CHAPTER I

INTRODUCTION

The genus *Fusarium* is linked to inhabitants of soil and of organic substrates and is widely distributed throughout the world. The species of *Fusarium* that were isolated from the seed samples of chilli include *Fusarium moniliformae, F. solani* (Mart.) sacc, *F. equesiti* (Gorda) Sacc., *F. oxysporum* Schl. Emend Sny and Hans and *Alternaria alternate* (Fr.) (Keissler & Hashmi, 1989). Therefore, many producers of seeds applied various methods in order to prevent growth of microscopic fungi on feed and consequent mycotoxin contamination. However, protection against toxic fungi was already started under field conditions. Thus, contamination risk can be reduced by crop rotation, an efficient use of fungicides, and proper fertilization (Supronienė et al., 2010).

The wilt plant disease has been well recognised in worldwide in chilli plantation because it caused serious economic loss due to *F. oxysporum* causative agent of wilt disease (Horst, 2008; Joshi et al., 2012; Hussain et al., 2013). Additionally, most severity of Crown disease recorded in oil palm plants in Malaysia were from 131 isolates of *Fusarium* and were identified in different oil palm plants from different areas. Based on the morphological identification, the *Fusarium* species identified were *F. solani*, *F. oxysporum*, *F. semitect, F. proliferatum* and *F.* sub *glutinans* (Hafizi et al., 2013). The phytopathogenic species affect a wide range and cause root rot, vascular wilting, yellowing, and foliar necrosis (Nelson & Hansen, 1997; Ramachandran et al., 1982).

These can be subdivided into *formae speciales*, characterised by their ability to cause ailments in specific hosts, and in race, agreeing to their reaction with a group of differentiating cultivars (Gordon & Martyn, 1997). In the culture of cowpea, one of the chief diseases was *Fusarium* wilt, caused by *F. oxysporum* Schl. f. sp. *tracheiphilum* (Antonia & Maria, 2006). According to Champion (1997) many fungi considered to be saprophytes, pathogens or others that lost their ability to cause disease, can survive in latency inside of the seeds, becoming active when these seeds germinate. The pathogenic forms may result in pre or post-emergence damping off. This, in turns, results in a poor plant stand in the field (Agarwal & Sinclair, 1997).

The *Fusarium* wilt could be avoided using microorganisms as biocontrol agents against fungal attack. For instance, lactic acid bacteria (LAB) strains that were isolated from rhizospheric samples of desert truffles and olive trees from diverse geographic regions include genera *Enterococcus, Lactobacillus, Pediococcus, Lactococcus* and they showed strong antifungal activity against phytopathogenic fungi of *Aspergillus niger*, *Penicillium expansum*, *Botrytis cinerea,* and *Verticillium dahlia* (Fhoula et al., 2013). Furthermore, *Lb. sakei*, *Lb. plantarum*, *P. acidilactici, P. pentosaceus, Lb. lactis* ITS type K, and *Lb. garvieae* showed antifungal activity against soil borne fungi of phytopathogenic fungi of *Aspergillus niger*, *Penicillium expansum*, *Botrytis cinerea,* and *Verticillium dahlia* (Fhoula et al., 2013). Similarly, Mataragas et al. (2003), Magnusson et al. (2003) & Stiles (1996) reported that environmentally isolates of LAB were excellent source for active metabolites to control different pathogenic fungi and bacteria. Besides *Lb. brevis* and *Lb. plantarum* that were isolated from healthy tomato samples could

inhibit *Aspergillus fumigatus* and some others *Fusarium* species, namely, *Fusarium solani*, *F. acuminatum,* and *F. funjikuroi* (Samuel et al., 2014).

Chilli (*Capsicum annuum* L.) is one of an important vegetable and spice crop in worldwide. Chilli fruits can be and consumed as fresh or processed from the chilli plant, belonging to Solanaceae family is as an annual herbaceous vegetable or perennial shrub of the (Amusa et al., 2004). It is cultivated in both tropical and sub-tropical regions (Than et al., 2008). It is high cash value due to consumption rate and the annual trade of chilli are nearly 17% of total spice trade in the world (Ahmed et al., 2000). It is one of the most important commercial crops in India with an annual production of 1.1 million tonnes, making the India consider the largest producer country of chilli in the world (Khan & Raj, 2006). Chilli pepper is also suitable for the diets either green or red and it is useful in cure of disease such as stomach and colon cancer (Pamplona-Roger, 2007). Chilli peppers are low in sodium, cholesterol free, rich in vitamins A and C, and are a good source of potassium, folic acid and vitamin E (Than et al., 2008). Fresh green chilli peppers contained more vitamin C than citrus fruits and fresh red chilli has more vitamin A than carrots (Than et al., 2008). In addition, Amusa et al. (2004) reported that *C. capsicum* fruits are used as flavouring agent and other purposes by consumers in worldwide.

However, numerous species of the genus *Fusarium* are known as phyto-pathogens for causing serious plant ailments on many economically important plants in the worldwide, such as in corn (Darnetty et al., 2008), in rice (Nur et al., 2008), in sugar cane (Siti et al., 2008), and in banana (Liew et al., 1998). Indeed, the family Solanaceae

such as chilli plant could also infected with *Fusarium solani* which recovered from the roots of chilli plant (Hussain et al. 2013). Other *Fusarium* species of *F. solani* is a cosmopolitan species and is classified into the section Martiella (Booth, 1971). *Fusarium solani* is also the causative agent of *Fusarium* root rot diseases (Taleb et al., 2011) in many vegetable crops in the worldwide. The infection can decrease the quantity and quality of major world crops and others economical plants (Parveen et al., 1993 and Ghaffar, 1995). Infection by *Fusarium* spp. are main cause of plant diseases in many countries such as, in Italy (Polizzi et al., 2010), Saudi Arabia (Perveen & Bokhari, 2010), Portugal (Felgueiras et al., 2010), and Turkey (Karaca & Kahveci, 2010).

Therefore, reduction of biomass of fungal contamination usually can be done using two methods such as use of pesticides and fungicides to avoid soil pollution and health problems. Alternatively, antifungal agents produced by microorganisms may be used as biocontrol agent on pathogenic fungi (Chitarra et al., 2003). Many investigators used LAB isolates to control fungal pathogens and plant protection. However, the comprehensive study on exploring the potential of LAB from soil and rahizospheric soil samples as the evidence on their inhibitory activity against *Fusarium* species on chilli plants protections were still lacking of information. The alternative method of use LAB to control the problem of *Fusarium* in chilli plantation were studied at the same time may provide additional nutrients to the plant.

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OBJECTIVES

Thus, the objectives of were to;

1 Isolate LAB from soil and rhizosphere soil and identify using API 50 CHL and 16S rDNA genotypic sequencing as well as to isolate the *Fusarium* from various plant sources.

2 Evaluate the effect of pH, temperature, and enzyme treatments on LAB supernatant activity and estimation of antifungal compound.

3 Determine the inhibitory activity of LAB as biocontrol agents against isolated *Fusarium* species *in vitro* and *in vivo* on chilli seeds germination.

4 Determine the effect cells of both *Lb. plantarum* isolated from different sources against *Fusarium* species on chilli plant system and isolation of endophytic LAB and fungi from treated chilli plant.

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