introduced to Asia via European and African traders. Chilli pepper grows well in Africa and Asia, and becomes a major vegetable crop and spice in their cuisine, especially in India and China (Andrews, 1995; Bosland, 1999).

Berke and Shieh (2001) defined chilli as pungent variety of *Capsicum* species, primarily *C. annuum*, while pepper is a generic term describing the fruits of *Capsicum* species, both pungent and non-pungent. Many cultivated forms of chilli pepper have been developed that include several species and varieties, in particular *C. annuum* variety *annuum*. These cultivated forms comprised "grossum group" (sweet peppers) and the "longum group" (chilli peppers), and any of the *Capsicum* plants varying in sizes, colours, and pungency.

Names given to *Capsicum* fruits in the UK are pepper, sweet pepper, chilli/chillies and chilli pepper. *Capsicum* fruits in the US are referred to as red pepper, bell pepper, sweet pepper, paprika, *Capsicum*, chili or chile, chili or chile pepper (Derera, 2000; De, 2003). Common names given to *Capsicum* fruits in Indonesia are lombok, cabé, and cabai, while in Malaysia it is known as lada merah (De, 2003).

Chilli peppers can be divided into two groups, sweet peppers (Figures 2.1) and hot peppers (Figure 2.2). Sweet peppers are non-pungent, mostly of the bell type, large, smooth, thick-walled flesh, mild in flavour, while hot peppers are pungent, elongated, cylinder, thin-walled flesh and tapering (Bosland *et al.*, 1996; De, 2003; Brummel and Pathirana, 2007).

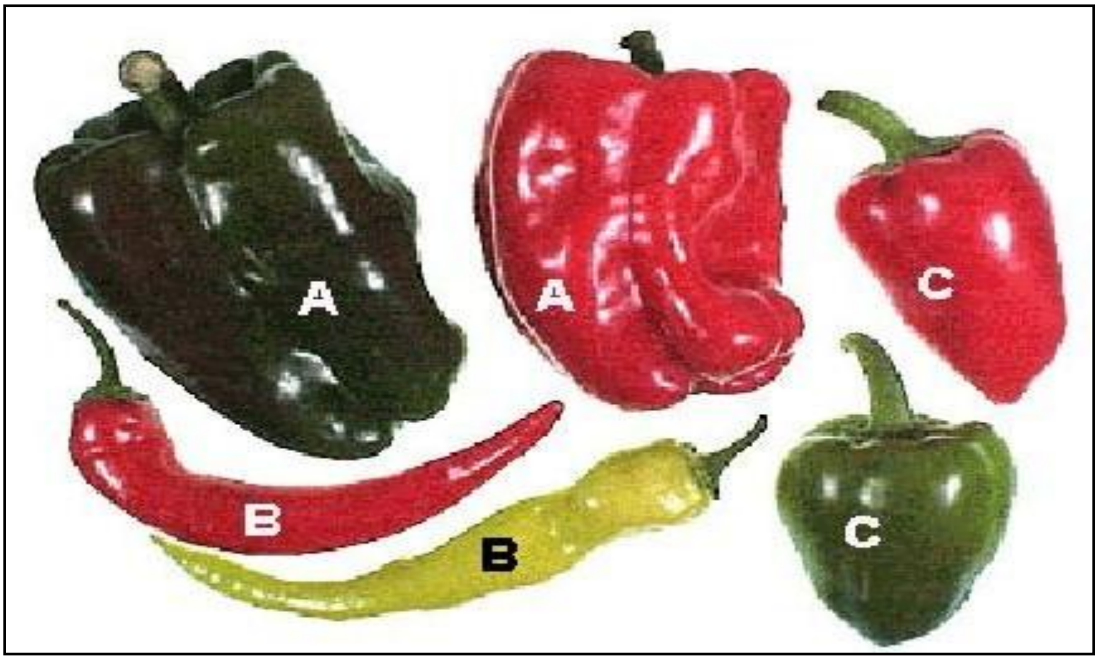

Figure 2.1 Sweet peppers: **A.** Bell pepper; **B.** Sweet banana; and **C.** Pimento (Source: *Armstrong*, 2000) (http://waynesword.palomar.edu/ww0401.htm; 10th October 2011).

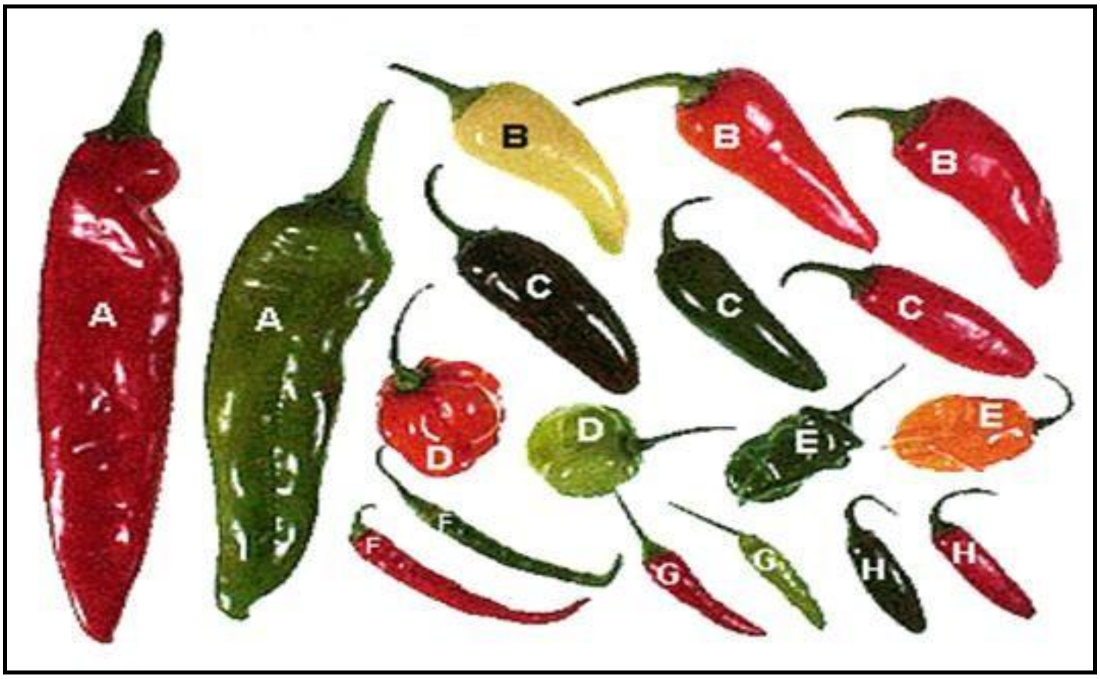

Figure 2.2 Hot peppers: **A.** Anaheim; **B.** Hungarian wax; **C.** Jalapeño; **D.** Caribbean; **E.** Habanero; **F.** Cayenne; **G.** Super chile; and **H.** Serrano (Source: *Armstrong*, 2000) (http://waynesword.palomar.edu/ww0401.htm; 10th October 2011).

According to plant database of United States Department of Agriculture (2012) (http://plants.usda.gov/classification.html; 24th April 2012), the classification of the genus *Capsicum* is as follows:

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Asteridae
Order	: Solanales
Family	: Solanaceae
Genus	: *Capsicum*
Species	: *annuum/ baccatum/ chinense,* etc.

Chilli pepper can grow as perennial and annual crops, and sometimes is also represents as shrubs and herbs but it is mostly cultivated as an annual crop all over the world. Most of chilli peppers are diploid with 24 chromosomes (2n = 24), and the flowers are complete with a calyx, corolla, male and female reproductive organs. All chilli peppers are both self-pollinating and cross-pollinating (DeWitt and Bosland, 2009; Bosland and Votava, 2012).

The cultivated chilli peppers usually grow less than 90 cm, the length of the leaf is 0.5 to 13 cm with ovate shape, the flower usually is pendant, and the colours of corolla are white or purple. The colours of immature fruits are green, yellow, white or purple, while the ripe fruits are red, orange, brown yellow, green or white.

The fruit can be globe-shaped, cone-shaped, elongated, and bell-shaped, with the size of the pods from 0.5 – 35 cm long (DeWitt and Bosland, 2009).

Chilli pepper is a source of vitamins A, B_1, B_2, B_3, C, and E (Bosland and Votava, 2012), carotenoids (Schweiggert *et al.,* 2007; Stummel and Bosland, 2007), and capsaicinoids. Capsaicinoids are the chemicals responsible for the well-known pungency of the chilli pepper (Ochoa-Alejo and Ramirez-Malagon, 2001; Tewksbury *et al.,* 2006). Capsaicinoids have antimicrobial properties (Molina-Torres *et al.*, 1999; Xing *et al.*, 2006), and the ability to protect chilli pepper seeds from infection by *Fusarium* (Tewksbury *et al.,* 2008).

2.2 Planting Technique

Chilli pepper is a warm weather crop, sensitive to low temperature at any growth stages. Optimum day temperatures of 20^o to 30^oC with night temperature about 24^oC are ideal for growth, and the tolerant temperature is about 32^oC. Temperature is a major factor for metabolism of chilli pepper plant. High temperature causes the rate of photosynthesis to decrease because the plant's enzymes such as RuBP carboxylase, PEP carboxylase, NADP-malate dehydrogenase, and NADP-G3P dehydrogenase are denatured (Kobayashi *et al.*, 1980; Wahid *et al*., 2007). Low temperature caused the rate of seed germination exceedingly slows. The temperature below 16^oC and above 32^oC caused the reduction of growth and yield, due to ineffective pollination and the fruit does not set. Chilli pepper is photoperiod-incentive plant, therefore day length does not influence the flowering and fruit set (Bosland and Votava, 2012; Berke *et al.,* 2005).

Chilli pepper is quite adaptable to grow in loam or silt loam soils with good drainage as well as in sandy soil. The soil pH from 5.5 to 6.8 is suitable for growth but if the soil is strongly acidic or alkaline it will inhibit the absorption of some essential nutrients as well as causing nutrients poisoning, that eventually stressing the growth of chilli pepper plant. The soil is selected and prepared well before planting by providing well drained by raising planting beds to provide good aeration of roots to minimize root diseases infection and flooding, well irrigated, and well fertilized by adding nutrients such as compost, animal manure, and green manure to the soil (Smith *et al.*, 1998; Berke *et al.*, 2005).

Chilli pepper proliferates by the seeds. The seeds are usually treated before sowing to avoid seed borne diseases. The chilli pepper seeds then sowed in seedling trays, which contain sterile sowing medium. It is grown in a greenhouse or a shading place, and protected with mesh netting to keep out the pests. After four to five weeks in the greenhouse the plants reach five-leaf stage and ready to be transplanted (Berke and Gniffke, 2006).

Chilli peppers are usually grown in double rows on raised beds about 30 cm high, with plastic mulch, and 1.5 – 1.8 m wide (furrow to furrow), and the rows are 55 cm apart, with 45 cm between plants within rows. Before transplanting, three or four granules of carbofuran (Furadan 5G) are placed in each hole to guard the seedlings against insect pests (Smith *et al.*, 1998; Berke *et al.*, 2005).

Chilli pepper plants are shallow-rooted, thus the plant cannot tolerate drought or flooding. Therefore, irrigation is important to manage the soil in moist conditions that are suitable for respiration and nutrients absorption around root areas to support the growth and fruit set of the plant. Furrow and drip irrigation are recommended to avoid the leaves and fruits from getting wet, which may stimulate the occurrence of diseases. The irrigation must be applied in the morning to avoid the leaves from desiccation (Berke *et al.*, 2005).

Chilli peppers required sufficient and balance fertilizers (Smith *et al.*, 1998). The amount of fertilizer depends on soil condition, soil organic matter, and requirement of the fertilizer N (Nitrogen), P (Phosphorus), and K (Potassium). Nutrient requirements for chilli pepper plant are as follows: N, 180 kg/ha, P, 22 kg/ha, and K, 200 kg/ha in which 40% of N and 50% of P and K are applied as basal fertilizer before transplanting. The remaining 60% of N is applied three times in equal amount at 2, 4, and 6 weeks after transplanting (WAT), and the remaining of P and K are applied at 4 WAT (Berke *et al.*, 2005). The excess or lack of nutrients can influence the chilli pepper plant growth, therefore the nutrient provided must be in the correct amount and in certain conditions, soil test is necessary.

Chilli peppers are harvested at green immature or red mature stages. The process of ripening of fruit takes about 55 – 60 days after flowering which varies, depending on the temperature, soil nutrient and variety of chilli pepper. Warmer temperatures and favourable conditions will hasten the ripening process. Chilli pepper can be harvested weekly for several months (Berke *et al.*, 2005; Berke and Gniffke, 2006). After harvest, fresh chilli pepper fruits are kept in cool conditions (10^{o}C), under shade and dry storage until sold (Smith *et al.*, 1998; Berke *et al.*, 2005).

2.3 Chilli Pepper Diseases

Diseases of chilli peppers are caused by biotic (infectious) and abiotic (non-infectious) factors which can reduce the production and quality of chilli pepper fruits. Biotic agents include fungi, bacteria, nematodes, and viruses, while abiotic disorders are sunlight, nutrient deficiency, and extreme temperatures (Snowdon, 2002). These diseases affect all parts of the chilli pepper plants including the foliage, stems, roots, fruits and young seedlings.

2.3.1 Diseases Caused by Fungi

Fungi are major pathogen of chilli pepper plants, causing a variety of diseases such as spotting, mildew, rotting, wilting and blight (Weiss, 2002; Koike *et al.*, 2007). Mildew generally showed the symptoms on the leaves, stems, and buds (Roberts *et al.*, 2004). Rot diseases usually infected the fruits, roots and young seedlings (Snowdon, 2002). Wilt showed yellowing of leaves, and finally the whole plant parts died (Weiss, 2002). Blight diseases usually showed brown to black lesion on the flowers, buds, and stems (Sherf and McNab, 1986).

Leaf spot diseases are caused by several fungi such as *Cercospora capsici*, *Stemphylium solani* and *S. lycopersici.* Infection of *C. capsici* on the leaves resulted in white, gray or tan circular spots, while the centre is surrounded by dark brown margin (Meon, 1990; Weiss, 2002). *Stemphylium solani* and *S. lycopersici* causing dark brown lesion, and later the lesion sunken with a light tan to white in the centre while surrounded by small lesion with reddish-brown margins, typically 3 to 5 mm in diameter (Koike *et al.*, 2007). Leaf spotting diseases infected chilli pepper plant at warm and wet conditions. Prevention of the spotting diseases infection can be done by using resistant cultivar and crop rotation. Good drainage and fungicides also can minimize the infection of the diseases (Goldberg, 1995).

Downy mildew on chilli pepper caused by *Peronospora tabacina* Adam, and powdery mildew, caused by *Leveillula taurica* (Lev) Arnaud. Downy mildew symptoms are observed on the underside of the leaves whereby the sporulated fungi penetrate the stomata and can be seen as white to grayish spots (Sherf and McNab, 1986; Roberts *et al.*, 2004). Powdery mildew produced powdery white spots on the leaf, stem and bud tissues, the leaf then curl upward, while on the top surface of the leaf showed yellow lesion with the brown necrotic centre (Bosland and Votava, 2012). The pathogens commonly infected chilli pepper during favourable conditions of the plant growth. Severe infection in the early season can reduce the productivity of the yield. Sanitation and chemical spraying are commonly used to control the diseases (Goldberg, 1995; Goldberg, 2004).

Common rot disease on chilli pepper fruits is anthracnose, caused by several species *Colletotrichum,* such as *C. capsici, C. coccodes,* and *C. gloeosporioides* (Koike *et al.,* 2007, Than *et al.,* 2008). Other fruit rot diseases are caused by *Alternaria alternate, A. solani*, *Botryotinia fuckeliana*, *Sclerotinia sclerotiorum*, *Cladosporium* spp., *Fulvia fulva* and *Pythium debaryanum*. The symptoms usually observed are lesion on chilli pepper fruits, covered by the mould and later the fruits become rotten (Snowdon, 2002). Fruit rot diseases commonly infect chilli pepper plant during rainy season and high humidity. Resistant cultivar, crop rotation and fungicides applied are several ways to manage the diseases (Goldberg, 1995).

Damping off and root rot which are seedling diseases are caused by several fungi including *Rhizoctonia solani* (Bosland and Votava, 2012), *Pythium* spp., *Phytophthora* spp. (Weiss, 2002; Sanogo and Carpenter, 2006), *Fusarium solani* (Snowdon, 2002) and *F. oxysporum* (Suryanto *et al.,* 2012). The symptoms depend on the growth stages of the chilli pepper plant, usually infected seedlings and the roots becoming brown and rotten. Seedling diseases infected chilli pepper plant during wet season after planting. To prevent seedling diseases, choosing high quality cultivar is recommended and applying seed treatment before sowing (Goldberg, 1995).

Wilt disease is commonly caused by *Fusarium solani* (Weiss, 2002), *F. oxysporum* (Sherf and McNab, 1986), *F. oxysporum* f. sp. *capsici* (Suprapta and Khalimi, 2012), *Sclerotium rolfsii* (Bosland and Votava, 2012), *Phytophthora capsici* and *Verticillium dahliae* (Sanogo and Carpenter, 2006). The symptoms usually showed yellowing and wilting of the leaves, later the infected stems become wilt and eventually died (Weiss, 2002). The fungi causing wilt diseases are commonly soil borne pathogens. The aggressiveness of the pathogens can cause various symptoms on the plant. Wilt disease is controlled by applying crop rotation and soil fumigation (Goldberg, 1995).

Blight disease on chilli pepper is not common, and it is caused by *Choanephora cucurbitarum.* Blight disease usually infected the plant at the blooming time. The disease symptoms showed water-soaked lesion on the leaves and the margins, then the leaf tips become blighted, flowers and buds turn brown to black and wilt, and sometime the young fruits can be infected (Chupp, 1994; Roberts *et al.,* 2004). The fungi has a wide host range. Severe infection of this diseases occur during extended rainy season and high temperatures. Blight disease is controlled by applying cultural practices such as increasing plant spacing, adequate drainage, irrigation and nutrition (Kucharek *et al.,* 2003).

2.3.2 Diseases Caused by Bacteria

Bacteria can infect the whole part of chilli pepper plants such as the leaves, stems and fruits, from the field until storage. Bacterial infections caused soft rot, spotting, wilt and canker, and are more severe during wet season and high humidity.

Bacterial soft rot is a post-harvest disease, caused by *Erwinia carotovora, E. carotovora* ssp. *atroseptica* and some strains of *Xanthomonas campestris.* The disease symptoms observed are lesions on the fruit that appeared water-soaked, and then the fruit is rapidly rotten (Snowdon, 2002). High moisture condition or wound created by insects on the fruits caused the bacterial infection. Several control methods are used to reduce bacteria soft rot infection such as controlling the insect population, avoid the injury of the fruits during storage and kept the fruits in cold storage (Goldberg, 1995).

Bacterial spot is caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye. The disease symptoms shown are small, circular, water-soaked spots on the leaves, stems, and fruits. These spots are then turn brown and usually about 5 mm in diameter, while in favourable condition, the spots may coalesce causing leaf blight (Koike *et al.*, 2007; Persley *et al.*, 2010). Bacterial spot is severe during wet season. To manage the disease, several methods are applied such as using resistant cultivars, crop rotation, soil sterilization, and using chemical control with a cooper-based spray (Goldberg, 1995).

Bacterial wilt (southern bacterial wilt) commonly caused by *Ralstonia [Pseudomonas] solanacearum*, showing leaf wilt from the youngest leaf, later the vascular system discoloured with a pale yellow to dark brown colour (Weiss, 2002). The pathogen is soil borne that widely distributed and has a wide host range (James *et al.*, 2003). The bacteria can persist in non-favourable conditions (Alvarez *et al.*, 2010). The disease usually is controlled by using chemicals and cultural practices, while biological control using commercial antagonist microorganisms such as effective microorganisms (EM) instant, EM preserved with vinegar and distilled ethyl alcohol (EM5), EM fermented plant extract (EM-FPE), EM fermented compost (Bokashi), and *Bacillus subtilis* can reduce the disease symptoms (Lwin and Ranamukhaarachchi, 2006).

Bacterial canker with bird's-eye spot is caused by *Clavibacter michiganensis* ssp. *michiganensis* (Smith). The symptoms on the fruits showed mature lesions being small scabby spot that surrounding by a white halo. The pathogen is dispersed by seeds and can persist on the weed hosts and debris. The disease is controlled by using pathogen-free seed, seed treatment, and sanitation (Snowdon, 2002).

2.3.3 Diseases Caused by Viruses

There are eight genera of viruses associated with chilli pepper that caused several diseases that transmitted through insects and mites, and have several weeds as hosts. The symptoms of viral infection are mosaic, leaf curl, chlorotic, necrotic, defoliation, and stunt. The viruses that infected chilli pepper plant are *Potyvirus, Tobamovirus, Topvirus, Cucumovirus, Begomovirus, Curtovirus, Alfamovirus,* and *Tombusvirus.* Viral diseases on chilli pepper plants are serious problem worldwide and no treatment can control the diseases, except to prevent the infection before chilli pepper plants are planted (Roberts *et al.,* 2004; Koike *et al.,* 2007).

The members of the *Potyvirus* are *Pepper mottle virus* (PeMoV), *Tobacco etch virus* (TEV) and *Potato virus Y* (PVY). The symptom of diseases caused by Potyvirus depends on chilli pepper cultivars and viral strains, generally showed mosaic or mottle on the leaves and dark green vein-banding (Sherf and McNab, 1986, Weiss, 2002). This genus distributed in North and Central America, Puerto Rico, India and Japan. The disease is transmitted by aphids from infected weeds in the chilli pepper plantation area. The disease can be controlled by using resistant cultivars, growing chilli pepper in insect-free greenhouse, chemical insecticides, and sanitation (Anon., 2009).

Tobacco mosaic virus (TMV), *Tomato mosaic virus* (ToMV) and *Pepper mild mottle virus* (PMMoV) are members of *Tobamovirus*. Infection by *Tobamovirus* shows symptoms such as chlorotic mosaic, leaf distortion, systemic necrosis and defoliation (Caciagli, 2009). The virus distributed worldwide and significantly reduces the yield (Martinez-Ochoa *et al.,* 2003). Prevention of the diseases infection can be conducted by eliminating weeds and infected plants (Goldberg, 1995).

Topvirus include *Tomato spotted wilt virus* (TSWV), which produced symptoms of stunted and hampered the fruit set at the early stage of infection, while in later stage showed chlorotic or necrotic rings on leaves and stems (Koike *et al.*, 2007; Caciagli, 2009). The disease has several hosts such as ornamental plants and weeds, and therefore is controlled by removing infected plants and weed hosts, and crop rotation (Goldberg, 1995).

Cucumber mosaic virus (CMV) is included in *Cucumovirus* and caused young chilli pepper plant to produce yellowing and narrowing of leaf, necrotic on the foliage and the fruit. CMV is one of the most important viral pathogens of chilli pepper worldwide (Sherf and McNab, 1986; Varma and Mandal, 2003; Koike *et al.*, 2007). Crop rotation and elimination of alternative hosts are common methods to control the disease (Goldberg, 1995).

Sinaloa tomato leaf curl virus (STLCV) from family *Geminiviridae* caused curl leaf and mild yellow mosaic virus disease on chilli pepper worldwide (Koike *et al.*, 2007), particularly in Aceh, and it is a serious problem. The disease is controlled by eliminating all infected plants and crop rotation with non-host plants (Goldberg, 1995).

Member of *Curtovirus* genus is *Beet curly top virus* (BCTV) which caused the leaves curl while the petioles curve downward. In the early stage the plants are stunted and chlorotic, later the plants produce only a few distorted fruits that ripen prematurely. BCTV is an important viral pathogen of chilli pepper worldwide which comprises several strains and infected a wide host range (Koike *et al.,* 2007). Controlling the disease can be done by crop rotation, and removing weeds and infected plants to reduce infection in the field (Goldberg, 1995).

Alfalfa mosaic virus (AMV) is an *Alfamovirus* and produced striking yellow or white blotches or mosaic leaf symptoms (Caciagli, 2009). The disease infect chilli pepper plant due to chilli pepper is planted after alfalfa in rotation. Therefore to control the disease is by avoiding chilli pepper planted in the same location as alfalfa plantation (Goldberg, 1995).

Tombusvirus which included *Tomato bushy stunt virus* (TBSV) stunting of the plants, mottling and deformation of the leaves, and no fruit set (Green and Kim, 1991). The virus is distributed in the USA, Europe, and North Africa and has various host plants (Green and Kim, 1994). The pathogen is soil borne transmitted by the seed, pollen, mechanical, vegetative propagation, grafting, and vectors such as fungi, thrips, and beetle (Lommel and Sit, 2009). The disease is managed by avoiding planting in the land that has been infected by TBSV and crop rotation every four years may help to reduce the infection (Davis *et al.,* 2008) (http://www.ipm.ucdavis.edu/PMG/r783102411.html; 15th August 2012).

2.3.4 Diseases Caused by Nematodes

Another pathogenic agent that caused several diseases to chilli pepper plant is nematode, which infected the roots. Nematodes invasion often interact with bacterial and fungal infection. Chilli pepper diseases caused by nematodes are root knot, sting, stubby and lesion diseases.

Root knot disease caused by *Meloidogyne* spp. showed the symptoms of wilting seedlings and defoliation with severe root galling (Sikora and Fernandez, 2005; Bridge and Starr, 2007). *Meloidogyne* spp. commonly recognized as major pathogen of solanaceous crops in temperate areas. Management of root knot disease is conducted by crop rotation, nematodes-free seedlings, and resistant cultivars (Bridge and Starr, 2007).

Sting disease caused by *Belonolaimus* spp. showed the symptoms of the damaged root that reduced the ability to absorb water and nutrients from the soil, and can cause chronic wilting of the plant (Sherf and McNab, 1986; Sikora and Fernandez, 2005). *Belonolaimus* spp. as pathogens on chilli pepper usually found in sandy soil and distributed in Americas and Australia (Crow and Brammer, 2001). Cultural practices, soil solarization, non-fumigant nematicides (Noling, 2009), and cover crop such as *Crotalaria* are used to control the nematode (Wang *et al.,* 2002).

Stubby disease caused by *Trichodorus* spp. suppresses the elongation of the root and produced stubby root, sometime become discoloured and necrotic. The stubby root nematodes have a wide host range and also known as important vectors of viral diseases. To reduce nematodes densities, several methods are applied such as ploughing, using cover crop (*Crotalaria spectabilis*), and applying non-fumigant nematicides such as carbamate and phosphate (Sikora and Fernandez, 2005).

Pratylenchus spp. produced lesion on the root cells and caused root necrosis (Sherf and McNab, 1986). *Pratylenchus* spp. are considered as important nematodes that associated with soil borne root rot fungi, causing severe damage on the root of chilli pepper plant. The nematodes can infect a wide range of vegetable crops, thus to manage the disease, short-term sanitation of fallow combined with root destruction between successive vegetable crops are recommended. Crop rotation, fumigant and non-fumigant nematicides sometime are ineffective to control the disease (Sikora and Fernandez, 2005).

False root-knot disease caused by *Nacobbus aberrans,* with the formation of galls along the roots which causing necrosis, stunting, poor growth and chlorosis of the plants (Sikora and Fernandez, 2005). The disease has been reported on chilli pepper in Mexico causing economic losses. The disease is widely distributed in Americas and it has infected various plants including weeds (Scurrah *et al.*, 2005). False root-knot is commonly controlled by using biological agent namely *Paecilomyces lilacinus*, crop rotation, resistant cultivars, mulching, organic amendments and soil solarization (Manzanilla-Lopez *et al.*, 2002).

2.3.5 Abiotic Disorders

Abiotic disorder is caused by non-living environmental conditions such as nutrient deficiency, extreme temperature, sunlight and water excess. Blossom end rot, chilling injury, sunscald and flooding are among abiotic disorders of chilli pepper plant.

Blossom end rot is a physiological disorder associated with a low concentration of calcium in the fruit. Water-soaked spots appeared on the blossom end of fruits, then turned to light brown and papery (Sherf and McNab, 1986; Snowdon, 2002; Weiss, 2002). The disorder can be controlled by adding calcium salt into the soil and frequent irrigation to ensure the availability of water during early fruit set (Snowdon, 2002).

Chilling injury occurs on chilli pepper fruit stored at 0 to 2^{o}C for 12 to 15 days. The disease symptoms are pitting and loss of firmness which are shown on the surface of the fruit, and usually associated with *Alternaria* rot and *Cladosporium* rot (Sherf and McNab, 1986; Snowdon, 2002). To avoid chilling injury, chilli pepper fruits are stored at 7 to 10^{o}C, and kept moist by sealing the fruits in plastic film (Snowdon, 2002).

Sunscald of chilli pepper occurs on parts of the fruit that are directly exposed to sunlight. The disorder showed the symptoms of soft spots, light-coloured, sometime slightly wrinkled, later becoming sunken, white and papery (Snowdon, 2002; Weiss, 2002). To control the disease, it is recommended to provide shade for chilli pepper plant and choosing cultivar with bushy leaf to provide cover for the fruits set (Snowdon, 2002).

Flooding on chilli pepper plantation areas occurs during extended rainy season that caused excessive soil moisture and reduce oxygen availability to the roots. The disorder symptoms showed discolored or water-soaked on the roots and finally the plant become wilt. The disease infection more severe caused by fungal invasion such as *Phytophthora* spp. The disorder can be controlled by managing good drainage of chilli pepper plantation areas and raising high beds to avoid the roots become rotten during flooding (Kennelly *et al.*, 2012) (https://www.apsnet.org/edcenter/intropp/PathogenGroups/Pages/Abiotic.aspx; 25th May 2013).

2.4 Chilli Pepper Plant Disease Management

Chilli pepper diseases management are conducted by using cultural, mechanical, biological, and chemical controls to allow the plants to stay healthy and increased yield (Roberts *et al*., 2004). The disease management is conducted before harvest and after harvest.

Cultural control include selection of healthy seedlings, growth condition, crop rotation to avoid continuously planted chilli pepper with another solanaceous crops, and pathogen-free soil with well ploughing, well-drained, proper irrigation, suitable soil pH, and proportionate or balance fertilization (Agrios, 2005; Burgess *et al.*, 2008). Cultural control is common method used for disease management on chilli pepper cultivation, and also can reduce the soil borne diseases infection.

Mechanical control is carried out by sanitation, soil sterilization, and the usage of mulching. Sanitation can reduce infection from the infected plants and weeds by burning the infected plants and manual weeding. Soil sterilization is conducted by heating the soil to sterilize the sowing medium to get healthy seedlings. The usage of mulching can keep the soil moist and cool which protect the plant against soil borne pathogens as well as weeds (Berke *et al.*, 2005; Burgess *et al.*, 2008).

Biological control to manage soil borne diseases of chilli pepper plant can be conducted by using compost that containing beneficial microbes such as antagonist fungi of *Trichoderma* and *Gliocladium,* or antagonist bacteria, *Pseudomonas fluorescens* and *Burkholderia cepacia* (Agrios, 2005). Pests as disease vectors can be managed by using natural enemy such as predator, parasite, and entomopathogen against insects and other pests, e.g. *Macrolophus pygmaeus* as nymph predator of whitefly (*Bemisia tabaci)* and *Stethorus punctillum* as mite predator (Perdikis *et al.*, 2008).

Chemical control by using fungicides, viricides, bactericides, and nematicides is used for seed treatment and to manage diseases by various pathogens. Many chemical products are applied to chilli pepper plants such as 100 µg/ml dimethomorph and 1,000 µg/ml fluazinam which control *Phytophthora capsici* (Matheron and Porchas, 2000) (http://ag.arizona.edu/pubs/crops/az1177/; 18th August 2012), malathion is good for controlling insect pests, methyl bromide is a good fungicide to control wilt diseases caused by *Fusarium*, *Rhizoctonia*, *Verticillium*, *Pythium*, and *Phytophthora*, and also effective to control root knot nematodes (Anon., 2004), and imidacloprid 17.8 SL (0.003%) is effective to manage insects caused leaf curl disease (Pandey *et al.*, 2010). Chemical control on chilli pepper plant can only be applied after identifying the causal agents of the plant diseases, and following the instruction carefully (Weiss, 2002; Berke *et al.*, 2005).

After harvesting of chilli pepper fruits, the storage and transportation conditions are critical to reduce the damage on the fruits. During storage, many fungal diseases can infect the fruits. Diseases on the field can be transferred to the storage and caused fruit rot. Controlling post-harvest diseases during storage and transportation can be conducted by sorting fresh chilli pepper fruits and remove diseased fruits, fumigation of the storage using the gases such as oxygen, carbon dioxide and nitrogen to manipulate the atmosphere of the storage, and storing the fresh chilli pepper fruits at 7 – 10^{o}C with humidity of 85 – 95% for 2 – 3 weeks. For long distance transportation, the fresh chilli pepper fruits are packed in waxed furrowed cardboard in refrigerated trucks. The storage conditions of fresh and dried chilli pepper can affect the pungency and colour of the fruits. Thus, dried chilli pepper is kept in sealed containers in the dark and cool conditions, after which dried chilli pepper is packed in boxes or corrugated cartons before transporting to the market (Weiss, 2002).

2.5 Biology of *Fusarium*

Fusarium is one of the most important genus of fungi that caused several types of diseases on agricultural crops, and some species are mycotoxin producer which affect humans and animals which can lead to economic losses. *Fusarium* species existed in natural and agricultural habitats and some species occurred depending on the climatic conditions and geographic regions. The climate could influence the diversity of the *Fusarium* species and can be used to predict diseases that caused by certain *Fusarium* species (Leslie and Summerell, 2006).

The genus *Fusarium* is known as saprophytic, endophytic, and parasitic fungi that distributed worldwide, in tropical, subtropical, cool and warm temperate regions. *Fusarium* species has a widespread distribution in natural soil, cultivated soil, organic materials, persist as chlamydospores or as hyphae in plant residues, and even some species can produce airborne conidia that colonized plant parts (Booth, 1971; Burgess *et al.*, 1994; Leslie and Summerell, 2006).

Saprophytic *Fusarium* commonly occurs in the rhizosphere soil and colonize diseased root and stem. Association between saprophytic and pathogenic strains of *Fusarium* is difficult to determine, therefore pathogenicity test should be conducted to determine pathogenic or saprophytic strains. Saprophytic *Fusarium* species have many applications such as can be used as an antagonist against plant pathogens, as biological control agents to control plant diseases, and some species are useful for bioremediation especially in areas contaminated with cyanides or nitriles. Several saprophytic species produced dimethylsulfoniopropionate lyase activity that can degrade leaf litter in salt-water marshes, and pectinase activity that can decompose flax as fibre crops by separating the fibres from pectin to produce textile fibres (Leslie and Summerell, 2006).

Endophytic fungi commonly colonized healthy plant tissues without causing any disease symptoms (Schulz and Boyle, 2006). *Fusarium* species as an endophyte can produce metabolite with antimicrobial and anti-canker activity. For example, *F. oxysporum* that associated with rhizomes of medicinal plant *Acorus calamus* produce antimicrobial activity against several fungal pathogens, Gram positive and Gram negative bacteria (Barik *et al.*, 2010).

Parasitic *Fusarium* species can cause several types of diseases on humans, animals and plants. Human pathogenic *Fusarium* can cause allergic to human, skin lesions, capable to infect cancer and HIV patients systemically (Nucci and Anaissie, 2007). On animals, *F. solani* has been reported to be pathogenic to crayfish, dogs, some insects, sharks, snakes and turtles. *Fusarium* is among the major plant pathogenic fungi and caused various types of diseases such as bakanae disease, damping off, dieback (Leslie and Summerell, 2006), stem splitting (Burgess *et al.*, 2008), vascular wilting, canker, and rotting on economically important crops (Leslie and Summerell, 2006; Burgess *et al.*, 2008).

Fusarium is one of the major storage fungi that contaminated food, feed, and seed that produced several types of mycotoxins such as fumonisin, beauvericin, fusaproliferin, fusaric acid, fusarins, and moniliformin which are very harmful to humans and animals. *Fusarium culmorum* produced several types of mycotoxin such as moniliformin, deoxynivalenol, trichotechenes, fusarin C, and zearalenone which are toxic to domesticated animals and experimental animals. *Fusarium fujikuroi* produced moniliformin which affected animal feeds and *F. proliferatum* produced moniliformin that contaminated grain and caused death, hemorrhage, and diarrhea to experimental rats. *Fusarium solani* is often associated with sweet potato toxins i.e. furanoterpenoids causing pneumonia to humans and animals. *Fusarium*