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Antagonistic Activity of *Trichoderma parareesei* and *Trichoderma harzianum* against *Colletotrichum* sp., a Causal Pathogen for Anthracnose Disease in Chilli.

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A report submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Agrotechnology) with Honours

Faculty of Agro-Based Industry

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled "Antagonistic Activity of *Trichoderma parareesei* and *Trichoderma harzianum* against *Colletotrichum* sp., a Causal Pathogen for Anthracnose Disease in Chilli" by Norhidayah Binti Yacob, matric number F15A0119 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agrotechnology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
°C	Degree Celsius
cm	Centimetre
g	Gram
ml	Millilitre
mm	Millimetre
Psi	Pressure unit standing for pound per square inch
PDA	Potato Dextrose Agar
PIRG	Percentage Inhibition Radial Growth

Antagonistic Activity of *Trichoderma Parareesei* and *Trichoderma Harzianum* against *Colletotrichum* sp., Causal Pathogen Anthracnose Disease in Chilli.

ABSTRACT

Chilli is an important vegetable crop in worldwide as for culinary substrate due to its nutritional, medical and economic value, while anthracnose disease caused by fungal pathogen *Colletotrichum* species that become major constraint for chilli production. Disease control by chemical and cultural practices is still not effective where chemical usage is not eco-friendly. Using biocontrol agent is an alternative optional that eco-friendly and more compatible. Thus, this study was aimed to isolate and identify the fungal pathogen of *Colletotrichum* sp. from infected chilli as well as control the pathogen by control agent of *Trichoderma* species which were *T. parareesei* and *T. harzianum*. The identification of *Colletotrichum* result showed that at the early stage of fungus growth, colour appear as white to greyish colour and then the colonies was bright orange conidial masses at late stage while for microscopic study found cylindrical-like conidia with ovoid, clavate and slightly irregular appressoria appeared which similar with *Colletotrichum gloeosporioides*. Hence, based on morphological and anatomical studies the isolated fungus has been identified as *C. gloeosporioides*. Antagonistic results shown that both strains of *Trichoderma* sp. were successfully inhibited the growth of *Colletotrichum gloeosporioides* by the basis of Percentage Inhibition Radial Growth (PIRG) analysis. The range values obtained from analysis were between 56.25% until 75%. Among these two strains of *Trichoderma sp, T. harzianum* showed the higher value of Percentage Inhibition Radial Growth (PIRG) which was 75% while *T. parareesei* shared the antagonistic activity which was 68.75%. In conclusion, the antagonistic results determined that both species of *Trichoderma* had important role to be used as a very potential biocontrol agent against *Colletotrichum gloeosporioides* for anthracnose disease in chilli.

Key words: Chilli, *Trichoderma*, antagonistic, anthracnose, *Colletotrichum gloeosporioides*

Aktiviti Antagonis *Trichoderma Parareesei* dan *Trichoderma Harzianum* terhadap *Colletotrichum* sp., Penyebab Penyakit Antraknos Pathogen di Cili.

ABSTRAK

Cili adalah tanaman sayur-sayuran penting yang digunakan di seluruh dunia untuk bahan masakan kerana nilai pemakanan, perubatan dan ekonomi, namun penyakit antraknos yang disebabkan oleh spesies patogen kulat *Colletotrichum* yang menyebabkan kerugian besar pada pengeluaran cili. Penyakit dikawal oleh amalan kimia dan budaya masih tidak berkesan di mana penggunaan kimia tidak mesra alam. Menggunakan ejen kawalan biologi adalah pilihan alternatif yang mesra alam dan lebih serasi. Oleh itu, kajian ini bertujuan untuk mengasingkan dan mengenal pasti patogen kulat *Colletotrichum* sp. dari cili yang dijangkiti serta mengawal patogen menggunakan agen kawalan biologi *Trichoderma* species yang dipilih iaitu agen kawalan biologi *T. parareesei* dan *T. harzianum*. Hasil pemerhatian *Colletotrichum* menunjukkan bahawa pada peringkat awal pertumbuhan kulat, warna kelihatan putih ke warna kekelabuan dan kemudian jajahannya adalah jisim conidial jingga yang cerah pada peringkat akhir manakala untuk kajian mikroskopik terdapat conidia silinder seperti dengan ovoid, clavate dan sedikit tidak teratur Appressoria muncul yang sama dengan *Colletotrichum gloeosporioides*. Oleh itu, berdasarkan kajian morfologi dan anatomi, kulat terpencil telah dikenal pasti sebagai *C. gloeosporioides*. Keputusan antagonistik menunjukkan bahawa kedua-dua jenis strain *Trichoderma* sp. berjaya menghalang pertumbuhan *Colletotrichum gloeosporioides* berdasarkan analisis Peratusan Pertumbuhan Jejari Perencatan (PIRG). Nilai julat yang diperoleh daripada analisis adalah antara 56.25% hingga 75%. Di antara dua jenis *Trichoderma* sp, *T. harzianum* menunjukkan nilai yang lebih tinggi daripada Peratusan Pertumbuhan Jejari Perencatan (PIRG) yang lebih tinggi iaitu 75% manakala *T. parareesei* berkongsi aktiviti antagonistik iaitu 68.75%. Kesimpulannya, keputusan antagonis menunjukkan bahawa kedua-dua spesis *Trichoderma* mempunyai peranan penting untuk digunakan sebagai agen kawalan biologi yang sangat potensial terhadap *Colletotrichum gloeosporioides* untuk penyakit antraknos dalam cili.

Kata kunci: Cili, *Trichoderma*, antagonistic, antraknos, *Colletotrichum gloeosporioides*

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Chilli (*Capsicum* spp.) also known as chilli is among the most popular vegetables through world and has been used for spices and condiments. It's also one of the most widely cultivated vegetable and spice crops worldwide and a major constituent in food products, pharmaceuticals and cosmetics (Pathirana, 2013). Chilli pepper belongs to the Solanaceae family. The genus *Capsicum* is closely related to tomato, eggplant, potato, and tobacco (Bashair, Javed, Atiq & Wakil, 2016).

India is the largest producer of chillies in the world market for chillies which contributing 25 per cent of the total world production, while four percent of it were exported because of high domestic consumption (THAMARAIKANNAN & SENGOTTUVE, 2011). Chilli can be exported in different forms: fresh chillies, stalk less chillies, green chillies, chilli powder which is the minimum level for the extraction market is 0.7 per cent of capsaicin and also as oleoresin. Beside India some other and major chilli producing countries are China (11%), Bangladesh (8%), Peru (8%), Pakistan (6%), Ethiopia (5%), Ghana (4%), Ghana (4%), Vietnam (4%), Mexico (3%) and Myanmar (3%) (Hussain & Abid, 2011). The productions of the chillies in Malaysia

are 47 015.0 tonnes in 2015 and have to import 47 670.7 tonnes to meet the Malaysian market (Statistik Utama Pemasaran FAMA 2017, 2017).

Chilli is affected by a number of pests and diseases that influenced losses in chilli production. Pests generally are more mobile and can reproduce rapidly than beneficial insects. Chilli is affected by number of pests attack on chillies crop on the growing season. For example aphids, thrips, leaf hoppers, ear wigs, crickets, mites, root grubs, pod borers, cut worms, flea Beatles, etc damage or destroy the crop. Other than pests, pathogens are also a serious threat for chilli crop such as fungi, bacteria, viruses and nematodes. Several fungi cause different diseases in chilli plants. Sometime these fungi can cause identical symptoms and mixed up to one another. There are some pre-harvesting fungal diseases such as anthracnose, cercospora (frogeye) leaf spot, charcoal rot, choanephora blight (wet rot), damping-off root rot, downy mildew, fusarium stem rot, fusarium wilt, gray leaf spot, gray mold, phytophthora blight, powdery mildew, southern blight, verticillium wilt and white mold that are grown in chilli crop (Hussain & Abid, 2011). In 2015, Malaysia face loss of 4577.4 tonnes of chillies due to pest and disease during pre-harvest and post-harvest (Statistik Utama Pemasaran FAMA 2017, 2017).

1.2 Problem Statement

Anthracnose disease in chilli plant is one of the dominant economic constraint fr the chilli global production, especially in tropical region. In the *Colletotrichum* patho-system, different *Colletotrichum* species can be related with anthracnose of the same host and subtropical regions (Than, Prihastuti, Phoulivong, Taylor & Hyde, 2008). According to Than, Prihastuti, Phoulivong, Taylor and Hyde (2008), anthracnose of chilli has been shown to be caused by more than one *Colletotrichum* species including

C. acutatum, C. capsici, C. gloeosporioides, and *C. coccodes*. As the anthracnose is mainly a problem on mature chilli fruits. Infection many times has lessened the market quality of intact red chilli fruits as well as chilli powder and paste in spices industry. There are four species of *Colletotrichum* involved in anthracnose throughout the world. Anthracnose is caused essentially by *Colletotrichum capsici* but, *C. gloeosporioides* is also an emerging problem in chilli (Pandey & Gupta, 2016). There are a lot of practice like resistant cultivars, use of disease free seeds and seedling or fungicide are not fully effective in order to control the disease, chemical control by fungicide such as Copper oxychloride, Mancozeb, Carbendazim, Tridimefon, Daconil, Guazatine, Imazalil, Thiophanatemethyl, Etaconazolc, Benomyl, Prochloraz, (Agnihotri, Prakash, Kishun, & Misra,1996) can control the disease while it caused harmful effect for the environment and another problem to produce resist strain against fungicide. Thus, to overcome the offensive effect, a biological approach is the best or eco-friendly way to control the disease. The species of *Trichoderma* is a most persuasive biocontrol agent to manage phytopathogen without negative effect for the environment (Bastakoti, Belbase, Manandhar & Arjyal, 2017).

1.3 Objectives

The objectives of this study were determined:

1.3.1 To isolate and identify the fungal pathogen of *Colletotrichum* species from anthracnose infected Chilli.

1.3.2 To determine the antagonistic activities as biocontrol agents of *Trichoderma parareesei* and *Trichoderma harzianum* against *Colletotrichum* sp.

1.4 Hypothesis

The pathogen of species of *Colletotrichum* can be isolated and identified from infected chilli as well as the identified *Trichoderma harzianum* and *Trichoderma parareesei* can be used as biocontrol agent to control the fungal pathogen *Colletotrichum* sp. that caused anthracnose disease in chilli.

1.5 Scope of Study

This study was focused on the isolation and identification of *Colletotrichum* sp. from infected part of chilli which is a causal pathogen for anthracnose disease. Fungal pathogen of anthracnose disease in chilli was isolated and cultivated in Potato Dextrose Agar (PDA) medium and identification was done based on microscopic and microscopic characters of fungi. Antagonistic activity of *Trichoderma* species, *T. harzianum* and T. *parareesei* against the *Collethotrichum gloeosporioides* were determined based on the radial growth rate for fungi, *Colletotrichum gloeosporioides* in the presence of *Trichoderma harzianum* and *Trichoderma parareesei* by plate assay experiment

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1.6 Limitation of study

First limitation in this study was on methodological process where to identification of microscopic characters for the fungi were hard to be done due to a limited of good microscope. Another problem was associated with the studying process

of fungal characteristics either in microscopic and macroscopic characters.it was difficult to identify and categorize the fungi due to lack of knowledge and skill.

1.7 Significance of Study

The anthracnose disease in chilli relied on high effective chemical approach where this approach can cause the harmful effects to human health, flora and fauna and also the environment for so many years. Therefore, biological control has been used as alternate way to cover the use of any organism as a control method of pathogen by environmental way. The concept was included the direct approach where the ability to suppress the activity of the pathogen by introducing the antagonist agent.

CHAPTER 2

LITERATURE REVIEW

2.1 Important of Chilli

Chilli comprises five domesticated and about 25 wild species. Mexico is believed to be the centre of origin of *Capsicum annuum*, whereas *Capsicum frutescens* and the other cultivated species (*Capsicum baccatum L.* var. pendulum (Willd.) Eshbaugh, *Capsicum chinense* Jacq., and *Capsicum pubescens* Ruiz & Pav.) originated in South America. Capsicum peppers were introduced to Asia in the 16th Century by Portuguese and Spanish explorers via trade routes from South America. Widespread geographic distribution of *C. annuum* and *C. frutescens* has taken placed on all continents, whereas the others are unusual outside South America (Ibiza, Blanca, Cañizares & Nuez, 2012; Orobiyi et al, 2013)

In the world, chilli is grown over 2020 thousand hectares of area, with a production of 3762 thousand tonnes (Geetha, & Selvarani, 2017). Leading countries growing chilli are India, Myanmar, Bangladesh, Pakistan, Thailand, Vietnam, Romania, China, Nigeria and Mexico etc. India is the huge chilli production followed by China, Thailand and Pakistan (Geetha, & Selvarani, 2017). The world trade placed second

position after black pepper which is 18 per cent of the total spice trade in the world (Geetha, & Selvarani, 2017). Chillies are used around the world as a spice and also in the production of beverages and medicines. The largest chilli exporters along with their percentage share in the world's total exports which are India is 25 per cent, China is 24 per cent, Spain is 17 per cent, Mexico is 8 per cent, Pakistan is 7.2 per cent, Morocco is 7 per cent and Turkey is 4.5 per cent. (Subbiah & JeyAkumAr, 2009)

In Malaysia, only two species *Capsicum annuum* (bell pappers) and *Capsicum frutescens* (Cayenne pepper) are grown. Cultivation of pungent chilli is mostly limited to the lowlands while the non-pungent bell peppers (*Capsicum annuum* var *grossum*), which prefers a cooler temperature, is the most cultivated in Cameron Highlands (Melor, 2008). In the lowlands, bell pepper is often grown ex situ which is under modified environments complete with fertigation systems. Total chilli production areas vary around 3000 ha of crop equivalent per year. Of this cultivated area, about 95% is planted with *C. annuum* and the remaining 5% is cultivated with *cili padi* (*C. frutescens*). Another species grown locally is *C. chinense* which is one of the spicy chilli species known. Locally, this species is panted as an ornamental, whereas elsewhere *C. chinense* is grown for capsaicin extraction (Melor, 2008).

Chilli is widely used spice and condiment in the world and is indeed priced for its pungency and adding as special flavour in many dishes around the world. In past it was mainly used for seasoning and also as medicinal plant, but nowadays chilli was used extended as raw and processed vegetable, spice, dried forms, used as food dye, bred as decoration plant and also production of extracts in various pharmaceutical and cosmetics industry (Than, Prihastuti, Phoulivong, Taylor & Hyde, 2008). Average world production of dry and green chillies are approximated at 3.9 and 34.5 million tons respectively and cultivated area that harvested from 1.8 and 1.9 million hectares for

both dry and green chillies respectively (Than, Prihastuti, Phoulivong, Taylor & Hyde, 2008)

2.2 Nutritional and medicinal value of Chilli

Chili peppers are contains various minerals, vitamins and amino acids which important for human health and growth. Pawar et al. (2011), discover that chillies have the entire best feature to be deliberate as a food. Chilli consists of wide array of phytochemicals such as vitamins, phenolics and flavonoids that are necessary for anti-oxidants that may weaken degenerative diseases. Chilli is rich in vitamin C, vitamin A, vitamin E mostly in B vitamins which is vitamin B5. They are also very high in potassium, magnesium, iron and rich in calcium and phosphorus. Chilli has many species and hundreds of varieties and types. They can be ingested as fresh unripe fruits, ripened red or other colours and dried forms. The different species, varieties and consumption forms are vary in the nutritional and the anti-oxidant contents (Saleh, Omer & Teweldemedhin, 2018). Chillies are cholesterol free, low sodium, low calorie, rich in vitamins A and C and a good source of folic acid, potassium and Vitamin E. In the past, chilli are used as a traditional medicine for, amongst others, anorexia and vertigo. They are scientifically known use to treat of asthma, arthritis, blood clots, cluster, headaches, postherpetic neuralgia (shingles) and burns (Mehta, 2017).

Capsaicin are an alkaloid in chili peppers which makes them hot, areis used as an analgesic in topical ointments, dermal patches, and nasal sprays to relieve pain. Fruit pungency is apparently important flavour trait of chilli as characteristic of the genus Capsicum and also due to an alkaloid compounds that known as capsaicinoids make it unique to Capsicum. The capsaicinoids are also called capsaicin because was common

among the seven capsaicinoids followed dihydrocapsaicin. Another five compounds which are norcapsaicin, nordihydrocapsaicin, nornordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin, are express as minor capsaicinoids. Capsaicin and its related compounds are the active ingredients in chilli are related to the anti-microbial and anti-carcinogenic and other medicinal characteristic in chilli. For measuring pungency, scoville organoleptic method and High Performance Liquid Chromatography (HPLC) are used. Pungency is dependent on gene of the chilli plant and some other environmental factors. *C.annuum* is the most inconstant in pungency among the cultivated chilli, and both *C.chinense* and *C.frutescens* are the highest and the lowest is *C.baccatum* while *C.pubescens* is mild (Saleh, Omer & Teweldemedhin, 2018).

In most developing countries, traditional medicine along with herbal medicine is still widely used, while complementary and alternative medicine is rapidly used in developing countries. The traditional medicine use in Eritrea is common to other countries with rich traditional medicinal use in many aspects. Chilli has been used for medicine for long period in different countries and civilizations. In old civilizations, the Mayas use chili to cure asthma, coughs, and sore and the Aztecs use chilli to relieve toothaches. In Eritrea chilli is part of the food culture that is mostly in daily consumption and Eritreans generally believe that chilli has health benefits, however, there are no information about its medicinal use (Saleh, Omer & Teweldemedhin, 2018).

2.3 Anthracnose infection in crops.

Anthracnose disease causes the wilting, withering, and dying of tissues in plant. It usually affects during development of shoots and leaves. The causal fungi usually *Colletotrichum* or *Gloeosporium* which are normally develop spores in tiny, sunken, saucer-shaped fruiting bodies known as acervuli. The symptoms of anthracnose are sunken spots or lesions (blight) of various colours in leaves, stems, fruits, or flowers, and some infections might causes cankers on twigs and branches. The severe of the infection depends on both the causal agent and also the infected species and can range from mere unsightliness to death ("anthracnose | Description, Symptoms, & Control", 2018). The prevalence as also the severity of anthracnose is influenced by excessive rains, heavy dews, high humidity and warm weather. During critical period of infection, there is profuse production of spores in slimy pink masses which ooze out of the acervuli. The spores are readily disseminated by rain water. Temperature ranging from, 10-30° C and a relative humidity more than 95% for 12 hours are considered optimum for quick proliferation of the pathogen. The anthracnose pathogen perpetuates on diseased plant parts and debris lying on soil surface. It is also carried as latent infection on fruit that further develops during transit and storage (Agnihotri, Prakash, Kishun, & Misra, 1996).

Anthracnose can be prevented by discard infected parts, using disease-free seed and disease-resistant varieties, applying fungicides, and controlling vector from that spread anthracnose fungi from infected plant to healthy plant. For infected in annual plants, such as tomatoes or melons, to limit the accumulation of fungal spores in the soil crop rotation is suggested ("anthracnose | Description, Symptoms, & Control", 2018).

Table 2.1. Anthracnose disease in plant crop and difference pathogens

PLANT	PATHOGEN	Ref.
Tomato	*Colletotrichum coccodes*	(Dillard & Cobb, 1998)
Potato (*Solanum tuberosum*)	*Colletotrichum coccodes*	(Lees & Hilton, 2003)
Chilli (*Capsicum annuum*)	*Colletotrichum* sp.	(Than, Prihastuti, Phoulivong, Taylor & Hyde, 2008)
Pigeon Pea (*Cajan cajan (*L.) Millsp	*Colletotrichum lindemuthianum*	(TUCKER, 1927)
Bitter gourd	*Colletotrichum gloeosporioides*	(Kim et al., 2015)
Soybean	*Colletotrichum truncatum*	(Network, 2018)
Cucurbit	*Colletotrichum orbiculare, Colletotrichum lagenarium*	(Goldberg, 2004; Zitter, 1987)
Cucumber	*Colletotrichum orbiculare (syn. C. lagenarium)*	(Palenchar, Treadwell, Datnoff, Gevens & Vallad, 2016)

2.4 Anthracnose disease in Chilli during pre-harvest and post-harvest and its losses

Diseases that commonly infected chilli such as damping-off, leaf spot, powdery mildew, anthracnose, die back, *Fusarium* wilt during growing season and aflatoxin contamination during a post-harvest. Farmers were dependent on pesticides as the insect pests, sucking pest, defoliator and fruit borer play a crucial role and the numbers of sprays often exceed 25 to 30 under irrigated conditions which automatically increase the cost of production apart from extreme damage to eco-system (Naik et al, 2011). There different species of *Colletotrichum* infect chilli plants at different development stages. In Korea leaves and stems are damaged by *C. coccodes* and *C. dematium* whereas *C. acutatum* and *C. gloeosporioides* infect chilli fruits. *Colletotrichum capsici* is usually found in red chilli fruits whereas *C. acutatum* and *C. gloeosporioides* source of infections both in young and mature chilli fruits (Kim, Yoon, Park, Park & Kim, 2004).

An annual loss was predicted about 29.5%, amounting masive figure of US$ 491.67 million has been reported only by India (Garg, Loganathan, Saha & Roy, 2014). In India, a calculated loss of 10–54% by the anthracnose disease has been reported in yield of the crop (Lakshmesha, Lakshmidevi & Mallikarjuna, 2005); Ramachandran & Rathnamma, 2006). In Vietnam amount of 20–80% losses has been accounted (Don, Van, Phuong Vy, & Kieu, 2007) and about 10% from Korea (Byung, 2007). The losses of the marketable yield of chilli fruits during post and pre harvest by pathogen causing a loss of 10–80% in industries (Than, Prihastuti, Phoulivong, Taylor & Hyde, 2008).

Circular or angular sunken lesions, with concentric rings of acervuli that are sometime wet and produce pink to orange conidial masses are typical fruit symptoms of anthracnose disease. Under intense disease pressure, lesions may coalesce. Conidial masses may also appear scattered or in concentric rings on the lesions. The disease

management practices are often deficient to defeat the diseases was concluded in many studies. Due to involvement of multiple *Colletotrichum* species in anthracnose infection breeding to establish the long-lasting resistant varieties has also not been successful (Than, Prihastuti, Phoulivong, Taylor & Hyde, 2008).

2.5 *Collentotrichum* sp. infected in plants of Malaysia.

The most problem crops infected by *Colletotrichum* sp. in Malaysia is chilli, mango, banana, guava and cocoa. Chilli that infected by *Colletotrichum gloeosporioides* and *Colletotrichum capsici* as the causative agent of anthracnose in Malaysia was first reported in Sabah (Yun, Ahmad, Muid & Seelan, 2009). The component of fruits is attacked by the pathogen early in their development. The fungi remain as germinated appressorium during quiescent period develop brown-black spots on the pericarp and soft rot in the mesocarp when fruit ripens for harvest. Infection of chillies by *Colletotrichum* sp. is mainly during fruit ripening due to ample nutrients and limited antifungal compounds formed (Prusky1996). The other crops that infected by *Colletotrichum* sp. in Malaysia is cocoa (*Theobromae cocoa, L*) which is leaf spot and pod rot of cocoa (*Theobromae cocoa, L*) infected by *Colletotnchum gloeosporioides* (Yee & Sariah, 1993). Mango (*Mangifera indica*) is economically important tropical fruit crops in Malaysia. Thus, *C. gloeosporioides sensu lato* and *C. asianum* was recovered from anthracnose lesion of Chokanan and Harum Manis mango varieties (Zakaria, Juhari, Vijaya & Anuar, 2015). Anthracnose of banana is caused by the Colletotrichum species and one of the most serious diseases on ripe banana. Symptoms of anthracnose include black and sunken lesions with spore masses or acervuli in the lesion. Affection on the banana normally starts during the development of the fruit but

remains quiescent until the fruit ripens and symptoms commonly revealed when storage and marketing (Prusky & Plumbley 1992). Thus, *Colletotrichum* sp. that causal anthracnose in banana and guava were identified as *Colletotrichum musae* and from water apple as *Colletotrichum gloeosporiodes* (Zakaria, Sahak, Zakaria & Salleh, 2009).

2.6 Controlling method of Colletotrichum

2.6.1 Cultural method

Anthracnose disease causal by *Colletotrichum* can be control with cultural method commonly used by farmer. Pathogen-free chilli seed are planted and weeds eliminated. Doing crops rotation in every 2-3 years with crops that are not alternative hosts of *Colletotrichum.* By controlling weeds and solanaceous volunteers all over the transplant houses to keep clean the transplant. The field should have good drainage and be free from infected plant debris. Crops should be rotated away from solanaceous plants for at least 2 years once the disease present (Than, Prihastuti, Phoulivong, Taylor & Hyde, 2008). Sanitation practices in the field include control of weeds and volunteer chilli plants. Selecting cultivars that bear fruit with a lessened ripening period may oblige the fruit to escape infection by the fungus. As wounds provide entry points for *Colletotrichum* spp. and other pathogens such as bacteria that cause soft rot, wounds in fruit from insects or other means should be reduced to possible extent. Removed or deep ploughed at the end of the season, it completely covers crop diseases from infected plant debris from the field (Agrios, 2005).

The uses of resistant varieties not only get rid of losses from diseases, but also reduce chemical and mechanical costs of disease control (Agrios, 2005). A few genetic resources resistant to anthracnose in chilli have been separately reported from different

countries and regions (Yoon and Park, 2001). In particular, some lines of *C. baccatum* show strong resistance to the pathogen, and pathogen inoculation appear in no or limited lesions on the chilli fruits (Yoon, 2003). However, in *Capsicum annuum*, which is the only species grown globally there no strong resistance has been found (Park, 2007). Mongkolporn et al. (2004a) accomplish a genetic research of anthracnose deficiance to *C. capsici*, which was conveyed in the interspecific cross of Thai susceptible C. *annuum* cv. 'Bangchang' and anthracnose deficiance *C. chinense* 'CM 021'. The genetic purity of the F1 was verified by using molecular marker analysis. Voorrips, Finkers, Sanjaya and Groenwold (2004) have discover one main quantitative trait locus (QTL) with high significance and large effects on resistance and three other QTLs with smaller effects on the F2 population (cross between *C. annuum* and *C. chinense*) on the traits they tested, such as infection frequency, the true lesion diameter and overall lesion diameter after inoculation with *Colletotrichum gloeosporioides* in the study of resistance to anthracnose disease in Indonesia.

2.6.2 Chemical method

Chemicals are the frequent approach that used to deal with anthracnose diseases. However, fungicide resistance usually derives quickly, if a single compound is relied upon too heavily. The anthracnose disease in chilli can be manage by using fungicide Manganese ethylenebisdithiocarbamate (Maneb) (Smith, 2000), eventhough it does not always control the acute form of anthracnose on chilli fruit. The strobilurin fungicides have recently been labelled for the control of anthracnose of chilli azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio), but only initial reports are available on the adequacy of these fungicides facing the severe form

of the disease (Ajithkumar, Savitha, Biradar, Rajanna, & Ramesh,2014). Under normal weather conditions the disease can be controlled reasonable spray program. However, negative effects of using chemicals on farmers' in-come and health, and toxic contamination to the environment, being reported numerously specifically in developing countries (Voorrips, Finkers, Sanjaya & Groenwold, 2004)

2.6.3 Biocontrol method

So far, biological control methods do not acquired much attention for chilli anthracnose disease. The possibility for biological control of *Colletotrichum* species had been suggested as early as in 1976 by Lenné and Parbery (1976). Jeger and Jeffries (1988) also emphasize the potential of biological control by using *Pseudomonas fluorescens* for post-harvest fruit diseases. *C. capsici*, the main anthracnose pathogen in Thailand can be completely control after established antagonistic bacterial strains (DGg13 and BB133) (Intanoo and Chamswarng, 2007). *Trichoderma* species also believed can successfully lessening pathogen infection that capable to fight for surface area, thereby lessening pathogen infection success (Jeffries and Koomen, 1992; Maymon et al., 2004). *Trichoderma* species have been used to control *Colletotrichum* species in chilli (Boonratkwang et al., 2007), strawberries (Freeman et al., 2001), and citrus in Belize (Moretto et al., 2001) with contributing disease decline. Other biological control agents such as Bacillus subtilis and Candida oleophila that have been tested for it efficacy against *C. acutatum* (Wharton and Diéguez-Uribeondo, 2004)

The control of chilli anthracnose fruit rot has, for many years, depend on chemicals and develop in many unwanted problems. There is a need to combine alternative control factor that are persuasive in field. Biological control of fruit rot and

dieback of chilli with plant products proven in many laboratories and field trials showed that the crude extract from rhizome, leaves and creeping branches of sweetflag (*Acorus calamus L.*), palmorosa (*Cymbopogon martinii*) oil, *Ocimum sanctum* leaf extract, and neem (*Azadirachia indica*) oil could inhibit growth of the anthracnose fungus (Than, Prihastuti, Phoulivong, Taylor & Hyde, 2008). Among the bio-fungicides used against the fungus *Colletotrichum* spp. on chilli fruit, Charigkapakorn (2000) found that the most effective control was sweetflag crude extract when tested in two intervals when the majority of the plants were at the first bloom stage and at the mature bloom stage

2.7 *Trichoderma* sp. as biocontrol agent

Trichoderma was used as biocontrol against soil borne pathogens, when demonstrated to be consistently effective, practical and economic, can serve as a model for the introduction and implementation. There are 9 species of Trichoderma recognised by Rifai (1969), *T. piluliferum, T. koningii, T. polysporum, T. hamatum, T. aureoviride, T. harzianum, T. longibrachiatum, T. pseudokoningii* and *T. viride*. *Trichoderma* spp. was generally used manage many soil borne plant pathogens in crops such as in black gram, brinjal, chilli, coconut, tomato, citrus and others.

Table2.2 *Trichoderma* species and its uses against differences plant pathogens.

Plant	**Causative Agent**	***Trichoderma* Species Used**	**Ref.**
Black gram	*Macrophomina phaseolina*, *alternate*	*T. harzianum, T. viride, T. virens*	(Rahman Kha & Shahid, 2016; Singh et al., 2015)

	Alternaria		
Brinjal	*Sclerotium rolfsii, Alternaria solani,*	*T. viride, T. harzianum*	(JADON & TIWARI, 2013; Ganie, Ghani, Nissar & Rehman, 2013)
Chickpea	*Fusarium oxysporum f. sp. Ciceris, Rizoctonia solani, F. solani*	*T. harzianum, T. viride*	(Bashir et al., 2017; BHAGAT & PAN, 2011; Mudawi, 2014)
Chilli	*Rhizoctonia solani, Pythium aphanidermatum, Meloidogyne incognita,*	*T. viride, T. harzianum, Trichoderma atroviride*	(Rini & Sulochana, 2006; Muthukumar, Eswaran & Sanjeevkumas, 2011; Herrera-Parra1, Cristóbal-Alejo & Ramos-Zapata, 2017)
Coconut	*Ganodermalucidum*	*T. harzianum, T. viride*	(Karthikeyan et al., 2006)
Coffee	*Phomopsisthaeae, Glomerellacingulata*	*T. harzianum*	(Deb, Deb & Dutta,1999)
Cowpea	*R. solani*	*T. koningii*	Latunde-Dada, 1991)
Groundnut	*Sclerotium rolfsii, Macrophomina phaseolina, Aspergillus niger , R. solani, P aphanidermatum, M.*	*T. harzianum T. viride, T. longibrachiatum*	(Biswas & Chitreswar, 2000; Karthikeyan, Sankaralingam & Nakkeeran, 2006; Kishore, Pande, Rao, & Podile, 2001; Bagwan,

	phaseolina		2011)
Pigeon pea	*Fusarium udum*	*T. virens*	(Chaudhary, Kumar & Kushwaha, 2017)
Tomato	*Meloidogyne javanica, Sclerotium rolfsii, Pythium aphanidermatum, Rhizoctonia solani,*	*T. harzianum T. viride T. longibrachiatum, T. virens*	(Lobna et al, 2016; Banyal, Mankotia & Sugha, 2008; Jayaraj, Radhakrishnan & Velazhahan, 2006; Sreenivasaprasad & Manibhushanrao, 1990).
Cauliflower	*Sclerotium rolfsii, Fusarium oxysporum, Pythium spp, Rhizoctonia solani, Phytophthora spp*	*T. harzianum,*	(Uddin, Akhtar, Islam & Faruq, 2011; Ahuja et al., 2012)
Citrus	*Pythium ultimum, Penicillium digitatum,*	*T. harzianum T. viride*	(Kean, Soytong, & To-anun, 2010; Abo-Elnaga, 2013)
Cotton	*Rhizoctonia bataticola, Fusarium oxysporum*	*T. harzianum*	(GAUR, SHARMA & SINGH, 2005; Sivan & Chet, 1986)
Ginger	*Pythium aphanidermatam*	*T. harzianum*	(Ayub, Sultana, Faruk, Rahman & Mamun, 2009)
Sesame	*F. oxysporium , M.*	*T. viride T.*	(Elewa, Mostafa, Sahab

phaseolina,	*harzianum*	& Ziedan, 2011)

The *Trichoderma* species was contain many of fungal strains that act as biological control agents, have activation of multiple mechanisms as the antagonistic properties. *Trichoderma* spp. has evolved many mechanisms that are involved in attacking other fungi and also can enhance plants and root growths which are competition for nutrient and space, mycoparacitisms, production of the inhibitory compound and inactivation of the pathogens enzyme. (Ozbay & Newman, 2004). Competition is the mechanisms of biological control activity of *Trichoderma* spp. against phytopathogenic fungi, *Trichoderma* species are generally consider being vigorous competitor. The observed inhibition action of *Trichoderma* was related with a high rate and extent of carbon dioxide aggregation. Thus, the plant pathogenic fungi that were more represented by slow rate of carbon dioxide production were more susceptible to the antagonistic (Ozbay & Newman, 2004).

Mycoparasitism, the lead attack of one fungus on another, is a very complicated process that contains subsequent events, including recognition, attack and subsequent penetration and killing of the host. *Trichoderma* spp. may apply direct biocontrol by parasitizing a territory of fungi, identify other fungi and growing towards them. When chitinases weaken fungal cell walls, they produce oligomers that persuade exochitinases, and attack begins (Benítez, Rincón, Limón, & Codon, 2004).

Major cause of biocontrol activity of *Trichoderma* is involved with production of chitinases to degenerate the cell wall of fungal phytopathogens (Anand & Reddy, 2009).Biological control of fungal diseases of plants including seed borne diseases using non-pathogenic fungi and bacteria has received increasing attention of plant

pathologists and soil microbiologists all over the world. Antagonistic microorganisms tested to seeds prior to planting, colonize rhizosphere of seedling and thus are existent at or near the pathogen's infection court, where they act by producing antifungal properties through hyperparasitism, or by aggressive colonizing spermosphere and rhizosphere substrate (Dandurand & Knudsen, 1993). Matroudi, Zamani and Motallebi (2009) tested 30 *Trichoderma* segregates and on the basis of maximum level of chitinase and determined that *T. atroviride* can be engaged in the field as biological control agents against *Sclerotinia sclerotiorum* .Woo and Lorito (2007)()studied that various species of *Trichoderma* belongs to soil microbes that have capability to antagonize the plant pathogens. These biocontrol species are known to produce different kinds of Cell Wall Degrading Enzymes such as chitinases, glucanoses, proteases and synergism (Benítez, Rincón, Limón, & Codon, 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of Anthracnose Infected Chilli

The chilli which is red and mature that infected with anthracnose were collected from local market at Jeli, Kelantan.

Figure 3.1: Black circular spot shows anthracnose infection on fruit of chillies

3.2 Preparation of Potato Dextrose Agar Medium

19.5 g of Potato Dextrose Agar (PDA) in powdered form was weighed and suspended in 500 ml of distilled water of media bottle. Next, the mixture heated with frequent agitation and boiled to make medium completely dissolved. The media bottle

was sterilized by autoclaving in 121°C at 15 psi for 15 minutes. After that, about 20ml/L of streptomycin added to media and mixed well. Then, the plates were prepared by pouring about 9ml of PDA media into Petri dish quickly in aseptic condition to prevent contamination later. All plates were sealed with Parafilm, labelled and put in chiller to allow the media be solidified to produce agar plate.

3.3 Isolation of *Colletotrichum sp.* from Infected Chilli Tissue

Isolation of *Colletotrichum* sp. from infected parts of chilli was made by surface sterilizing the disease area. The advancing margin of lesion was selected from infected part and cut into small pieces that containing both diseased and healthy tissue. Both of the tissues were put into surface sterilizing agent solution (10% sodium hyperchlorite) for 30 seconds. The tissue section was washed thoroughly in three times with distilled water to free them from chemical residue and dried with the tissue to remove excess water. Then sterilized pieces were put in PDA plate. The plates were sealed, labelled and place in room temperature.

3.4 Pure culture of *Colletotrichum* sp.

A thin piece of agar from isolates *Colletotrichum* sp. plate was put into the centre of PDA plate in order to obtain a pure culture of *Colletotrichum* species. In order to the culture to remain as pure culture, the culture was observed in order to ensure there was no contamination from other fungus or bacteria.

3.5 Preparation of Slide Culture for Identification

A bent glass rod was placed in sterile petri plate side, and a sterile glass slide was put on the glass rod. A 1-by-1-cm block potato dextrose agar (PDA) cut with a sterile scalpel was then transferred to the glass slide. Using sterile wire needle, the fungus would then be inoculated from culture plate to the four sides of the agar block. Sterile coverslip was put over the block with slight pressure to ensure adherence. Approximately 2 mL of sterile water was put on the bottom of petri plate, and then the plate cover was replaced. The whole procedure was repeated for each of culture used in this study. When everything set, the plates were put on clean plastic basket and incubated at 30° Celsius (ROSANA, MATSUZAWA, GONOI & KARUNIAWATI, 2014).

3.6 Identification of *Colletotrichum* under Microscope

For identification and characterization of *Colletotrichum* species, both microscopic and macroscopic characters were determined. As macroscopic characters emphasized more to the colony characteristic like colony outline, texture and growth rate. As for microscopic identification, slide culture technique by Johnson (1946) was used as a method of preparation of *Colletotichum* colonies for examination and identification of *Colletotrichum* species. It allowed the fungi to be studied virtually. A thin film of agar from PDA media contained *Colletotrichum* species was prepared on microscopic slide and observed under microscope. The identification was done based on their morphological characterization like the shape of conidia and appressoria, type of hyphae were observed under compound microscope (LEICA DM750).

3.7 Sources of *Trichoderma* spp.

Selected Trichoderma species which were *T. hazianums* (TRUJT5) strains and *T. parareesei* (TRUJG1) strains were subcultured in aseptic condition. All the sources were provided from previous study that had been done by Universiti Malaysia Kelantan. Both fungi were subcultured into PDA medium to get pure culture.

3.6 Antagonistic activity of *Trichoderma* spp. against *Colletotrichum* sp.

The antagonistic activity of *Trichoderma* species of *T. hazianum* (TRUJT5) strains and *T. parareesei* (TRUJG1) strains were evaluated against *Colletotrichum* species. Two steps were involved in dual culture method where a thin piece of agar was taken from the periphery of pure culture of *Colletotrichum* sp. and inoculated 1cm away from the edge of PDA plate. After 24 - 48 hours, a thin piece of *Trichoderma harzianum* (TRUJT5) strains and *Trichoderma parareesei* (TRUJG1) strains were transferred at the opposite of *Colletotrichum* sp. continuity with 1cm away from the edge plate in separately. Plate that only contained with *Colletotrichum* sp. was served as control.

The radial growth was measured and the percentage of growth inhibition of *Colletotrichum* sp. was determined by using Percentage Inhibition of Radial Growth (PIRG) that developed by Skidmore and Dickinson (1976). The formula as follow:

$$PIRG = \frac{R1 - R2}{R1} \times 100$$

Where,

PIRG = Percentage Inhibition of Radial Growth

R1 = Pathogen radial growth in control plate absent of *Trichoderma* sp.

R2 = Pathogen radial growth in presence of biocontrol agent of *Trichoderma* sp.

3.7 Analysis of Data

The collected PIRG data were analysed to determine the antagonistic activity of each strains of *Trichoderma* species, *T. hazianum* strain (TRUJT5) and *T. parareesei* strain (TRUJG1) against *Colletotrichum* species.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Identification of *Colletotrichum* sp.

The anthracnose disease caused by *Colletotrichum* sp. is an important disease that primarily cause fruit rot in chilli. The *Colletotrichum* species were obtained from infected chilli in local market at Jeli, Kelantan. The chilli chosen were mature chilli that shown anthracnose symptom which is black circular or angular sunken lesions, with concentric ring of acervuli.

In this study, pure culture of isolated *Colletotrichum* sp. was done where the colony of fungi was grew from the centre of 90 mm Petri dish and let in room temperature. The observation of *Colletotrichum* cultures showed a slow growth of mycelium where the fungus took 14 days to fully grown in 90 mm of Petri dish. Identification and characterization of the fungi were based on macro and microscopic characteristics where macroscopic identification was highlighted the colony colour, colony reverse and colony outline while for the microscopic identification was emphasized more to shape of conidia and appressoria and also type of hyphae.

4.1.1 Macroscopic characters

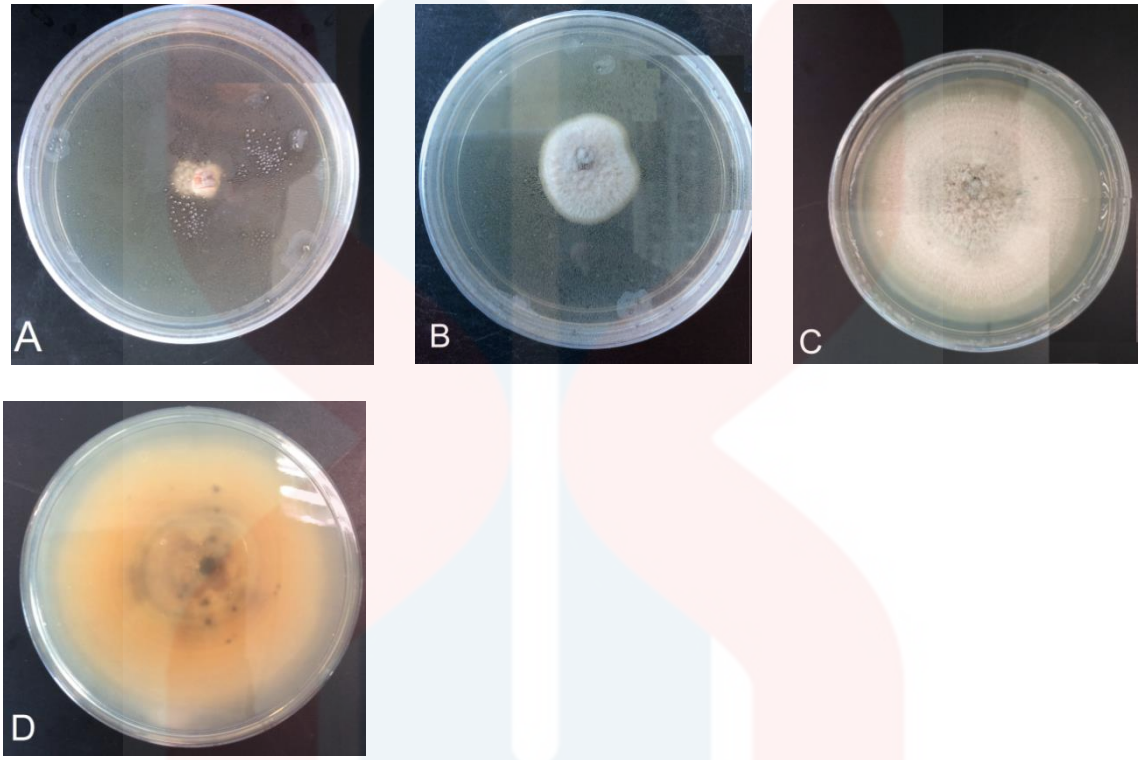

Figure 4.1: Macroscopic Characteristic of *Colletotrichum* sp. A: From front side on Day 3 B: From front side on Day 5 C: From front side on Day 14 D: From back side on Day 14

The colony characters were observed under dissecting microscope. Based on Figure 4.1, colour appeared with pale grey colonies, with sporadic white aerial mycelia. The back side of the colonies was bright orange conidial masses were observed near the inoculum point. Based on the previous study, all listed characters were shown similarities with *Colletotrichum gloeospioides* where Photita, Taylor, Ford, Hyde and Lumyong (2005) stated that the colonies contained sparse, pale grey to black mycelium with a few orange conidial masses near the inoculum point while Liu et al., (2016) found that the colony produced pale yellowish colonies, with sparse white aerial

mycelia. The reverse side of the colonies was white, and many bright orange conidial masses were observed near the inoculum point.

4.1.2 Microscopic characters

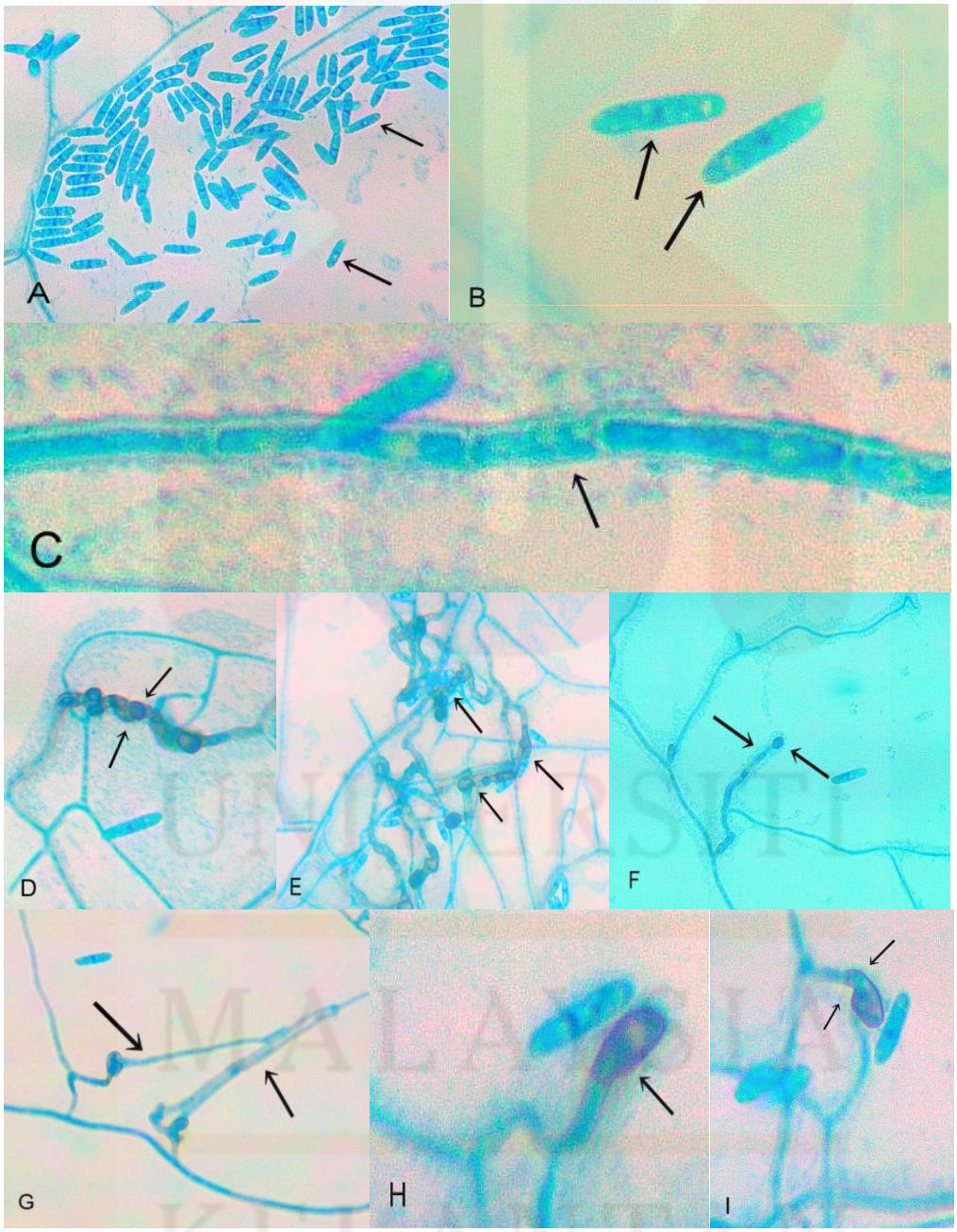

Figure 4.2: Microscopic Characteristic of Colletotrichum sp. A:Mass Of Conidia; B: Conidia; C: septate hyphae; D: Mycelial appressoria; E: Mycelial appressoria; F: Hyphal appressoria; G: Hyphal appressoria; H: Hypha appressoria; I: Hyphal appressoria

Identification results were based on the observation under compound microscope by using oil immersion technique. Based on Figure 4.2, shape of conidia were cylindrical with obtuse to slightly rounded end. Hyphal structure was tubular and dividing wall called septum were existed. Ovoid, clavate and slightly irregular appressoria were observe where the formation were existed before the fungus infecting the host plant. Appressorium used to infect or penatrate the host plant in order to develop colonisation of cell.

As result from previous study, Liu et al. (2016) found that the Colletotrichum gloeosporioides were cylindrical conidia with obtuse to slightly rounded end. The conidia were aseptate, but they often develop a septum after germinating and forming appresoria. The conidia appressoria were varied from ovoid to slightly irregular in shape and from brown to dark black in colour. The mycelial appressorium were varied from ovoid, clavate and slighty irregular to irregular, smooth or slightly lobed and they were light brown and brown in colour. The conodiosphore were hyaline to pale brown, simple or septate, rarely branches and smooth wall and also nearly cylindrical but narrower toward the end.

4.2 Antagonistic Activity of *Trichoderma* spp. against *Colletotrichum* sp.

For the antagonistic activity of *Trichoderma* against *Colletotrichum* sp. a plate away experiment was conducted. The actively growing mycelia of *Colletotrichum* sp. was transfer first into the edge of petri dish and after 24 hour, the *Trichoderma* mycelia was transfer on opposite of *Colletotrichum* species. To provide a uniform antagonistic assessment, *Trichoderma* sp. was inoculated within 24 hours after *Colletotrichum* sp.

was put in the same Petri plate. This was because of the growth of *Colletotrichum* sp. was slower compared to *Trichoderma* sp. based on collection average radial growth of *Colletotrichum* sp. and *Trichoderma* sp.

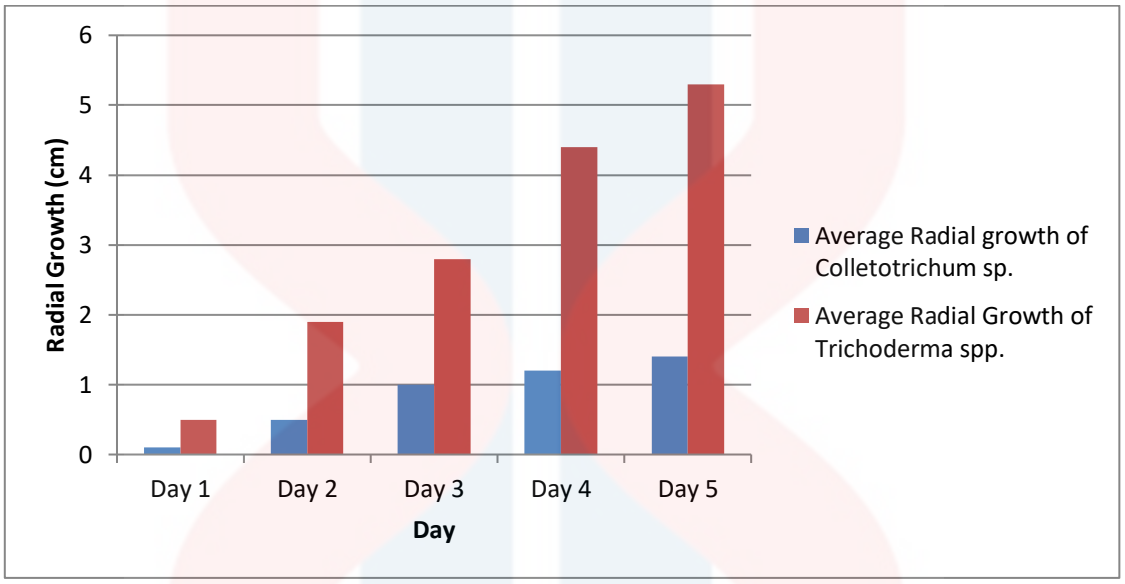

Figure 4.3: Average Radial Growth of *Colletotrichum* sp. and *Trichoderma* sp.

Dual culture result shown that the concavity that the depression oriented more towards to the colony of *Colletotrichum* sp. where all ten strains of *Trichoderma* sp showed rapid growth compared to *Colletotrichum* species. Contact zone between these two fungi was observed to see that biocontrol agent, *Trichoderma* sp. able to control fungal pathogen, *Collethtrichum* sp. and also determine effectiveness of *T. hazianum* and *T. parareesei*.

In order to determine the antagonistic activity of *Trichoderma* sp. from *T. hazianum* and *T. parareesei* against *Colletotrichum* sp., radial growth of *Colletotrichum* sp. in both control plate and dual culture were both measured for 7 days and the Percentage Inhibition Radial Growth (PIRG) analysis were conducted.

Table 4.1: Mean Percentage Inhibition of Radial Growth (PIRG) of *Trichoderma hazianum* and *Trichoderma parareesei* against *Colletotrichum* sp.

Antagonistic isolates	**Radial Growth of *Colletotrichum* sp. (cm)**	**Mean PIRG (%)**	**Scale of Antagonistic Activity**
TRUJT5(1)	**0.4**	**75.00**	**+++**
TRUJT5(2)	0.6	62.50	+++
TRUJT5(3)	0.6	62.50	+++
TRUJT5(4)	**0.5**	**68.75**	**+++**
TRUJT5(5)	0.6	62.50	+++
TRUJG1(1)	**0.5**	**68.75**	**+++**
TRUJG1(2)	0.7	56.25	+++
TRUJG1(3)	0.6	62.50	+++
TRUJG1(4)	0.7	56.25	+++
TRUJG1(5)	0.7	56.25	+++

Descriptive assessment of antagonistic was scale as follow

++++ = Very high antagonistic activity (>75 PIRG)

+++ = High antagonistic activity (61 – 75 PIRG)

++ = Moderate antagonistic activity (51 – 60 PIRG)

\+ = Low antagonistic activity (<50 PIRG)

Antagonistic result found that average Percentage Inhibition Radial Growth (PIRG) of *Trichoderma* sp. against *Colletotrichum* sp. (Table 4.1) showed values between 62.5% until 75% in seven days. It was proven that all strains successfully inhibited the colony growth of *Colletotrichum* sp. especially *T. hazianum* strains TRUJT5(1) obtained the highest value of Percentage Inhibition Radial Growth (PIRG) which is 75% while *T. parareesei* strains TRUJG1(1) and *T. hazianum* strains

TRUJT5(4) obtained same value which 68.75% of PIRG that categorized as high antagonistic activity.

According to Soytong (1988), Percentage Inhibition Radial Growth value from 61% - 75% was categorized as a high antagonistic activity. However, PIRG value of all strains as categorized as a high antagonistic activity which grown from 62.5% - 75%.

Despite the analysed Percentage Inhibition Radial Growth in 7 days found that all strains of *T. hazianum* and *T. parareesei* successfully controlled the fungal pathogen, *Colletotrichum* sp. in anthracnose disease of chilli as TRUJT5(1), TRUJT5(4) and TRUJG1(1) obtained three highest value of PIRG analysis compared to other strains. Hence, continuous antagonistic assessment for *T. hazianum* strain TRUJT5(1), TRUJT5(4) and *T. parareesei* strain TRUJG1(1) were done.

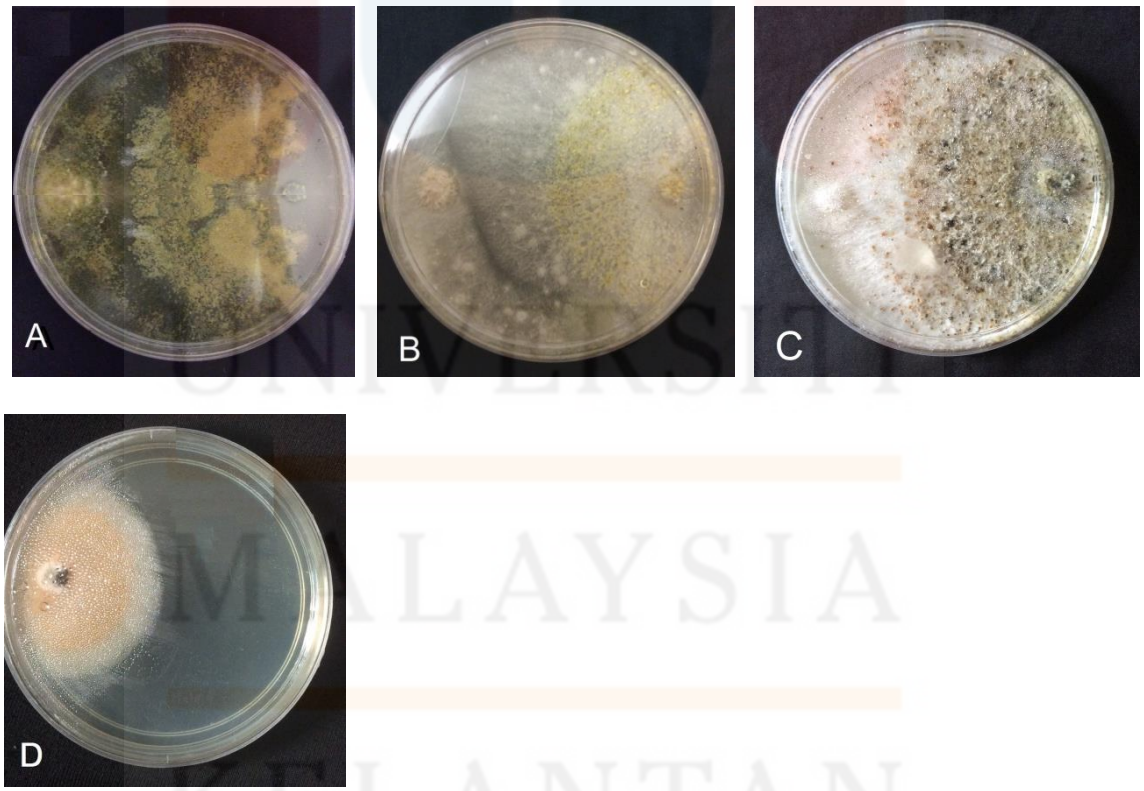

Figure 4.4: Dual Cuture of Antagonistic Activity on Day 14 of *Trichoderma* sp (right side) and *Colletotrichum* sp (left side). A: *T. parareesei* strain TRUJG1(1) B:*T.*

hazianum strain TRUJT5(1) C: *T. hazianum* strain TRUJT5(4) D: *Colletotrichum* sp. control strain

Based on the figure above, antagonistic results found that on day 14, three strains of TRUJG1(1), TRUJT5(1) and TRUJT5(4) were monopolized each plate where the colonies of *Colletotrichum* sp. in all three plates were suppressed and unable to grow I the same plate of *Trichoderma* species. These phenomena shown the inhibition growth of *Colletotrichum* sp. in culture plate. It was proved that the presence of *Trichoderma* sp. strains had a important role in inhibit and suppress the growth of Colletotrichum sp. in culture media and able to be a good biocontrol agent against the anthracnose disease in chilli.

CHAPTER 5

CONCLUSION

The collection and isolation of pathogenic fungi from the infected red mature chilli by anthracnose disease were done. Then, the identification and characterization of the fungus were done according to both macro and micromorphological properties. As the results obtained from this study, there were pale grey colony, with sporadic white aerial mycelia. The back side of the colonies was bright orange conidial masses were observed near the inoculum point. The shape of conidia were cylindrical with obtuse to slightly rounded end. Ovoid, clavate and slightly irregular appressoria were observed. Similar observations were found from previous study of isolated *Colletotrichum gloeosporioides*. Unfortunately, the species cannot be confirmed 100% accurate due to poor identification and characterization process in this research. As for the antagonistic activity of *Trichoderma sp., T. harzianum* strain (TRUJT5) and *T. parareesei* strain (TRUJG1) against *Colletotrichum* sp. was proved that *Trichoderma* sp. were good biocontrol agent in disease management of anthracnose in chilli where strain *T. harzianum* TRUJT5(1) obtained the highest value of Percentage Inhibition Radial Growth (PIRG) which 75% while *T. harzianum* strains TRUJT5(4) and *T. parareesei* TRUJG1(1) obtained same value which 68.75% of PIRG that categorized as high antagonistic activity. The presence of *Trichoderma* sp. strains clearly had important role in inhibit and suppress the growth of *Colletotrichum* sp. in culture and able to be good biocontrol agent, *Trichoderma* sp. needed to be produced and used in large scale

production not only as an alternative option to control anthracnose disease in chilli, but also can be used to control disease in other crops.

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