CHAPTER II

LITERATURE REVIEW

2.1 Lactic acid bacteria

Lactic acid bacteria are generally the group consists of Gram-positive bacteria, cocci or rods that are non-sporulating, non-respiring and produce lactic acid as the major end product during fermentation of carbohydrates. These bacteria were first described as milk-souring organisms, due to the sour milk that arose from their production of lactic acid. LAB isolates are comparatively different group of bacteria, however, they have a number of typical metabolic and physiological features. Previously, the core group involves the genera *Pediococcus, Streptococcus, Leuconostoc* and *Lactobacillus*, but the major LAB genera important in food-technology also comprise *Carnobacterium Aerococcus, Oenococcus, Lactococcus, Tetragenococcus, Vagococcus* and *Weisella* (Stiles & Holzapfel, 1997; Sabia et al., 2004). Soil-borne LAB is obtained from soil and the rhizosphere of different plant species and moderately easy to isolate. The soil-borne LAB can be a novel source for LAB strains (Matthias et al., 2012). For identification of LAB using the API system that was good system to identify rapidly more than seventy nine strains were satisfactorily identified using API system (Bill et al., 1992; Klinger, 1992). Based on fermentation of hexoses under non-limited growth conditions, LAB strains are divided into two categories: homo-fermentative and hetero fermentative

The glycolysis (Embden-Meyerhof-Parnas) pathway of LAB is considered as homo-fermentative and produce lactic acid as the main end product. Other category produces lactic acid, carbon dioxide and ethanol (or acetic acid) as the major end products in the 6-phosphogluconate/ phosphoketolase pathway (6-PG/PK), which is known as hetero fermentative. Some species are regarded as facultative hetero-fermenters due to their production of certain fermentation products under certain conditions such as available carbon source is a pentose. However, homo-fermentative and hetero-fermentative LAB cannot be exclusively distinguished because both categories can stimulate 6-phosphogluconate/ phosphoketolase pathway, resultant in heterolactic fermentation (Axelsson, 2004).

2.2 Lactic acid bacteria have ability to control pathogenic fungi

Lactic acid bacteria (LAB) are easily isolated from fermented foods, but limited study has been reported on the isolation of LAB in soil that can be used as biocontrol of soil borne phyto-fungi (Kohl et al., 2011). The microorganisms LAB produce a variety of antimicrobial compounds and effective substances such as lactic acid, acetic acid, propionic acid, antibiotics, bacteriocins as well as hydrogen peroxide and carbon dioxide (Ouwehand, 1998; Trias et al., 2008; Samuel et al., 2014). The use of LAB is not limited to the production of fermented foods, but it can also be used as biocontrol agents in plants. Several *in vitro* studies have reported that LAB has potential application as biocontrol against phytopathogenic fungi (Stephane et al., 2005; Ashgar & Mohammad 2010; El-Mabrouk et al., 2012; Rosalia et al., 2008 & Wang et al., 2011).

Certain microbes known as plant growth promoting bacteria (PGPB) such as LAB can have multi-functions, both as biocontrol and plant growth regulator (Hamed et al., 2011). Most of the physical and chemical methods that applied for the detoxification of agricultural products contaminated with mycotoxins are restricted because of problems concerning safety issues, feasible losses in the nutritional quality of treated commodities, coupled with limited efficacy and cost implication (Kohl et al., 2011). Therefore, LAB with their evidence as antifungal agent (Muhialdin & Zaiton, 2011) are considered as one of the most prominent non-pathogenic strains that can be used as biocontrol agent against fungi. The thesis reports on the new finding related to the *in vitro* study of LAB isolated from soil and other fermented foods used as a biocontrol agent agaist *Fusarium* species.

2.3 Factors influencing the inhibitory activity of LAB

2.3.1 Effect of heat treatment on inhibitory activity of LAB metabolites

Based on previous studies, Magnusson et al. (2003) and Mataragas et al. (2003) have reported that environmentally isolates LAB are excellent source for active metabolite to control different pathogenic bacteria and fungi due to different types of compounds which are produced by LAB such as organic acids, hydrogen peroxide, cyclic dipeptides, phenolic and proteinaceous compounds which could be responsible for the antifungal activity.

Effect of heat on inhibitory activity of LAB substances could be decreased or increased after heating treatment. It was observed that heating the Cell Free Supernatant (CFS) of

Lb. brevis at 60°C was effective against *A. furmigatus*, *F. solani, F. acuminatum,* and *F. fujikuroi* (Samuel et al., 2014). The LAB compounds antifungal activity was affected either decreased or lost the antifungal activity after heat treatment (Samuel et al., 2014). In other study, the LAB-CFS of LAB isolates *Lactococcus, Lactobacillus, Entorococcus, Streptococcus, Leuconostoc* and *Pediococcus* species metabolites was heat-stable either decreased or increased of heating temperatures (Assefa et al., 2008). In other study, *Lb. pentosus* G004 and *Lb. fermentum* Te007 supernatant heated at low temparature (90°C) antifungal activity reduced against *A. niger* and the antifungal activity of *P. pentosaceus* Te010 supernatant was destroyed at high temparature (121°C). However, the antifungal activity of the isolates *Lb. fermentum* Te007 and *Lb. pentosus* G004 increased when the supernatant were heated high temparature (121°C) (Muhialdin et al., 2011). Similarly, heating effect on compounds of *Lb. plantarum* LB20 and *Lb. plantarum* LB54 was not affect the inhibitory activity against *Aspergillus* spp. and *Fusarium roseum* (Laref & Guessas, 2013).

2.3.2 Effect of pH on inhibitory activity of LAB metabolites

Cell Free Supernatant of LAB produce organic acids and also activate other antifungal compounds such as peptides by lowering the pH and the metabolites show the antifungal activity against fungi is depending on pH treatments. The antifungal substances were most active at low pH 2 and the pH value between 3.0 and 4 was found to be stable of peptide, however, the antifungal activity quickly decreased between pH 4.5 and 6.0 (Magnusson and Schnurer 2001). In contrast, the antifungal activity of *Lb. plantarum*

LB52 and *Lb. plantarum* LB20 strains remained active at pH 6.0 and 7.0 (Laref & Guessas, 2013). Therefore, the antifungal activity of LAB-CFS did not simply due to undissociated organic acids, but also by dissociated with organic acids which are presence in LAB supernatants (Laref and Guessas 2013). In other study, De Muynck et al. (2004) recommended that the neutralisation of the supernatant of *Lb. acidophilus* LMG 9433, *Lb. amylovorus* DSM 20532, *Lb. brevis* LMG 6906 and *Lb. coryniformis* subsp. *coryniformis* LMG 9196 isolates to pH values of 5.0, 5.5 and 6.0 indeed dismissed the antifungal activity against fungi.

2.3.3 Effect of enzymes on inhibitory activity of LAB substances

Lactic acid bacteria (LAB) exert a strong antagonistic activity with many food-contaminating microorganisms due to production of organic acids, hydrogen peroxide, diacetyl, inhibitory enzymes and bacteriocins (Atta et al. 2009). Inhibitory activity of substances can be changed after reacting with enzymes. Proteolysis of antifungal compounds was determined by agar diffusion assay of trypsin enzyme treated with LAB extracts where the antifungal activity was almost completely lost by trypsin digestion against *Aspergillus parasiticus* CBS 971, *Aspergillus versicolor* CBS 117286, and *Penicillium bialowiezense* CBS 110102 (Rossana et al., 2011).

The obtained results revealed that the inhibitory effectiveness of bacteriocins was higher on Gram-positive bacteria than Gram-negative bacteria. Further, the largest inhibition zone was obtainedby *Lb. acidophilus* bacteriocin against *Bacillus subtilis* while

the smallest one was against *E.coli.* The extracted bacteriocin exhibited broad spectrum of inhibition at concentration 6400AU/mL against *Staph aureus*, *Bacillus subtilis* and *E.coli.* The antimicrobial activity of crude supernatant fluid was stable after heating at 100°C for 30 min and declined thereafter. Stability of antimicrobial activity was observed at pH ranged from 2.0 to 8.0. Its active principle was proteinaceous in nature since the bacteriocin was inactivated by proteinase K enzyme (Atta et al., 2009).

The effect of proteolytic enzymes on *Lb. plantarum* and *Lb. farciminis* were evaluated with the chymotrypsin and pepsin enzymes. The results showed stronger effects on inhibitory activity against *Penicillium* species, *Fusarium roseum*, *Stemphylium* spp. and *Trichoderma* spp. rather than *Aspergillus* species (Laref & Guessa, 2013). Ndagano et al. (2011) reported that the treatment of supernatant by proteolytic enzymes (pronase, pepsine. Proteinase K and α chymotrypsin) showed that inhibitory activity of CFS of LAB against pathogenic strains was not affected by the enzyme treatments. However, the concentrated supernatant of *Lb. plantarum* treated with proteinase K essentially reduced their antifungal activity (Rouse et al., 2008). Similarly, Mauch et al. (2010) and Guo et al. (2011) showed that the proteolytic treatment reduced the antifungal activity of *Lb. brevis* and *Lb. reuteri* R2. Currently, Marwa et al. (2015) demonstrated the stability of the bacteriocin extracted from *Lb. acidophilus* treated proteinase K enzyme noticed that inhibitory effectiveness of bacteriocins was higher on Gram-positive bacteria than Gram-negative bacteria. Because of, the largest inhibition zone was obtained by *Lb. acidophilus* bacteriocin against *Bacillus subtilis* while the smallest one was against *E.coli.* The extracted bacteriocin exhibited broad spectrum of inhibition at concentration 6400AU/mL

against *Staph aureus*, *Bacillus subtilis* and *E.coli*. Its active principle was proteinaceous in nature since the bacteriocin was inactivated by proteinase K enzyme.

2.4 Important antimicrobial metabolites produced by LAB

Microorganisms such as LAB are non-pathogenic and easily can grow in anaerobic conditions; have showed ability to produce organic acids during fermentation of carbohydrates (Hammes & Hertel, 2003; Hammes & Tichaczek, 1994; Asmahan, 2010). The human dietary enrichment could be increased by LAB to development of a wide diversity of flavours, aromas and textures during the food fermentation process and, enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids and vitamin (Caplice & Fitzgerald, 1999). For food and feed storage bio-preservation by microorganisms; such as LAB are recognised for long time and they are known to produce different antimicrobial compounds that are able to control pathogenic, spoilage bacteria, undesirable spoilage yeast and spoilage fungi (Dalie et al. 2009; Messens, 2002; Lindgren & Dobrogosz, 1990). The detail description of LAB compounds are described in the following:

2.4.1 Organic acids

Lactic acid bactria produce variety of antimicrobial organic compounds such as lactic and acetic acids, ethanol and diacetyl (Gould, 1992; De Vuyst & Vandamme, 1994; Holzapfel et al., 1995) and these microorganisms have been used in food and feed preservation for

centuries. During the fermentation systems of the raw materials from food sources, organic acids are the main product of LAB fermentation. Depending on the strain of LAB, useful acids produced by LAB are lactic acid and acetic acid, beside other acids (El-Ziney, 1998) and mechanism of organic acid against antimicrobial is described by Piard & Desmazeaud (1991). The inhibition activity of LAB against the growth of pathogenic microorganisms is utmost likely due to the production of organic acids and bacteriocins (De Vos, 1993; Klaenhammer, 1993). Corsetti et al. (1998) observed that *Fusarium, Penicillium, Aspergillus* and *Monilia* were subdued by a mixture of acetic, formic, propionic, caproic, butyric and n-valeric acids. These compounds were detected from obligate hetero-fermentative *Lactobacillus* species and *Lb. sanfrancisco* CB1 was the largest antifungal spectrum.

2.4.2 Carbon dioxide

Heterofermenters LAB were recognised to produce carbon dioxide (CO_2) and the activity of CO_2 creates anaerobic condition and replaces the existent molecular oxygen in the products. It also showed antimicrobial activity and this compound important in the vegetable fermentation to prevent the growth of spoilage fungi (Lindgren & Dobrogosz, 1990). It provides protection against common fruit spoilage organisms such as *Botrytis, Rhizopus* and *Penicillum.* According to Clark and Takles (1980) and Bliekstad et al. (1981), concentrations of CO_2 between 20 and 50% showed strong antifungal activity.

2.4.3 Hydrogen peroxide

Most of the LAB were showed inability to produce hydrogen peroxide (H_2O_2) when oxygen is available (Kandler, 1983) and anti-pathogenic ability of LAB were examined against pathogenic microorganisms based on agar diffusion and broth dilution methods. Furthermore, growth of fungi, *Penicillium expansum* was inhibited entirely by using the concentration of 5% H_2O_2 solution on agar diffusion assay (Venturini et al., 2002). Ponts (2006) reported that the rate of the spore germination of *F. graminearum* may be affected by H_2O_2. Recommendation by previous researchers was to prove that small quantity of hydrogen peroxide is an alternative for fungicides inhibition against *P. expansum*. According to Condon (1987) and Magnusson (2003), the mode of action of hydrogen peroxide was well studied. The effect of hydrogen peroxide as strong oxidizing agent is mainly on the bacterial cell and destruction of basic molecular structures of cellular proteins.

2.4.4 Hydroxyl fatty acids

Many LAB strains could produce antimicrobial fatty acids that improve the sensory quality of fermented products (Earnshaw, 1992). Caproic acid isolated from *Lb. sanfrancisco* CB1 was by far the main powerful antifungal substance produced by this isolate (Corsetti et al., 1998). This compound could act in synergy with other acids such as butyric, propionic, and valeric acids. In addition, the occurrence of hydroxylated fatty acids (C3) with a great deal of fungal inhibitory activity has been described by (Sjogren et

al., 2003). These fatty acids, the most vigorous was shown to possess a 12-carbon atom chain length. Hydroxylated fatty acid compounds present a very broad inhibition spectrum and are efficient against yeasts and moulds.

2.4.5 Lactic acid

Lactic acid is the major metabolite compounds of LAB fermentation where it is in equilibrium with its undissociated and dissociated forms, and the extent of the dissociation depends on pH. At low pH, a large quantity of lactic acid is in the un-dissociated form and it is toxic to many fungi, bacteria, and yeasts. However, different microorganisms vary considerably in their sensitivity to lactic acid. At pH 5.0, lactic acid was inhibitory toward spore-forming bacteria however, it was ineffective against yeasts and moulds (Woolford, 1975).

In other study, it was potential to grow *Aspergillus parasiticus* NRRL 2999 in a medium containing 0.5 or 0.75% lactic acid at pH 3.5 or 4.5 (El-Gazzar et al. 1987). In different study by Lindgren and Dobrogosz (1990), at different pH ranges, the minimum inhibitory concentration (MIC) of the undissociated lactic acid was varied against *Clostridium tyrobutyricum*, *Enterobacter* species and *Propionibacterium freudenreichii* ssp. *shermanii*. Furthermore, the stereo isomers of lactic acid also vary in antimicrobial activity; L-lactic acid is strong inhibitory than the D-isomer (Benthin & Villadsen, 1995).

2.4.6 Diacetyl

Diacetyl production was observed in the end product of *P. pentosaceus* INT02 fermentation could make a good defensive system. This strain produced a relatively higher concentration of antimicrobial substances than other strains. Production of a relatively high diacetyl concentration has been observed to contribute significantly to exertion of antagonism by *Pediococcus* against most unwanted organisms (Jyoti et al., 2003; Jay, 1982; Lanciotti et al., 2003). The LAB were isolated and identified from Nigerian beef. The LAB was observed to produce diacetyl in the early stage of incubation by the *Pediococcus* strains which observed the maximum production of the antimicrobial substances between 15-20 h (Olaoye & Onilude, 2011).

2.4.7 Phenolic compounds

Natural phenolic compounds are low molecular weight naturally occurring organic compounds which contains one or more phenolic group. They are naturally produced by plants and microorganisms (Abadet al., 2007). There is only a study has dealt with the involvement of a phenolic compound in the antifungal activity of LAB (Mandal et al., 2007). This phenolic compound, which remains to be identified, was produced by *P. acidilactici* LAB 5 and showed variable degrees of antifungal activity against a number of plant pathogenic fungi and food-borne moulds.

2.4.8 Antifungal proteinaceous compounds

A novel proteinaceous compound was isolated from *Lb. plantarum* VTT E78076 that was inhibitory against *Fusarium avenaceum* (Niku-Paavola et al., 1999). Okkers et al. (1999) reported that purified and characterized a peptide TV35b from *Lb. pentosus* with antifungal effect against *Candida albicans* species. The strains *Lb. coryniformis* sub sp. *coryniformis* produces different kinds of antifungal dipeptides such as cyclic specifically, cyclo (Phe-OH-Pro) and cyclo (Phe-Pro) that were mostly effective to the reduction of mass growth of *Aspergillus* species (Strom et al., 2002; Magnusson, 2003). The antifungal dipeptides cyclo (Phe- OH-Pro) and cyclo (Phe-Pro) are highly heat stable based on an estimated molecular weight 3 kDa (Roy et al., 1996; Magnusson and Schnürer 2001). Some *Lactobacillus* species produce specific compounds that were tested for antifungal properties (Stiles et al., 1999; Plockova et al., 1999, 2001).

2.4.9 Phyto-hormones and nutritional substances production by LAB

2.4.9.1 Phyto-hormones or plant regulators

The various crops are nowadays well established by applying beneficial effects of variety of soil microorganisms. These microorganisms may promote plant growth directly by providing nutrients or growth factors or indirectly by antagonizing soil-borne phyto-pathogens through secondary metabolites (Okon & Hardar, 1987; Davison, 1988). The

bacteria were isolated from rhizospheric soil of crops have potential to promote phyto-hormone and identified as plant growth-promoting rhizobacteria.

These rhizobacteria are responsible to enhance the growth of the plant with two actions either directly or indirectly (Kloepper et al., 1980). The direct mechanisms involve phosphorus, nitrogen fixation and solubilisation production. The indirect mechanism is to suppress the fungal infection with the help of producing antimicrobial metabolites on plants. It was observed that the production of phyto-hormones such as auxins, gibberellins and cytokinins could lower the ethylene concentration (Kloepper et al. 1989; Glick and Ibid 1995; Glick et al., 1999). During the pot trials with LAB I and LAB II applied as seed treatment and soil drench were revealed their ability to enhance the growth of plant (Narasimha et al., 2012). The LAB also produced indole acetic acid (IAA) in the presence of different concentrations of tryptophan and the highest amount of IAA was produced by LAB isolate of KLF01 (Anupama et al., 2014).

2.4.9.2 Nutritional substances or bio-fertilisers

Microorganism *Lb. plantarum* strains isolated during fermentation of the wild forest and fruits improved the availability of extra plant nutrients such as Cu, Zn and Fe (Duangporn et al., 2009). Availability of extra plant nutrients were reported by Prachyakij et al. (2008) in a fermented seaweed beverage.

2.4.9.3 Phyto-hormones interaction in plants

Kloepper and Schroth (1981) reported that plant growth promoting rhizobacteria (PGPR) mediated plant growth promotion by the alteration of the most microbial community in rhizosphere niche through the production of a variety of substances. Bacteria in soil rhizosphere namely, *Bacillus* species, *Pseudomonas* species, *Azotobacter* spp. and *Rhizobium* species are reported responsible to generate indole acetic acid (IAA), ammonia production (Joseph et al., 2007). Other researchers reported *Pseudomonas fluorescens* induced systemic resistance, antifungal activity in crops (Saravanakumar et al., 2007); *P. chlororaphis* showed inhibitory activity against pathogenic microorganisms (Liu et al., 2007); *Azotobacter chroococcum* produced gibberellins, kinetin, IAA (Verma et al., 2001), and *Lb. plantarum* strain produced plant nutrients such as Cu, Zn and Fe (Prachyakij et al., 2008). Generally, the PGPR promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of different pathogens on plant growth and development in the forms of biocontrol agents (Glick, 2012). It may be possible the presence of LAB MSS and FF11 in the soil and in the plants as endophytic modulate plant growth regulator. However, this was studied in this experiment and therefore, needs to be further elucidated.

2.5 Antifungal activity of LAB

Various studies reported the antifungal activity of LAB from *Lb. casei* (Gourama, 1997), *Lb. pentosus* (Okkers et al., 1999), *Lb. coryniformis* sub sp. *coryniformis* (Magnusson and Schnurer 2001), *Lb. lactis* sub sp. *lactis* (Roy et al., 1996) and *Lb. plantarum* (Niku-Paavola et al., 1999; Strom et al., 2002; Lavermicocca et al., 2003). Suzuki et al., (1991) reported inhibitory activity of *Leuconostoc mesenteroides* sp. that used in cheese to have anti-mould activity, but the compound was not isolated and identified. Earlier report from Batish et al. (1989) observed the antifungal activity of *S. lactis* sub sp. *diacetilactis* DRC1 against spectrum range of fungi and suggested the activity to be feasibly due to proteinaceous compounds. Isolates of LAB produce numerous compounds that compounds recognised as more effective antifungal compounds against harmful pathogenic fungi.

2.6 Inhibition of mycotoxins biosynthesis with Lactic acid bacteria

Most of publications dealing with the inhibition of mycotoxin biosynthesis by LAB have been focusing on aflatoxins (Thyagaraja & Hosono, 1994). At the time of fungi cells are lysed, it is possible that LAB release molecules that potentially prevent the growth of mould, therefore, it leads to a lower accumulation of their mycotoxins (Gourama & Bullarman, 1995). These anti myco-toxicogenic metabolites can also be produced during the growth of LAB. Report from (Gourama, 1991) showed using a dialysis assay, the occurrence of a metabolite that inhibits the aflatoxin accumulation in *Lactobacillus* cell

free extracts. It is suggested that this inhibition of aflatoxin biosynthesis is not the result of a hydrogen peroxide production or pH decrease (Karunarane et al., 1990). These findings are found to be reliable with report by Gouraman (1990) who showed that the inhibition of aflatoxin biosynthesis by *Lactobacillus* cell free supernatants (CFS) are probably due to the specific bacterial metabolites.

Coallier Ascah & Idziak (1985) reported a significant decrease of aflatoxin biosynthesis by *Lactobacillus* CFS and even though they suggested that this inhibition is related to a heat-stable of low molecular weight inhibitory compounds. Although *Lactobacillus* is initiated to delay aflatoxin biosynthesis, other LAB isolates such as *Lactobacillus lactis* are also found to stimulate aflatoxin accumulation (Luchese & Harigan, 1990).

2.7 Mechanisms of the inhibitory compounds produced by LAB strains

Antimicrobial compounds produced by LAB have been proved by many researchers, however, mechanisms and interactions of different antimicrobial compounds are not yet completely understood, particularly on the description of existing of bacterial organic acids on their inhibitory activity against pathogenic microorganisms. Generally, the effect of organic acids on inhibitory activity against microorganism is associated to the pH value. In the case of dilute solutions, pH is directly interrelated to the concentration of hydrogen ions. The release of hydrogen ions from an acid is dependent on the strength of the acid. Acids produced from fermenting bacteria are weak organic acids, such as

propionic acid, acetic acid and lactic acid (Helena, 2010). These types of acids only relatively release their hydrogen ions in the pH range of foods. The plasma membrane of most microorganisms restricts penetration by charged molecules. But, undissociated molecules can simply diffuse (Stratford, 1999).

Different microorganisms have shown different response to the weak acid. The action of lactic acid and other organic acids with some other mechanism is not clear, meanwhile, it is not linked to the decrease in internal pH and degree of lactic acid inhibition (Freese et al., 1973). The mode of action of bacteriocins produced by LAB is the formation of holes in the membrane and damage to the target pathogenic microorganisms' cells, causing leakage and damage of the trans-membrane potential (Moll et al., 1996; Todorov, 2009). An innovative plasmid with antimicrobial activity protein digalacticin produced by *Streptococcus* sub sp. *equisimilis* also interrupts the membrane and inhibits glucose uptake process (Swe et al., 2009). Most of the mechanisms of bacteriocins are related to forming of pore on the surface pathogenic microorganism's membranes which damages or pore forming on cells membranes resulted to the leakage of the cytoplasm (Jack et al., 1995; Jose et al., 2007). Furthermore, bacteriocins produced from *Escherichia coli* with molecular weight of 40 to 80 was found to cause nuclease/pore-forming which lead to the damage of cells membranes via forming pore, thus leakage of nuclease content causing the death of microorganism (Margaret & John, 2002).

2.8 Pathogenic fungi

The genus *Fusarium* link comprises inhabitants of the soil and of organic substrate and it is widely distributed throughout the world (Burgess, 1981). Genus *Fusarium* was firstly introduced (Link, 1809) and it is notorious for harbouring a variety of phytopathogenic fungal species (Zhang et al., 2012). Many soil fungi, this genus is capable with several means of survival, amongst which is its hasty capacity for change, both physiological and morphological, when faced with environmental changes (Booth, 1971). Many pathogenic isolates are involved in the *F. oxysporum* Schlechtend Fr. These can be subdivided into *formae speciales*, characterized by their ability to cause ailments in specific hosts, and in race, agreeing to their reaction with a group of differentiating cultivars (Gordon and Martyn, 1997). The development of biomolecular method such as Polymerase Chain Reaction or PCR made precise and reliable identification of causal organisms possible. The PCR assay used here is suitable for detection of *F. oxysporum*. Using the primers, a specific band at 700 bp was obtained by PCR for nine isolates. Edel et al. (1995) used a number of molecular techniques (RFLP, ERIC-PCR, REP-PCR) in the identification of *F. oxysporum* species.

2.8.1 Mycotoxin compound produce by phytopathogenic fungi

Numerous species of the genus *Fusarium* are known as phyto-pathogens for causing serious plant diseases on many economically important plants worldwide. Reasons of that genus *Fusarium* also produce harmful secondary metabolites known as mycotoxins in

food and feeds (Desjardins, 2006). Pathogenic fungi produce several mycotoxins compounds these are caused economic losses and responsible for increased many diseases which affect to human health. Filamentous fungi, mainly *Fusarium* species, *Aspergillus* species, and *Penicillium* species are responsible to produce mycotoxins in suitable environmental conditions which are toxic secondary metabolites (Bennett & Klich, 2003).

Most of the species of *Fusarium* are recognized that four specific types of mycotoxins produced namely zearalenone (ZEN), moniliformin (MON), fumonisin B1 (FB1) and beauvericin (BEA) (Leslie et al., 2004; Logrieco et al., 2002; Sopterean and Puia, 2012). It is potential health risks on both animals and humans that have evoked universal concern over food safety. Therefore, several research works have been focused on the toxicology of *Fusarium* mycotoxins (Lee et al., 2010; Negedu et al., 2011; Tan et al., 2012; (Dacasto et al., 1995). Mycotoxin ZEN has always been suggested to cause infertility among mammals, feminization in males and mammary hypertrophy in females and with swine being the most susceptible animal towards this toxin (Smith et al., 1994).

Some studies have been demonstrated moniliformin (MON) is toxic, causing muscular weakness, distress, coma, respiratory and even lead to fatality in tested animals (Engelhardt et al., 1989; Ledoux et al., 1995). Reports from Jonsson et al. (2012) and Sharma et al. (2012) showed that the heart is the main target tissue of moniliformin (MON) toxicity in rats and avian. Among series of fumonisins, FB1 is the most prominent and frequently found in foods and feeds. Phytopathogenic strains of *Fusarium* like *F. semitectum, F. solani, F. equiseti, F. compactum, F. subglutinans, F. sacchari, F.*

oxysporum, F. proliferatum, F. chlamydosporum, F. lateritium, F. incarnatum-equiseti species complexes. *F. nelsonii* isolated from different species of asymptomatic grasses or non-cultivated grasses Malaysian samples in Peninsular Malaysia and these strains have ability to produce four major types of mycotoxins, i.e. moniliformin (MON), fumonisin B_1 (FB_1), zearalenone (ZEN), beauvericin (BEA) and one strain represented *F. incarnatum-equiseti* species complex was able to produce moniliformin (MON). The results of this study may indicate a potential health risk for ruminants that feed on these grasses and consequently for humans who consume these animals as source of protein (Azliza et al., 2014).

2.8.2 Fungal pathogenicity incidence on seeds

The disease is responsible for losses of just as in the soil, the presence of *Fusarium* in cowpea seeds does not essentially result in transmission of ailment. The fungi can remain endophytic in the seeds as dormant mycelium or chlamydo-spores without causing infection (Menezes, 1988). According to Champion (1997), many fungi considered to be saprophytes, pathogens or others that lost their ability to cause disease. These fungi can survive in latency inside of the seeds before they become active again when those seeds are germinated. The pathogenic forms may result in pre or post-emergence damping off (Agarwal & Sinclair, 1997).

Phytopathogens were isolated from plants *Capsicum frutescens* mainly *Fusarium moniliformae* Sheldon, *Colletotrichum capsici*, *Curvularia lunata* (Wakker) Boedijn, and

Alternaria tenuis Nes ex Pers. Based on Asalmol et al. (2001), they reported that the seed borne fungi in the chilli seeds with causative agent of *Fusarium entmoniliformae, Aspergillus flavus, Rhizopus stolonifer, Colletotrichum capsici* and *Aspergillus niger.* Severity of *Fusarium* spp. infection is mostly observed in the seeds, where more than 211 isolates were obtained from cowpea seeds. Isolates were identified in the following relative frequency: *F. semitectum* (47.39%), *F. equiseti* (22.27%), *F. oxysporum* (16.59%), *F. solani* (3.79%), *F. anthophilum* (2.37%), *F. sporotrichioides* (1.42%), *F. moniliforme* (0.95%), and *Fusarium* species (Antonia & Maria, 2006). Reports from many country with the prevalence of pathogenic fungi were isolated from seed samples of chilli such as *Fusarium moniliformae, F. solani* (Mart.) Sacc., *F. equesiti* (Gorda) Sacc., *F. oxysporum* Schl. Emend Sny and Hans, *Alternaria alternata* (Fr.) (Keissler, 1989).

Chilli seeds were collected from diseased fruits which without surface sterilized has yielded most severity of fungi isolates of *Fusarium, Colletotrichum capsici* and *Alternaria* Nees ex Fr. However, from apparently healthy fruits, severity were observed associated with *Alternaria*, *Aspergillus flavus*, *Fusarium* species and most possibility of *C. capsici* (Padaganur & Naik, 1991). Seeds samples of different chilli cultivars were showed the strains of *F. moniliformae*, *C. capsici*, *A. niger, A. flavus* Linkex Fries, *Alternaria alternate* and *Curvularia lunata.* The germination rate of bean, red gram, green gram and black gram seeds had strongly reduced when seeds treated by culture filtrated of the toxic fungal strains (Janardhan et al., 2011). On the other hand, microorganisms such as LAB, *Lb. fermentum* and *Lb. rhamnosus* assayed showed growth inhibition of the mycotoxin producing *Aspergillus* strain (Munoz et al., 2010).

2.8.3 Fungal pathogenicity incidence on plants

The phytopathogenic species affect a wide range of hosts and cause root rot, vascular wilting, yellowing and foliar necrosis (Ramachandran et al., 1982; Nelson & Hansen, 1997). There are pathogenic and non–pathogenic forms. The latter can colonize the cortex of roots of plants without causing any symptoms of disease (Appel & Gordon, 1994) and survive in living tissue as also exercising antagonism interaction between the pathogenic forms in the soil (Edel et al., 1997).

In the culture of cowpea, one of the chief diseases is *Fusarium* Wilt, caused by *F. oxysporum* schl. f. sp. *tracheiphilum* and symptoms in the plants begin with a small change in the colouring of the older leaves, from green to yellow, on one of the sides of the leaves or of the plant. When the contamination progresses, defoliation occurs as does the darkening of the vessels and later death of the plants (Kendrick, 1931; Kendrick & Snyder, 1942). Maize ear rot disease reduced the maize production in Kenya and this disease is caused by most of fungi that belongs to several genera which include *Fusarium* spp., *Stenocarpell*a spp., *Penicillium* species and *Aspergillus* spp. (Kedera et al., 1992, 1998; MacDonald & Chapman, 1997).

Significant losses were caused by fungal infections in many economical crops in worldwide (Agrios, 2005). The phytopathogenic fungi *F. oxysporum* schlecht cause leaf spots *Fusarium* wilt diseases on a wide variety of agricultural crops (Yaqub & Shahzad, 2005; Abdel-Fattah et al., 2011; Alwathnani & Perveen 2012). Most fungal diseases

controlled by chemicals are effectively and extensively used for reduction of phyto-pathogenic fungi. But, the use of chemical fungicides may not always be desirable. Excessive and improper use of these fungicides presents a danger to the health of humans, animals, and the environment. Therefore, extensive needs for bio-fungicides that are environmentally safe and easily biodegradable have been carried out during the last two decades (Gnanamanickam, 2002). When plants are contaminated by *Fusarium* species, they produce fumonisins which could cause physiological damage on plants such as growth inhibition and death in plants (Abbas & Ocamb, 1995). *Fusarium* species are found to be associated with maize several phytopathogenic *Fusarium* species such as *F. proliferatum*, *F. verticillioides*, *F. anthophilum* and *F. graminearum* (Scott, 1993; Munkvold & Desjardins, 1997).

2.8.4 Types of fungi pathogenicity and their effects on plant

Wilt disease is an economically significant plant-pathogenic disease which is caused by *F. oxysporum* in worldwide and this fungal disease infected the plants parts, such as in vascular tissues and causes wilting of plant (Rai et al., 2011). Fungi *F. oxysporum* is a complex species and different host specific individual within this complex is termed as *formae specialize* and abbreviated as "f. sp." (Beckman, 1987). The fungi *F. equiseti* is a cosmopolitan fungus spread across regions with cool through to hot and arid climates (Leslie & Summerell, 2006). Simple method such as crop rotation is not much effective on pathogenic infections. Use of resistant crop varieties is a good strategy against wilt, but their use is limited due to the location specific pathogen races (Singh et al., 2006).

The fungi *F. solani* can be distinguished into 50 sub-specific lineages and most of them have not been further pronounced formally (O'Donnell, 2000). The species is among the well-known plant pathogen, causing various types of infections on a wide range of plants. There are at least 111 plant species from 87 genera that are generally infected by *F. solani* (Kolattukudy & Gamble, 1995). *Fusarium* species are ubiquitous in soils and are considered as field fungi invading more than 50% of maize grains before harvest and these species cause to root rot pathogens, attacking on beans at all growth stages and also cause damping-off at the seedling stage, yellowing of the leaves, stunted growth, and death when the population of pathogens in highly in soil (Robledo, 1991).

Cereals are the dietary main in most temperate regions. Inappropriately, they can become colonised by *Fusarium,* often resulting in severe crop disease, strongly reduced yields, and the accumulation of secondary metabolites toxic to humans and animals. *Fusarium* head blight (FHB) of small grain cereals is an ailment complex that includes several *Fusarium* species producing basically hazy symptoms. The species mostly associated with FHB in Europe are *F. graminearum, F. avenaceum* and *F. poae* (Somma et al., 2010; Xu et al., 2008). Climate change scenarios predict variations in water availability and increasing temperatures could encourage changes in the profile of FHB species on cereals. Meanwhile, each species has a characteristic of mycotoxin profile, thus, the risk of mycotoxin contamination of cereals may also be increased (Miraglia et al., 2009). The some pathogenic fungi *Fusarium* species were found to infected specific crop as mentioned in the following sections.

2.8.4.1 *Fusarium oxysporum* f. sp. *lycopersici*

Fungi such as *F. oxysporum* f. sp. *lycopersici* was caused vascular wilt in plant (Krishnan et al., 2009; Mishra & Das, 2003). Tomato plant, an economically important crop which is severely damaged by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), is mainly known as pyto-pathogenic fungi of Solanacae crops (Suarez et al., 2007). Tomato yield and plants are significantly losses by *F. oxysporum* f. sp. *lycopersici* because it can destroy roots of tomatoes at the growth stages, thus the productivity of plant's growth was affected. Eventhough numerous strategies have been implemented, the attack of this pathogenic fungi was remained a major issue (Ahmed, 2011; Biondi et al., 2004).

2.8.4.2 *Fusarium solani*

The *F. solani* was recovered from the roots of chilli plant which naturally infected the plants and *Fusarium* wilt disease (Hussain et al., 2013). Bean production decrease because of root rot ailment is caused by *F. solani* f. sp. *phaseoli*, *Rhizoctonia solani*, and *Pythium* species in Kenya especially where soil fertility is low and bean production is intensive (Otsyula et al., 1998; CIAT 1992). Phytopathogenic fungi *F. solani* f sp. *phaseoli*, *Rhizoctonia solani*, and *Pythium* species are responsible to root rot disease in the primarily plant (Nderitu et al., 1997).

2.8.4.3 *Fusarium acuminatum*

Fungi *F. acuminatum* is widely spread around the world, although chiefly in temperate regions. It behaves as a soil saprophyte, however, it is also found associated with the crowns and roots of plants (Leslie & Summerell, 2006; Pitt & Hocking, 2009). Its presence has recently been reported in Southern Europe, predominantly Spain (Marin, 2010). Fungi *F acuminatum* has been testified to produce trace levels of trichothecene toxins such as monoacetoxyscirpenol (MAS), diacetoxy-scirpenol (DAS), HT-2 toxin (HT-2) and neosolaniol (NEO) (Adejumo et al., 2007; Wing et al., 1994). Furthermore, it produces moniliformin and enniatin B, steroids (Leslie & Summerell, 2006).

2. 8.4.4 *Fusarium proliferatum*

Mohammed et al. (2014) reported that more than 62% of *F. proliferatum* was isolated from date palm trees tissues, which cause damaged to the date trees in Iraq. Many *Fusarium* spp. isolated from bakanae infected rice plant samples that are identified as *F. proliferatum.* All isolates were found to be pathogenic, but there was variation in the degree of infection observed among the isolates and this is the first report of *F. proliferatum* as the causal agent of bakanae disease in Malaysia (Quazi et al., 2013). *F. proliferatum* is an endophytic fungus was isolated from inner stem bark of *Dysoxylum bi-nectariferum* (Patel et al., 2011). Fungi induced to sporulate by inoculating mycelia on the surface of autoclaved green gram seeds (Shweta et al., 2010) and the fungus produced

only conidia and not ascospores. The isolate was inferred to be *F. proliferatum* (Leslie et al., 2006).

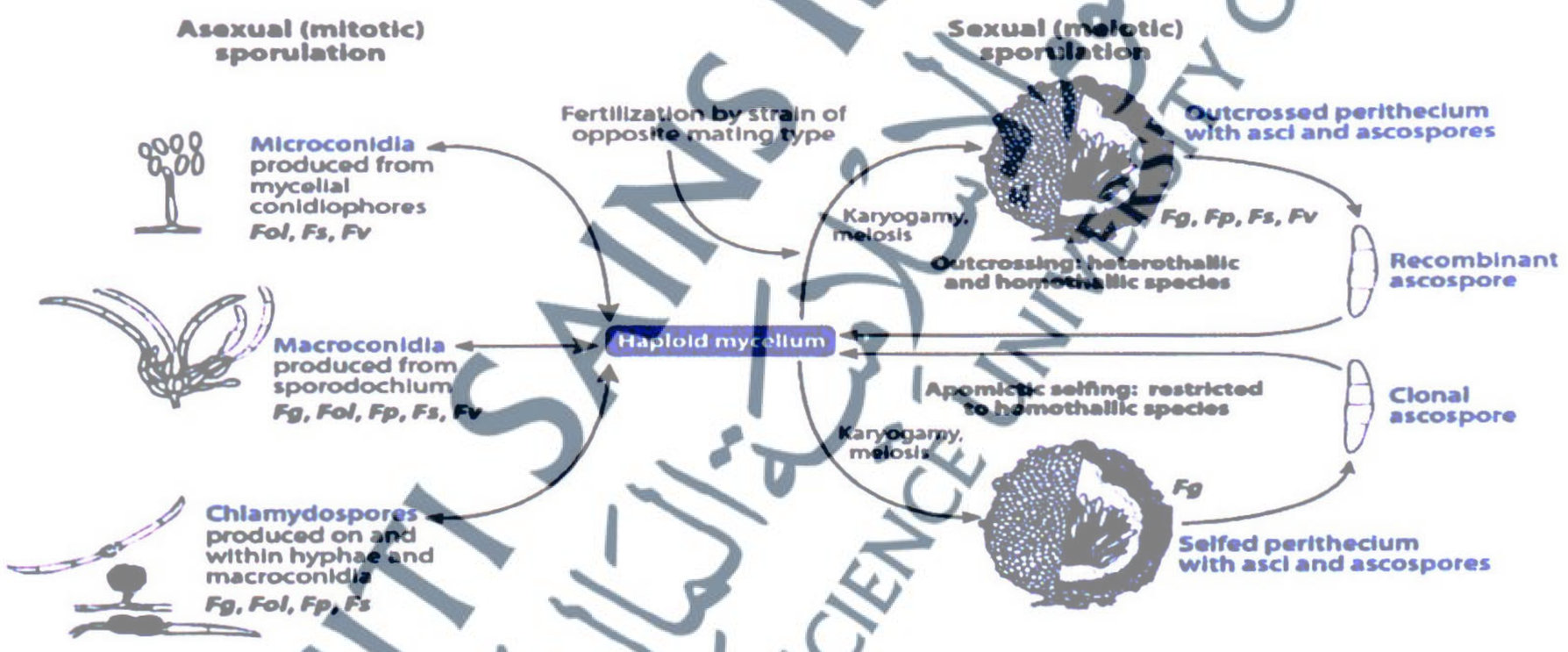
2.8.5 General life cycle of fungi *Fusarium*

Fusarium is a cosmopolitan genus of filamentous ascomycete fungi Sordariomycetes, Hypocreales and Nectriaceae that includes many toxin-producing crops pathogens of agricultural importance. *Fusarium* diseases include rots, wilts, blights and cankers of many horticultural, area, ornamental, and forest crops in both natural ecosystems and agriculture systems. *Fusaria* also produce a diverse array of toxic secondary metabolites (mycotoxins), such as fumonisins and trichothecene (Woloshuk & Shim, 2013) that can contaminate agricultural products, making them unsuitable for feed or food.

Mycotoxin such as Trichothecene can also act as virulence causes in plant diseases (Bai et al., 2002; Desjardins et al., 1996; Desmond et al., 2008; Ilgen et al., 2008; Proctor et al., 1995). Some *Fusarium* species generates meiotic (sexual) spores and three types of mitotic (asexual) spores. Conversely, not all spore types are known to be produced by all *Fusarium* species and less than 20% of *Fusarium* species has been known as sexual cycle. As phyto-pathogens, *Fusarium* species employ a broad range of infection strategies. Most can be loosely classified as hemibio-trophs, because infection initially resembles that of a pathogen that relies on a living host cells (bio-trophic), however ultimately the transitions to killing and consuming host cells (necrotrophic). *Fusarium* diseases may initiated in roots from soil-borne inoculums or in above-ground plant parts

by water or air and *Fusarium* species have ability to infect plant leaves and areal part of many plants resulting wilting disease. In this study observed wilting in chilli plant which caused by *F. solani*-CS. The general life cycle of *Fusarium* species described in the following (Figure 1). In some cases, *F. oxysporum formae speciales* consist of multiple, independent lineages that developed polyphyletically through convergent evolution (Baayen et al., 2000 and O'Donnell et al., 1998). All human pathogenic *Fusarium* species produce micro-conidial phases, and many produce bio-films on plumbing surfaces (Short et al., 2011).

FIGURE 1: General life cycle of *Fusarium* species



Adapted from (Li-Jun Ma et al., 2013)

Notes:- General life cycle of *Fusarium* species; the organism breeds as a haploid colony of hyphae, excluding for short dikaryotic (each parental haploid nuclei containing two cells) and diploid phases preceding meiosis and the production of haploid, sexually produced spores (ascospores). Ascospores are produced in groups of eight in a sac (ascus) enclosed within a flask-shaped structure (perithecium). Homothallic *Fusarium* species is competent of self-fertilization, producing clonal ascospores progeny (apomixis); heterothallic *Fusarium* species are self-sterile (Li-Jun Ma et al., 2013)

2.8.6 Morphological characteristics of fungi *Fusarium* species

Fusarium species are among the fungal genera that can cause contamination or spoilage on vegetable and infect rotting tissues of different vegetable crops (Snowdon, 1990; Tournas, 2005a,b; Naureen et al., 2009; Nurul et al., 2014). *F. oxysporum* f. sp. *cubense* (*Foc*) cannot be morphologically distinguished from other *formae speciales* that cause wilting in other hosts and other non-pathogenic *F. oxysporum* endophytic, saprophytes and opponents (Booth, 1972; Leslie & Summerell, 2006). In Malaysia, occurrences of *Fusarium* species are specifically on red chilli, tomato, cucumber, loofah, okra, bitter gourd, moringa, long bean and brinjal. The most possibility of *Fusarium* species found that are *F. oxysporum*, *F. semitectum*, *F. solani*, *F. proliferatum*, *F. pseudo-circinatum*, *F. sacchari*, *F. equiseti* and *F. verticillioides* in Malaysia (Nurul et al., 2014).

The morphological characteristics of some *Fusarium* species as shown in the Table 1. The fungi *F. oxysporum* f. sp. *cubense* (*Foc*) is an anamorphic fungus without a known sexual stage (teleomorph). The fungus produces microconidia, macroconidia and chlamydo-spores for reproduction and diffusion. Microconidia and macroconidia are produced in orange structures called sporodochia. Sexual stage (teleomorph) has not been found yet in isolates carrying genes Mat 1 and Mat 2 (Fourie et al., 2011). Macroconidia (27-55 × 3.3-5.5 µm) are abundant, falcate to erect approximately straight of thin walls with 3 to 5 septa (generally 3 septa). Apical cell is attenuated or hook-shaped of some *Fusarium* isolates. Basal cells are foot-shaped. Macroconidia are developed in single phialids in hypha. Microconidia (5-16 × 2.4-3.5 µm), regularly without septa, can be oval,

elliptic to kidney shaped and developed in large quantities in false heads in short mono-phialides. Chlamydo-spores (7-11 μm), are plentifully formed in hyphae or in conidia, single or in chains, typically in pairs, other than that, their development can be slower in some isolates.

Mycelia can be hairy to cottony, spaced or plentiful and capricious from white, salmon, to pale violet. Black to violet *Sclerotia* can be produced in some pathogenic fungi *Fusarium* species. On potato-dextrose-agar (PDA) medium, colonies have a changeable morphology. Phytopathogenic fungi *F. oxysporum* usually produces pale violet to dark red color pigments in PDA (Stover, 1962; Ploetz, 1990; Perez et al., 2003). Some *F. oxysporum* isolates change quickly from pionnotal (with abundant greasy or brilliant conidia aggregates) to flat humid mycelia of white-pale yellowish to peach colour on a PDA culture (Stover, 1962; Ploetz, 1990). In modified Komada media (K2), pathogenic fungi *Fusarium* species of TR4 develop laciniated radial colonies, which are not found in isolates of races 1 and 2 (Qi et al., 2008). This characteristic is not a determinant of a *Foc* TR4 diagnostic.

TABLE 1: The morphological characteristics of some vegetable crops *Fusarium* species

Species	Characteristics of morphology				
	Micro-conidia	Macro-conidia	Conidio-genous cell	Chlamydo-spore	Pigmentation
F. proliferatum	Abundant, club shape with flattened base, in chain (10- 15 conidia) and false head	Abundant, slender, almost straight, curved apical cell and poorly developed foot-shaped basal cell	Polyphailides and monophailides	Absent	White to purple
F.oxysporum	Abundant , formed in aerial mycelia, oval to kidney-shaped produced in false head	Abundant in sporodochia, slightly sickle-shaped, thin walled, tapered apical cell, foot-shaped basal cell	Monophailides	Present, singly or in pairs	White to purple.
F. solani	Abundant, oval to kidney-shaped produced in the agar and carnation leaf	Abundant, stout, cylindrical, blunt apical cell, distinct and rounded foot-shaped basal cell.	Long monophailides	Present singly or in pairs	Cream to white
F. sacchari	Abundant, oval, produced only in false head. Presence of mesoconidia in false head	Abundant, slightly sickle-shaped to almost straight, curved apical cell and poorly developed basal cell.	Polyphailides and monophailides	Absent	White to purple.

(Adopted from Nurul et al., 2014)

2.9 Control disease on plants

According to the Food and Agriculture Organization (FAO) of the United Nations, approximately more than 25% food crops in the world are vanished yearly because main reasons of mycotoxin contamination, with the *Fusarium* species contributing significantly to food contamination (Chelkowski, 1998).Therefore, removal for fungal contamination usually can be done using heat treatment and chemical treatment. Alternative, antifungal agents produced by microorganisms may be used as biocontrol against pathogenic fungi (Chitarra et al., 2003). Crop rotations which contribute to minimization of *Fusarium* species inoculums in soils are not feasible due to scarcity of land and cultural values (Hall & Phillips, 1992).

2.10 Cultural performance

The seeds that are to be sown and weed free is not possible because it is clear that the fungus can remain endophytic and saprophytic in the seeds as dormant mycelium or chlamydo-spores without causing infection (Menezes, 1988). It is recommended that the same culture is used for 2 to 3 year rotation with crops that are free from plant pathogens. The area must be kept clean by planting a good control of weeds and solanaceous around the implants field. The field must have a good drainage and it also must be autonomous from the remaining infected plants. If the presence of the infection has already confirmed, it should be turned away from the culturing Solanacae at least for two years (Roberts et al., 2001).

The first practice of the field of sanitation can include the measure of controlling weed and volunteer pepper. The varieties that can produce fruit with the maturity not too time consuming can be selected, to let the fruit escape fungal disease. The wound should be moderated in fruit from insects or other means as the wounds are the entrance points for *Fusarium* spp. as well as other pathogens such as bacteria that are the cause behind of soft rot. At the end of season, the remains of the plants contaminated with the field should be eliminated (Agros, 2005).

2.11 Use of resistant cultivar

The resistant varieties work as two actions to reduce the lost from diseases, but also eradicated chemical and mechanical disease control expenses (Agrios, 2005). Some genetic resources resistant to anthracnose in chilli have been recorded in many countries and regions, taken from various sources (Kim et al., 1986, 1987; Hong & Hwang, 1998; Pae et al., 1998; Park et al., 1987; Yoon & Park, 2001). In particular, some lines of *F. oxysporum* has demonstrated strong resistant to the pathogen, and pathogen inoculation has brought about no or limited lesions on the chilli fruits (Ali, 2006; Joshi et al., 2012). However, as it is no strong resistance is manifested in *Capsicum annum*, which is the only one species cultivated worldwide (Park, 2007).

A genetic study of anthracnose resistance to *C. capsici*, expressed in interdetailed cross of Thai susceptible *C. annum cv.* Bangchang and anthracnose resistant *C. chinense*

CM 021 (Mongkolporn et al., 2004). The genetic purity of the F1 is confirmed under the molecular marker analysis. The Voorrips et al., (2004) have discovered on major quantitative trait locus (QTL) and large effects on resistance and three other (QTLs) with lesser effect on the F2 population (cross between *C. annum* and *C. chinense)* on the traits they have put to test, such as disease frequency, the true lesion diameter and overall lesion diameter having inoculated with *C. gloeosporirdes* concerning the resistance to anthracnose disease.

2.12 Use of chemical against pathogens

Fusarium wilt, anthracnose and other fungal diseases mostly are controlled by the use of chemical method in the field, soil and storages, whereas fungicides which have active ingredients, hymexazol, azoxystrobin, fludioxolin and quinoline, was used to control infection caused by *Fusarium* wilt (Fakher et al., 2006). The fungicide traditionally suggested for anthracnose administering in the chilli manganese ethylene bisdithiocarbamat (MANEB) (Than et al., 2008b). However, fungicide tolerance on surfaces quickly, if a single compound is chosen, it will be the be-all and end all (Staub, 1991). Even though, it does not control more serious form of anthracnose on chilli fruit.

The strubilurin fungicides azoxystrobin (Quadris), pyraclostrobin (Cabrio) and trifloxystrobin (Flint) have been classified to control the anthracnose of chilli, however, only earlier reports on the efficacy of these fungicides against the severe form of the disease are accessible (Alexander and Waldenmaier, 2002; Lewis & Miller, 2003). The

disease can be monitored under normal weather conditions with a sensible spray programme. However, there are numerous reports using chemical and its impact on farmers' income and health, and the consequences of toxic contamination to lead the environmentally, mainly in developing countries in the world (Voorrip et al., 2004).

2.13 Use of bio-fungicides to control pathogens

Wilt is an important disease of brinjal crop causing significant reduction in yield by infection of fungi *Fusarium solani* f. sp. *melongenae*; wilt disease is able to control with plant extracts viz., *Azardiachta indica, Artemessia annua, Eucalyptus globulus; Ocimum sanctum and Rheum emodi* (Babu et al., 2008). For the control of chilli anthracnose, fruit rot has been clinging onto chemicals and this has resulted in many uninvited issues. There is a need to combine the alternative control components that are rendered successful in field. The biological control of fruit rot and dieback of chilli with plant products tested in various laboratories and field trial have pointed out that the crude extract from rhizome, leaves and creeping branches of sweet flag (*Acorus calamus* L.), palmorosa (*Cymbopogon matinii*) oil, neem (*Azadirachia indica*) oil *Ocimum sanctum* leaf extract could impede the anthracnose fungus from growing (Jeyalakshmi & sheetharaman, 1998; Korpraditskul et al., 1999). Among the bio-fungicides used against the fungus *Colletrotrichum* species on chilli fruit has found that the most successful control was the sweet flag crude extract when applied in two intervals when most of plants were still at the first bloom stage and at a much later mature bloom stage (Than et al., 2008b).

2.14 Biological control with microorganisms

It is unfortunate that biological control for chilli wilt disease do not seem to gain much attention. The potential for biological of Genus *Fusarium* was first introduced by Link (1809). This genus is notorious for harboring a variety of pathogenic fungal species (Zhang et al., 2012). The fungi *F. proliferatum, F. culmorum, F. verticillioides* and *F. graminearum* are *Fusarium* species that are important field or pre-harvest pathogens that cause large crop damage because of root, stalk and ear rot diseases. The main reason of harm to crops infected by *Fusarium* species is due to their ability to produce a variety of fungal toxins, which threaten the health of humans and animals that consume them (Julian et al., 1995; Azliza et al., 2014). The various crops are nowadays well established by applying beneficial effects of variety of soil microorganisms. These microorganisms may encourage to plant growth directly by providing nutrients or growth factors or indirectly by antagonizing soil borne phyto-pathogens through secondary metabolites (Okon and Hardar, 1987; Davison, 1988). Prema et al., (2008) suggested that *Lactobacillus plantarum* is a superior producer of a broad spectrum of antifungal compound that is include 3-phenyllactic acid. Niku-Paavola et al. (1999) has been confirmed that *Lb. plantarum* VTT E78076 is a creator of novel proteinaceous compound that showed inhibitory against *F. avenaceum*. It is widely confirmed that for biological control by antagonistic microorganisms, as a more encouraging action to control the station of post-harvest diseases (Janisiewicz & Korstn, 2002).

2.15 CHILLI

2.15.1 History of chilli

Chilli (*Capsicum annuum* L.) is belonging to Family Solanaceae and it is one of the most important in human food (NHB, 2011; Dias et al., 2013; Wahyuni et al., 2013). The varieties of chilli their common name, scientific and horticultural group is shown in Table 2. India is a major chilli producing countries in the world. It is a spicy, a fruit vegetable widely cultivated in the world. It is globally covering the areas of the tropical, subtropical and also produces in temperate zones (Pickersgill; 1997). The fruit of this plant knows various names, for example chilli pepper or chilli based on origins and the type of fruit concerned (Than et al., 2008b). The term pepper in different world regions refers especially to the kind of small chillies (Jeff McCormack, 2006; Than et al., 2008b). Chilli was first derived in South America, are common in the tropics and subtropics (Purseglove, 1987). Archaeological proof of chilli seeds annum L. before 5000 BC was discovered in the Tehuacan, Mexico. It was first transported to the European countries with the help of Christopher Columbus. Chilli had been sold in markets in all over the world, including on the tropical, subtropical and temperate trade paths and among the Spanish and Portuguese (Stummel & Bosland, 2007) who had simply been drawn to this spicy products.

TABLE 2: Botanical classification of commonly cultivated species of peppers

Common Name	Scientific Name	Horticultural Group
Chili pepper	*Capsicum annuum* var. *annuum*	Longum
Bird pepper	*Capsicum annuum* var. *aviculare*	Unknown
Aji pepper	*Capsicum baccatum* var. *baccatum*	Baccatum
Bell pepper	*Capsicum annuum* var. *annuum*	Grossum
Cone pepper	*Capsicum annuum* var. *annuum*	Conoides
Cayenne (Red cluster pepper)	*Capsicum annuum* var. *annuum*	Fasciculatum
Rocoto and Manzano	*Capsicum pubescens*	Unknown
Habanero	*Capsicum chinense*	Unknown
Tabasco and Squash pepper	*Capsicum frutescens*	Unknown
Aji pepper	*Capsicum baccatum* var. *pendulum*	Pendulum

(Adopting from Jeff McCormack, 2006)

Chilli (*Capsicum annum* L.) is very well established as red pepper and red feature can be described by the pigment capsanthin and its pungency influenced by an alkaloid capsaicin. Capsaicin has been considered effective in the pharmaceutical and cosmetic industries for the healing of colds and throat and chest congestions (Muthukummar et al., 2010). Chilli pepper (Capsicum spp.) is one of the essential vegetable crops, with features considered attractive to numerous; aesthetic aroma quality, good taste, soft texture, fascinating colours, and also the fact that it has a variety of nutrients, like minerals, vitamins A, C, and E and antioxidant properties and throughout the world. Chili

is consumed fresh, dried or in powder (Muthukummar et al., 2010; El-Ghoraba et al., 2013).

At the global level, chili is one of the spices that produce huge revenues for producers and therefore contributes to poverty alleviation and improvement of women's social status (Karungi et al., 2013). Although it is economic, food and medicinal importance, chili remains in several countries a neglected crop that is rarely of national priority in terms of agricultural development (FAO, 2010).

Chilli fruits of (*Capsicum annum* L.) is one of the Indian's leading commercial crops. With a yearly production of 1.1 million tones, India has been quick to stand out as the major chilli manufacturer in the world (Khan & Raj, 2006). Chilli contains around 20 to 27 species and out of this figure, five of which are domesticated namely, *C. annuum* L., *C. chinense* Jacq., *C. frutescens* L , *C. baccatum* L. and *C. pubescens* Keep. These plants cultivated in various regions all over the world. From the five types of chilli grown, *C. annum* is the most popular (Tong & Bosland, 1999; Bosland & Botava, 2000; Costa et al., 2009) followed by *C. frutescens*.

There are various chemicals in this fruit, including volatile oils, fatty oils and steam capsaicinoids, carotenoids, vitamins, protein, fibre and minerals (Than et al., 2008b; (Nsabiyera et al., 2013). The compounds and capscinoids found in chilli Capsicum are capsaicin (C 69%), homocapsaicin (HC 1%) and homodihydrocapsaicin (HDHC 1%), di-hydrodcapsaicin (DHC 22 %), non-hydrocapsaicin (NHDC 7%), the

namely C and DHC are the chemicals which cause the sensation of rapid bite at the back of the plate and the throat, while the others bring the eaters to along, low intensity bite on the tongue and mid plate capsaicinoids that are very important in preventing chronic diseases such as cancer, asthma, coughs, sore throats, toothache, diabetes and cardiovascular diseases (El-Ghoraba et al., 2013; Wahyuni et al., 2013). The consumption of fresh fruits facilitates starchy food digestion (Masada et al., 1971; Bhattacharya et al., 2010).

2.15.2 Effect of biological control against wilt disease on chilli

Chilli is the most important source of income for poor farmers in India, but it experiences from many diseases, one of them described a wilt disease of chilli caused by *Fusarium* species. The symptoms of *Fusarium* wilt included leaf chlorosis, vascular discolouration, and wilting of chilli plants. High temperature and high moisture were conducive to the symptom development of wilt (Sanogo, 2003). This wilt disease had been making the chilli difficult to be grown in hot humid conditions. Therefore, chilli farmers have experienced extensive economic loss due to *Fusarium oxysporum* (Joshi et al., 2012). Fungi *F. solani* is the causative agent for *Fusarium* Wilt of chili pepper where the underground stems are dry, brown, but the roots soft and water-soaked, plants wilt and die rapidly. Spores are spread in irrigation water and with wind-blown particles of soil. Heavy and poorly drained soils should be avoided (*Savary* et al., 2006; Montesinos, 2007; Horst, 2008).

If economically justified, soil fumigants and solarization can be used to reduce pathogen populations in soil, but increasing use of pesticides has led to several problems, such as environmental degradation health hazards for human, pest resistance and decrease in the population of beneficial insects, which has direct impact on the disease resistance (Groenewald, 2005). There is a worldwide need to adopt the practice of sustainable agriculture, using strategies that are environmental-friendly, less dependent on agricultural chemicals, and less damaging to soil and water resources. One of the key elements of such sustainable agriculture is the application of biological controlling strategies for plant protection (Joshi et al., 2012). LAB is one of the successful biological control agents against *Fusarium* species (Hamed et al., 2011) and *Colletotrichum* species (El-Mabrouk et at., 2014).