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Antagonistic Activity of *Trichoderma harzianum* and *Trichoderma koningii* Against *Colletotrichum* sp.

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

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DECLARATION

I hereby declare that the work embodied in this report is entirely the result of my own work and that where any material could be construed as the work of others, it is fully cited and referenced with appropriate acknowledgement given.

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LIST OF ABBREVIATION AND SYMBOLS

ANOVA	Analysis Of Variance
PDA	Potato Dextrose Agar
PGI	Percent Growth Inhibition
sp.	Species

Aktiviti antagonistik *Trichoderma harzianum* dan *Trichoderma koningii* Terhadap *Colletotrichum* sp.

ABSTRAK

Kajian ini adalah mengenai aktiviti antagonistik *Trichoderma harzianum* dan *Trichoderma koningii* terhadap *Colletotrichum* sp. yang mana penyakit bintik berpusar telah menjadi punca penurunan hasil dan pengeluaran cili oleh petani. Tujuan kajian ini adalah untuk mengetahui sama ada kedua-dua kulat *Trichoderma* sp. boleh merencat pertumbuhan *Colletotrichum* sp. dalam satu tempoh masa. Eksperimen yang dijalankan dengan cili yang dibeli dari pasaran Jeli yang telah dijangkiti penyakit bintik berpusar. Kemudian, bahagian yang dijangkiti akan dibiakkan pada dektros kentang (PDA). Selepas itu, *Colletotrichum* sp. yang awalnya perlu dibiakkan lagi ke PDA baru untuk memperbanyakkan populasi kulat. *Trichoderma* sp. yang disediakan dari stok makmal juga perlu dibiakkan dari hasil awalnya ke PDA baru untuk memperbanyakkan populasi kulat. Selepas ketiga-tiga pembiakkan kulat, aktiviti antagonistik *Trichoderma hazianum* dan *Trichoderma koningii* terhadap *Colletotrichum* sp. boleh diteruskan dengan membiakkan *Colletotrichum* sp. dan *Trichoderma koningii, Colletotrichum* sp.dan *Trichoderma harzianum* dan hanya *Colletotrichum* sp. sebagai kawalan dalam plat PDA baru. Data diambil setiap hari dengan mengambil panjang *Colletotrichum* sp. pertumbuhan *Colletotrichum* sp. dari bahagian belakang plat. Hasilnya adalah seperti yang dijangkakan di mana *Trichoderma* sp. boleh merencatkan pertumbuhan *Colletotrichum* sp. dengan peratusan PGI *Trichoderma harzianum* adalah 78% dan *Trichoderma koningii* adalah 83% terhadap *Colletotrichum* sp..

Kata kunci: *Trichoderma harzianum, Trichoderma koningii, Colletotrichum* sp., Cili, Antagonistik

Antagonistic Activity of *Trichoderma harzianum* and *Trichoderma koningii* Against *Colletotrichumsp.*

ABSTRACT

This study is about the antagonistic activity of *Trichoderma harzianum* and *Trichoderma koningii* against *Colletotrichum* sp. which antrachnose disease has been the cause of decline in chili yield production and concern by the farmer. The aim of this study is to observe whether the both *Trichoderma* sp. fungi can inhibit the growth of fungus, *Colletotrichum* sp. within period of time. The experiment carried out by bought chili fruits from Jeli market which has been infected with antrachnose disease. Then the infected part will be cultured and growth on Potato Dextrose Agar (PDA). After that, the pure culture of *Colletotrichum* sp. need to be subcultured into new PDA for multplying the fungus population. Same goes to the *Trichoderma harzianum* and *Trichoderma koningii* need to be subcultured from pure culture into new PDA which provided from laboratory stock. After the three fungus growth, the antagonistic activity of *Trichoderma* sp. against *Colletotrichum* sp. can be proceed by culture *Colletotrichum* sp. and *Trichoderma koningii*, *Colletotrichum* sp.and *Trichoderma harzianum* and only *Colletotrichum* sp. as control in new PDA plate. The data were collected everyday by taking the length of the *Colletotrichum* sp.growth from backside of plates. The result outcome is as expected where the *Trichoderma* sp. fungi can inhibit the growth of *Colletotrichum* sp. with PGI percentage of *Trichoderma harzianum* is 78% and *Trichoderma koningii* is 83% against *Colletotrichum* sp..

Keywords: *Trichoderma harzianum, Trichoderma koningii, Colletotrichum* sp., Chili, Antagonistic.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Chili plant has been cultivated and grown since 3500 B.C and was reported used in Mexico as spices for their food. In 1492, Christoper Columbus was the person who brought chili in to the rest of the world where he traveled from Spain to bring the spices from India. After that chili start to spread to other countries became indispensable spice and popular spices for other country to cultivate and have. About quarter of the earth population on the Earth consume chili every days (Jodi, 2018). In the Capsicum genus there are about 25 species of chili also the spiciness and pungent are variety. Other than that, there are also variety colour and shape of the chili that can consume variety way (Steve, 2008).

Colletotrichum sp. is a fungi which also known as a worldwide plant pathogen that causing anthracnose. Anthracnose is a disease that attacked cereals, vegetables, legumes and fruits which the most important crop for economic. Generally, the disease symptoms appeared on the chili fruits such as sunken necrotic tissues with acervuli concentric rings usually wet. This disease has reduce the marketability of chili which

also cause a big loss to the farmer. The *Colletotrichum* species that has been known to cause anthracnose disease are *C.capsici, C.gloeosporioides, C.acutatum and C.coccodes*. The *Colletotrichum* sp. identification is by the morphological characteristic and cultural characteristic (Than et al, 2008).

Trichoderma sp. is soilborne fungi which is green spored ascomycetes that easily can be discovered globally. There are many studies has been done regarding *Trichoderma* sp. characteristics and applications. They have been known in their habitat as successful colonizer because they could confront their competitors efficiently. *Trichoderma* sp. also have been use for agricultural management such as biopesticide like fungicide where *Trichoderma* sp. potentially act as novel antibiotics also they also acts as defense mechanism in against the enemy (Andre & Monika, 2010).

This study concentrate on the antagonistic activity of *Trichoderma hazianum* and *Trichoderma koningii* against *Colletotrichum* sp.. The aim of this study is to observed the growth of the *Colletotrichum* sp. could be inhibited by the presence of *Trichoderma* sp. which could help in anthracnose disease management in agriculture industry.

1.2 Problem Statement

Chili is a crop that been consume globally and the cultivation of chili plant need a systematically management to produce high yield production. Chili plant may suffer from many disease that cause by fungi, bacteria or insect. For example, antrachnose is a disease cause by fungi which is a major disease problem in chili cultivation. This due to the reason that this disease could cause about 50% of yield loss where it not available for marketability. This disease will produce dark spot and expand rapidly in the field which difficult to control especially during rainy season or excessive irrigation presence (Faisal & Muhamad, 2011).

Trichoderma sp. is a beneficial fungi that has been used in many studies and resulting to positive finish. This fungi has been reported can help in against pathogen where they could induce systemic resistance (Andre & Monika, 2010). The aim of this study is to observed whether the *Trichoderma* sp. could inhibit the growth of *Colletotrichum* sp. by in-vitro experiment. The observation of this research could prove and used in order to prevent from the growth or spread of *Colletotrichum* sp. that has been attacking chili plant and cause loss of yield production.

1.3 Objectives

The objectives of the study are:

1. Identification of *Colletotrichum* sp. in the antagonistic activity

2. To observe the antagonistic activity of *Trichoderma harzianum* and *Trichoderma koningii* against *Colletotrichum* sp.

1.4 Scope Of Study

Anthracnose is a soil-borne disease cause by *Colletotrichum* sp. that really need to be concerned by the agricultural industry since this disease cause decrease in yield of production. This disease potentially wipes out the yield production of chili cultivation. A treatment to prevent from loss of yield production is needed in order reduce this disease to spread widely. The scope of the study is to observe the antagonistic activity of *Trichoderma* sp. in against *Colletotrichum* sp. by in-vitro method within a period of time.

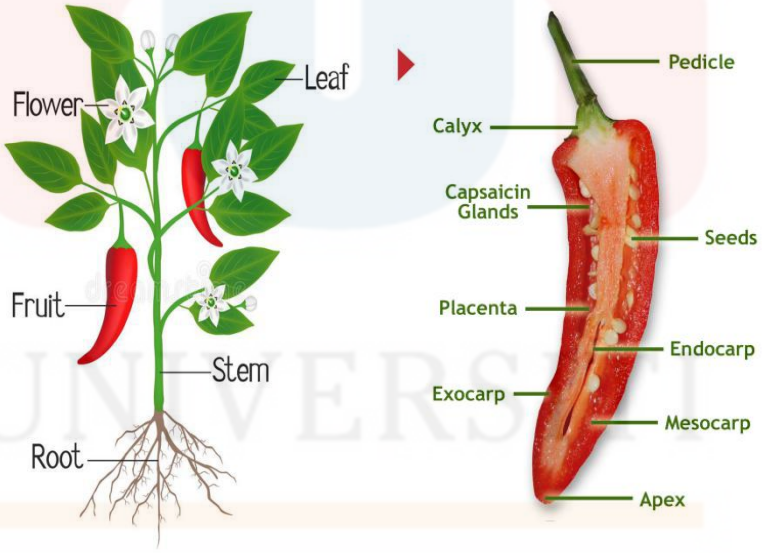
1.5 Significant Of Study

As mentioned before, *Trichoderma* sp. is a beneficial fungi that has been used as biological agent. For example, the usage of *Trichoderma* sp. could control prevent disease growth since they contain systemic resistance so by using this fungi in agricultural industry such as biopesticide and biofertilizer they could enhance the yield production of crops. Other than control disease growth they also act as plant growth promoter which their strains could solubilize phosphate and micronutrients. This study also provides opportunity to advance the understanding about the anthracnose disease and fungus behavior which can be related to chili plantation.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction Of Chili



(a) (b)

Figure 2.11: (a) Chilli plant morphology (Nadiia, 2000), (b) Chilli fruit anatomy (Sarah, 2014)

Chilli has been use in daily life of people around the world and become a very important value as a vegetable and spices. It also become an ingredient for culinary

purpose for it unique pungency. Chilli or known as genus Capsicum is define from Latin which mean box or chest because the fruit shape encloses the seed nicely. Chilli mainly cultivated in sub-tropical and tropical countries such as India, Mexico, Japan and others. There are about 4000 varieties of chilli around the world with variety or range of heat from mild to super hots. The varieties of chilli keep growing by breeder which produce to unexpected result. The chilli been identified based on their morphology like flower colour, fruit surface, fruit shape, or flower per axil (Prem & Jessina, 2016). There are also varieties of colour which range from racing green to purple also bi-colour and tri-colour. Other than that, they also come with different sizes and shapes from tiny round to elongated which can reach about 30 cm long, some chilli are long, thin, fat, round, wedges, spiral, mushroom or heart shaped.

There are five most known or popular chilli species which are *Capsicum chinense* which is the hottest among the five species, *Capsicum annum* can be found most of the places or can be known as worldwide species, *Capsicum frutescens* has shrub appearance, *Capsicum baccatum* grow vine-like plant, and *Capsicum pubescens* has black seed. The chilli fruit body part consist of several distinct part that can be found by dissect them such as pedicle or known as stalk to hold the fruit, calyx which joins the chilli and the stem, capsaicin glands where the hotness of the chilli placed, placenta function by holding the capsaicin glands, apex which is the chilli pod tip, seeds, exocarp is the outer surface of chilli, mesocarp is the mid-pod and endocarp the inner flesh of chilli pod (Sarah, 2014).

2.2 Antrachnose Of Chilli

Figure 2.21: Chilli infected with anthracnose disease (Philip & Premavathi, 2015)

Anthracnose is a disease cause by fungi known as *Colletotrichum* sp. which is a very concerned and serious disease that affecting the production yield. Anthracnose is define from Greek which is mean coal because the disease characteristic dark, sunken lesions, and contain spores. This disease cause major damage to the chilli fruits during pre-harvest and postharvest which not acceptable for market value. *Colletotrichum* sp. cause latent pathogen which mean its a very important plant phatogens to be aware. This disease not only attacking the fruit but it also can occur on the plant leaves and the stems.

Like other fungus *Colletotrichum* sp. also have varities of species and different species infecting different part of the chilli plant. For example, *C.acutatum* and

C.gloeosporioides only infect the chilli fruits, *C.coccodes* and *C.dematium* usually infect the plant leaves and stems and *C.coccodes* could damage the seedling (Po,Haryudian&Kevin,2008). Sakarindr and Narong (2014) has reported that *C.gloeosporioides C.capsici* and *C.acutatum* are the major species of *Colletotrichum* sp. cause anthracnose disease. Different type of species also play different role during the mature phase such as *C.capsici* growth widely in red chilli while *C.acutatum and C.gloeosporioides* usually widespread on young and mature green chilli.

The survival ability of *Colletotrichum* sp. has been reported that they can survive in or on the chilli seeds as acervuli and micro-sclerotia where the seeds are coated. It also can survive over the winter on the alternative host like rotten fruits or plant debris in the field. They produce micro-sclerotia naturally in order during winter or stress condition it could allow dormancy and survive for many years. While during wet and warm surrounding the conidia located on micro-sclerotia and acervuli could be splashed by rain or water from excessive irrigation from infected to healthy fruit and foliage of the plant. Infected fruits with disease could be the sources or host for the fungi to spread from plant to other plant in the field (Po, Haryudian & Kevin, 2008).

2.2.1 Identification Of *Colletotrichum* Species

Generally, the identification of *Colletotrichum* species are based on their morphological characteristic like shape and size of appressoria and conidia, setae, teleomorph state or the cultural characteristic like colony colour, rate of growth and the

texture. However the criteria alone not enough to prove or identify the species correctly because error might happen such as overlap in morphological characteristic or the variation of phenotypic (Po, Haryudian & Kevin, 2008). There are varieties of shape of the conidia such as round to elongate, cylindrical and falcate shaped. Some species has presence sclerotia such as *C.coccodes* with globose shaped, *C.dematium* with conical scletoria and *C.graminicola* has irregular shape while some of other species has absence of sclerotia. Other than that, the appressoria for some species might be entirely margin, crenate margin with deeply lobed or irregular lobed.

2.2.2 *Colletotrichum Capsici*

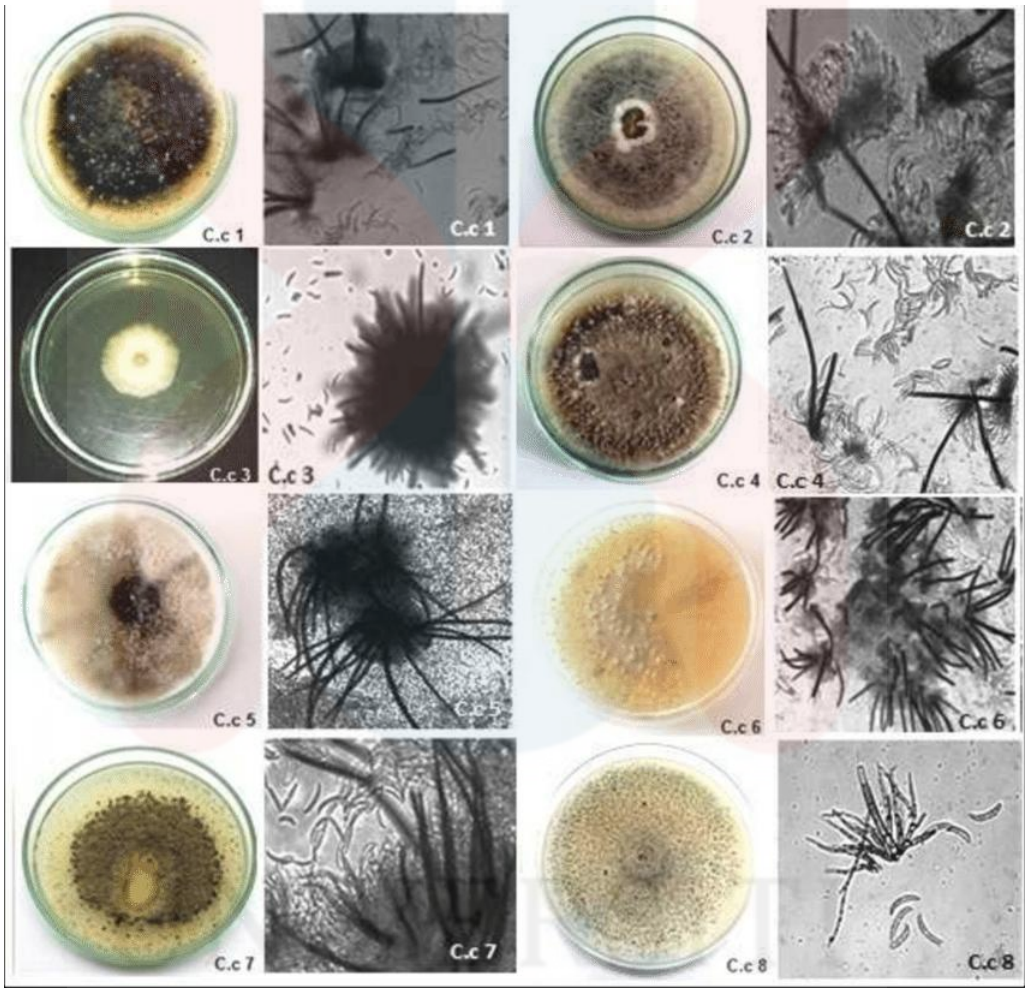


Figure 2.2.21: Macro and microscopic view of eight different isolates of *Colletotrichum capsici* species (Srideepthi, Lakshmisahitya, Peddakasim, Suneetha & Krishna, 2017)

Different species of *Colletotrichum* sp. may have different behaviour or role such as during the chilli mature phase. *C.capsici* play it part by spreading in red chilli fruits. There are also report that *C.capsici* has been wide spread in India, Indonesia, Papua New Guinea, Thailand and Vietnam. Generally, *Colletotrichum* sp. can survive on or in

seed of the fruits and also can be transferred through transplant that infected with the disease. There are two pathway infection of *C.capsici* to the chilli fruits which are through the invasion of the seed coat and the testa opening. This fungi causing the seedling to suffer from root rot. Furthermore, there are studies of antagonistic of certain bacterial strain to control *C.capsici* which is dominant disease causal of anthracnose were succeed. *Thrichoderma* sp. also able to reduce the disease infection to control *Colletotrichum* sp. in chilli, strawberry and citrus (Than et al, 2008).

Saket, Vinay and Vineeta (2015) have reported that the causal disease of antrachnose in chilli is *C.capsici* where the fungi causing severe damage to the chilli fruits during pre-harvest and post-harvest. The infected part of the chilli pod will appear brown lession on them and will turn to black due to setae and sclerotia formation. They also stated that the culture of the colonies are white pale grey or pale orange also might producing purple or pinkish pigment and poor development of conidiomaeta with no setae or few only.

2.3 *Trichoderma s*p.

Trichoderma sp. is a fungi with green spore ascomycetes that can be discovered globally. It is a beneficial fungi that have been studied and resulting as a efficiently fungi against their opponent. They also beneficially as deleterious interaction with the hosts, production and secretion of enzyme, development of sexual and response to the environmental. Furthermore, they also beneficial in biocontrol where the strains of the

genus development can be promising biopesticide for agricultural industry. *Trichoderma* sp. can be found anywhere decaying plant is present and in rhizosphere of plants. This fungus are differentiate by the growth rate, greenish colour of conidia and conidiophore branch structure. They are capable of survive in different environment and diversified habitat as well dark place or sterile laboratory setting. Even with all these condition they still able to grow and capable to modify their lifestyle which can be used for beneficial things (Andre & Monika, 2010).

Trichoderma harzianum is a biocontrol agent that is effective in counter several fungus that are soilborne plant pathogen. The antagonistic activity of this fungi is widely recognized as a promising biocontrol agent in counter several plant pathogen. It grow out into the surrounding from the roots and forming a network of external hyphal which function as mineral nutrients uptake also help in enhance the plant growth (Helge, John, Pal, Dan & Iver, 1999). *T.harzianum* also the first established of biofungicide in the market with proven in controlling some common soil-borne diseases such as Rhizoctonia, Pythium and Fusarium. *Trichoderma koningii* is also fungi use as biocontrol agent in counter plant disease which commonly used to counter fungus-induced plant disease. Reported that *T.koningii* and *T.harzianum* able to kill root knot nematode totally. *T.koningii* benefits to the plant health and uptake of nutrient with highly active in calcium oxalate crystal biomineralizing in the soil (Gary, Sarah & Irina, 2006).

CHAPTER 3

METHODOLOGY

In order to determine the parameter value of percentage growth inhibition (PGI) value of the antagonistic activity of the *Trichoderma* sp. against *Colletotrichum* sp. an appropriate experimental data is needed. Laboratory work had been done to observed the growth of fungus. Below are the chronological steps in the study:

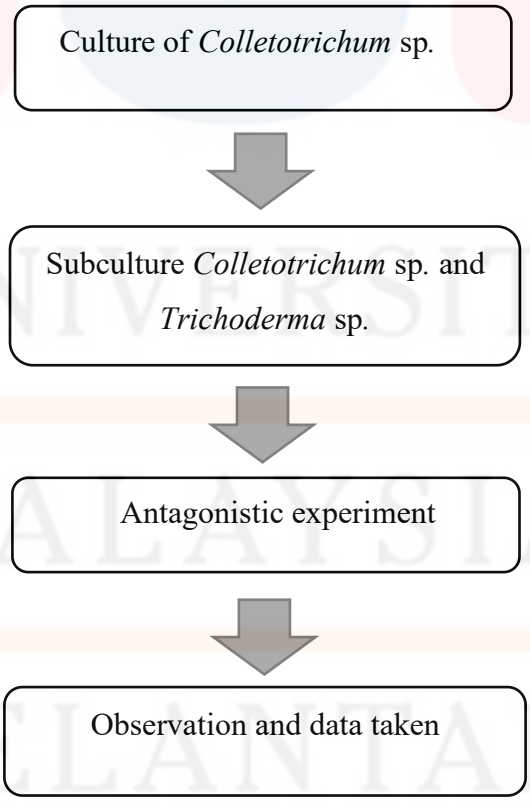


Figure 3.01: The flow chart of the experiment

3.1 Culture

3.1.1 Culture Of *Colletotrichum* sp.

The chillies (*Capsicum annuum L.*) infected and has symptom of anthracnose disease was bought from Jeli market. Then, the work done in laminar flow in order to keep save from the fungus spreading. The infected part of chilli pod with the anthracnose disease was cut off into small pieces using sterilize surgical knife. After that, the small pieces of infected chilli pod will be immersed in sodium hypochlorite or known as chlorox for a minute then rinsed with distilled water two times. Next, tranfer and blot them with filter paper to dry the excess water and cultured on to Potato Dextrose Agar (PDA) (Maria, Kurt, Maria, Rosa, 2001). The petri dishes were sealed with parafilm in order to prevent from contamination and kept in room temperature.

3.1.2 Subculture Of *Colletotrichum* sp. And *Trichoderma* sp.

After *Colletotrichum* sp. from the pure culture growth larger in the plate about one to two weeks then subculture them into new plate. This is to multiply the population of fungus in the pure culture. The *T.harzianum* and *T.koningii* were multiplied the population by subculture both of the fungus in new petri dish contain PDA media (Svetlana et. al, 2010). The petri dishes were sealed with parafilm in order to prevent from contamination and kept in room temperature. The *Trichoderma* sp. were taken

from Universiti Malaysia Kelantan laboratory stock. After about one week the colony of the three fungus are ready for the antagonistic experiment.

3.2 Antagonistic Activity Of *Trichoderma* sp. Against *Colletotrichum* sp.

After around one week after the subculture of *Colletotrichum* sp. and *Trichoderma* sp. the fungus can be used and ready for the antagonistic experiment. For this phase, there are three different component which are plates for control which consist of *Colletotrichum* sp. only, plates consist of *Colletotrichum* sp. and *T.harzianum* and plates consist of *Colletotrichum* sp. and *T.koningii*. The antagonism was performed on PDA by dual culture method (Shovan, Bhuiyan, Begum & Pervez, 2008). Every pieces of culture must be inoculated 1 cm from the plate side and seal the petri dish with parafilm in order to prevent from contamination and put in room temperature. The data was observed and collected everyday within a period of time. The percent growth inhibition was calculated using the formula:

$$PGI(\%) = (T1-T2)/T1 \times 100$$

T1 is represent the distance of *Colletotrichum* sp. growth from the point of inoculation to the margin of the colony of control petri dishes while T2 represent the distance of *Colletotrichum* sp. growth from the point of inoculation to the margin of the colony of the treated petri dishes in the direction of the antagonist (Svetlana et. al, 2010).

3.3 Anova Analysis

Analysis of Variance (ANOVA) is a statistical method that has been used when there are two or more factor or means. It is function for general test rather than specific difference of the means. The benefits of using ANOVA is help in analyze complicated types of analysis and ANOVA has been highly used because easy to understand the research report especially for comparing means. There are two type of ANOVA analysis which are one-way ANOVA and two-way ANOVA where one-way is used when the research only has one independent variable while two-way is used when there are two or more of independent variable (Stephanie, 2014). For this experiment, one-way ANOVA has been used in order to get the desired result.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 The percent growth Inhibition (PGI)

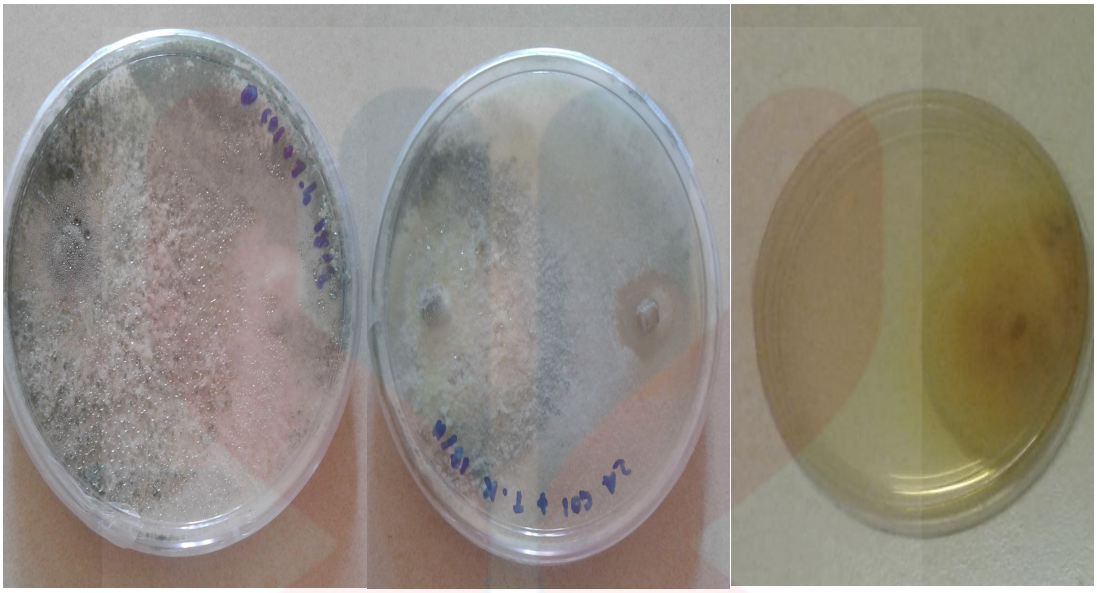
Table 4.11: Antagonistic activity of *Trichoderma* sp. against *Colletotrichum* sp. within 15 days.

DAY	CONTROL (CM)	*T.HARZIANUM* (CM)	*T.KONINGII* (CM)
1	0.0	0.0	0.0
2	0.0	0.0	0.0
3	0.5	0.3	0.3
4	1.0	0.5	0.6
5	1.3	0.7	0.7
6	1.5	0.9	0.7
7	1.6	0.9	0.7
8	1.9	0.9	0.7
9	2.1	0.9	0.7
10	2.4	0.9	0.7
11	2.9	0.9	0.7
12	3.2	0.9	0.7
13	3.4	0.9	0.7

14	3.7	0.9	0.7
15	4.0	0.9	0.7

Table 4.12: Percent growth inhibition (PGI) of antagonistic activity.

DAY	*T.HARZIANUM* (%)	*T.KONINGII* (%)
1	0	0
2	0	0
3	40	40
4	50	40
5	47	47
6	40	53
7	44	56
8	53	63
9	57	67
10	63	71
11	69	76
12	72	78
13	74	79
14	76	81
15	78	83



(a) (b) (c)

Figure 4.13: (a) Antagonistic acitivity of *T.harzianum* against *Colletotrichum* sp. (b) Antagonistic activity of *T.koningii* against *Colletotrichum* sp. (c) Control consist of *Colletotrichum* sp. only.

Based on the result data the antagonistic activity of *Trichoderma koningii* against *Colletotrichum* sp. is faster than the antagonistic activity of *Trichoderma harzianum* against *Colletotrichum* sp. with the antagonistic of *T.koningii* is 0.7 cm while value for antagonistic *T.harzianum* is 0.9 cm also the control sample where only consist of *Colletotrichum* sp. is 3.4 cm for the final result. The data of the *Colletotrichum* sp. growth for antagonistic activity for the cultures is 15 days. From the observation the *Colletotrichum* sp. start to growth in the petri dishes on the third days and the *Trichoderma* sp. also start growing on third days. From the Table 4.11 the *Colletotrichum* sp. growth rate is slower than the growth of *Trichoderma* sp. and growth of *T.koningii* and *T.harzianum* in the plate whereas the reason why *Trichoderma* sp. could control the growth of *Colletotrichum* sp. from spreading.

From the result, on days 5 *T.koningii* has fully covered inside the petri dish and control the growth the *Colletotrichum* sp. while *T.harzianum* still growing and not fully cover the inside of the petri dish. However, on days 6 *T.harzianum* has fully covered the inside of the petri dish which also mean it also able to control the growth of *Colletotrichum* sp. in then plate. From this result, it shown that *T.koningii* able to control *Colletotrichum* sp. faster than *T.harzianum* because the growth of *T.koningii* mycelial more faster than the mycelial growth of *T.harzianum* (Maria, Kurt, Maria & Rosa, 2001).

The reason why the percent growth inhibition (PGI) for *T.koningii* is higher than *T.harzianum* is because the result of mycelial growth of *T.koningii* is faster than *T.harzianum* make the PGI is higher. However, even though T.koningii mycelial growth faster than *T.harzianum* it does not mean *T.harzianum* not capable to control *Colletotrichum* sp., *T.harzianum* also capable to control the growth of *Colletotrichum* sp. which has been proved in many studies that it able to control many plant pathogen and has been used in agricutural industry (Svetlana et. al, 2010). From this study, it shown that *T.koningii* and *T.harzianum* could against the *Colletotrichum* sp. with different mycelial growth rate. This also mean it is proven that *Trichoderma* sp. are promising biocontrol agents that have systemic resistance to help counter or control plant pathogen and can used in agricultural industry.

4.2 Identification Of *Colletotrichum* sp.

Figure 4.21: Chilli fruit infected by anthracnose

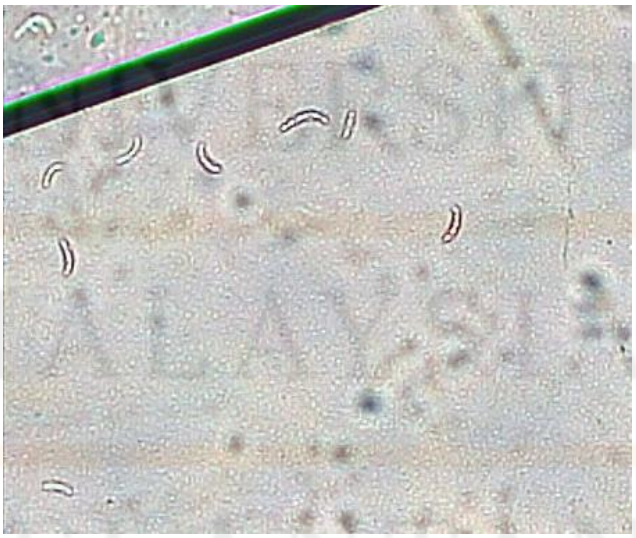


Figure 4.22: *Colletotrichum capsici* under 10X0.22 mignification.

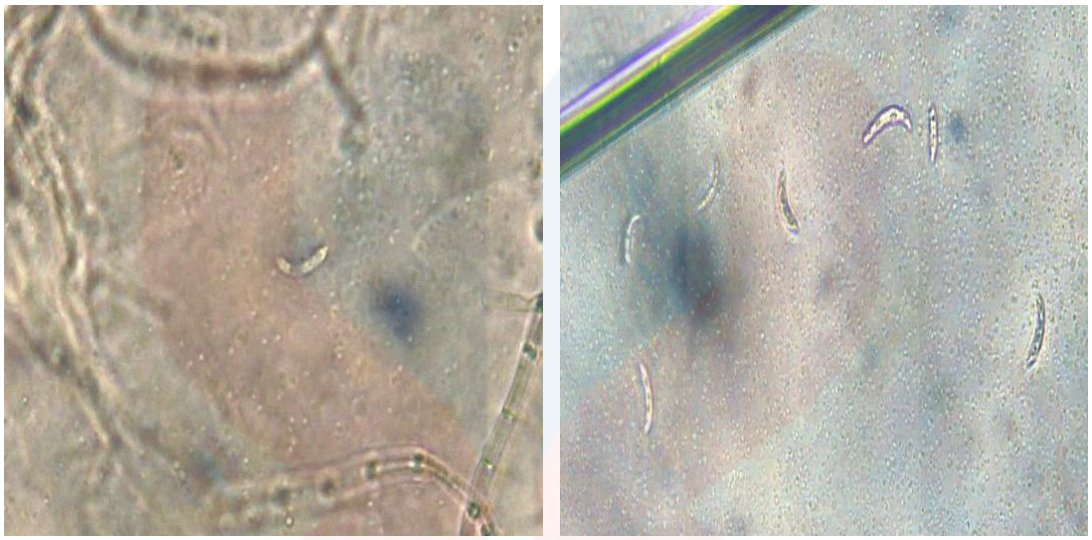


Figure 4.23:*Colletotrichum capsici* under 40X0.65 magnification

Figure 4.24: *Colletotrichum capsici* culture

The identification of *Colletotrichum* species for the experiment is *Colletotrichum capsici*. This is because this experiment has used *Capsicum annuum L.* as in Figure 4.21

which is red pepper where *Colletotrichum capsici* is the major cause of anthracnose disease in this chilli (Po, Haryudian & Kevin, 2008). The mycelial from the *Colletotrichum* sp. culture plate was took a little bit and spread on slide and cover it with cover slit then, the slide was observed under microscope to identify the *Colletotrichum* species by morphological characterization (Saket, Vinay & Vineeta, 2015). The isolated *Colletotrichum* sp. grown on the plate also has coloured varied from light to dark and the colony has cottony or fluffy mycelial growth with irregular margin also shown *Colletotrichum* sp. morphology (Srideepthi, Lakshmisahitya, Peddakasim, Suneerha & Krishna, 2017).

The growth rate of the fungus which take about less than 2 weeks to growth as big as Figure 4.23 which mean the the radial growth of this *Colletotrichum capsici* is very slow growing. The reason of the fungus is very slow might be due to the surrounding temperature which is at the moment was a rainy season and could related to the very slow growth where the fungus suppose to be keep in 27±2 ℃ and under darkness where the colony will growth on the second day (Saket, Vinay & Ruchi, 2015). The morphological characteristic of the *Colletotrichum* sp. for conidia shape is falcate shaped which also can been seen in the Figure 4.22 and 4.23 which also shown characteristic of *Colletotrichum capsici* morphology (Lubna, Ali, Shahzad, Sofi, 2013).

The *Colletotrichum* sp. isolated has shown pathogenicity establish of *C.capsici.* Moreover, the fungus was isolated from chilli pepper which is *Capsicum annuum L*. or also known as red chilli where the major infected chilli with *Colletotrchum capsici.* Based on the Figure 4.21 the *Capsicum annuum L* shown anthracnose diseases where

infected with the *Colletotrichum* sp. and show symptom that can been seen such as circular dark sunken lesions as Figure 4.21 (Than et al, 2008).

4.3 Anova Analysis

Table 4.31. Mean value of antagonistic activity of *T.harzianum* and *T.koningii* against *Colletotrichum* sp.

Sample	Mean	Standard deviation	Significant
Sample A - Control	1.964[a]	1.270	*0.000
Sample B - *T.harzianum*	0.769[b]	0.614	
Sample C - *T.koningii*	0.547[b]	0.547	

Based on the result, the data collected for the growth of the *Colletotrichum* sp. for antagonistic activity of the *Trichoderma* sp. and *Colletotrichum* sp. are synthesize using one-way analysis of variance (ANOVA). From the table below, the result are for control, 1.964 which has highest value than Sample B, 0.769 and Sample C, 0.547. The reason is because the growth of *Colletotrichum* sp. for control sample within the 15 days are increasing while the growth of *Colletotrichum* sp. in the Sample B and Sample C were stopped and controlled by the *Trichoderma* sp. and resulting as Table 4.31 shown. From this result, it shown that *Trichoderma harzianum* and *Trichoderma koningii* are capable of inhibit the growth of *Colletotrichum* sp. during the antagonistic experiment.

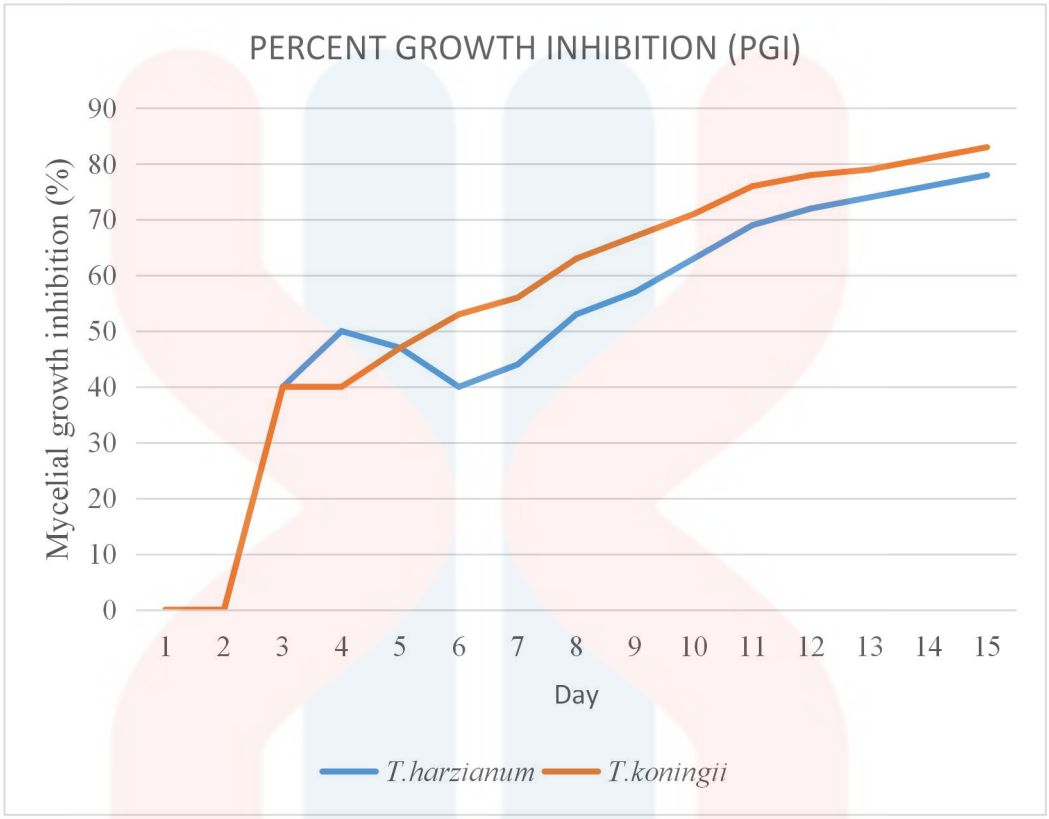


Figure 4.32: Percent Growth Inhibition of *Trichoderma harzianum* and *Trichoderma koningii*

The Percent Growth Inhibition (PGI) data were calculated using formula from the methodology to get the result in the Figure 4.32. From the chart in the Figure 4.32 above shown the PGI value of *T.harzianum* is lower than *T.koningii* whereas the final percentage for *T.harzianum* is 78% while *T.koningii* is 83%. This is because, the growth of *T.koningii* is faster than the growth of *T.harzianum* which mean *T.koningii* has controlled the growth of *Colletotrichum* sp. to spread in the petri dishes earlier than *T.harzianum* to controlled the growth of *Colletotrichum* sp. in the plate. *T.koningii* has controlled the growth of *Colletotrichum* sp. on day 5 while *T.harzianum* able to controlled the *Colletotrichum* sp. on day 6 which is the reason of the differences

between the two *Trichiderma* sp. percent growth inhibition (PGI). The hypotheses of this experiment is accepted because *Trichoderma harzianum* and *Trichoderma koningii* can inhibit the growth of *Colletotrichum* sp. during the experiment. The inhibition growth of the mycelial increase as the growth of *Trichoderma* sp. also increase.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

To conclude the experimental data shown that *Trichoderma* sp. are beneficial fungal that can be used to counter against plant pathogen such as anthrachnose. During the 15 days of data collected the *T.koningii* inhibit the growth of *Colletotrichum* sp. faster than *T.harzianum* which on days 5 while *T.harzianum* able to inhibit the *Colletotrichum* sp. growth on days 6. The PGI of the the antagonistic activitiy for *T.harzianum* against *Colletotrichum* sp. are around 78% while for *Trichoderma koningii* against *Colletotrichum* sp. are around 83% which mean *T.koningii* has higher PGI than *T.harzianum*. This is because the growth rate of *T.koningii* is more faster than *T.harzianum*. The identification of *Colletotrichum* sp. is resulting to *Colletotrichum capsici* the fungus was identify using slide and observed under microscope. The identification also based on morphological characteristic such as the shape of the conidia and cultural characteristic like the growth behavior of the fungus.

For further study, there are some recommendation that could help improve the experiment such as by using more varieties of *Trichoderma* species in order to identify the best species could be used to produce a product for biocontrol agent in a comercial way for management agriculture industry.

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APPENDIX

Figure 1: Pure culture of *Colletotrichum* after 7 days.

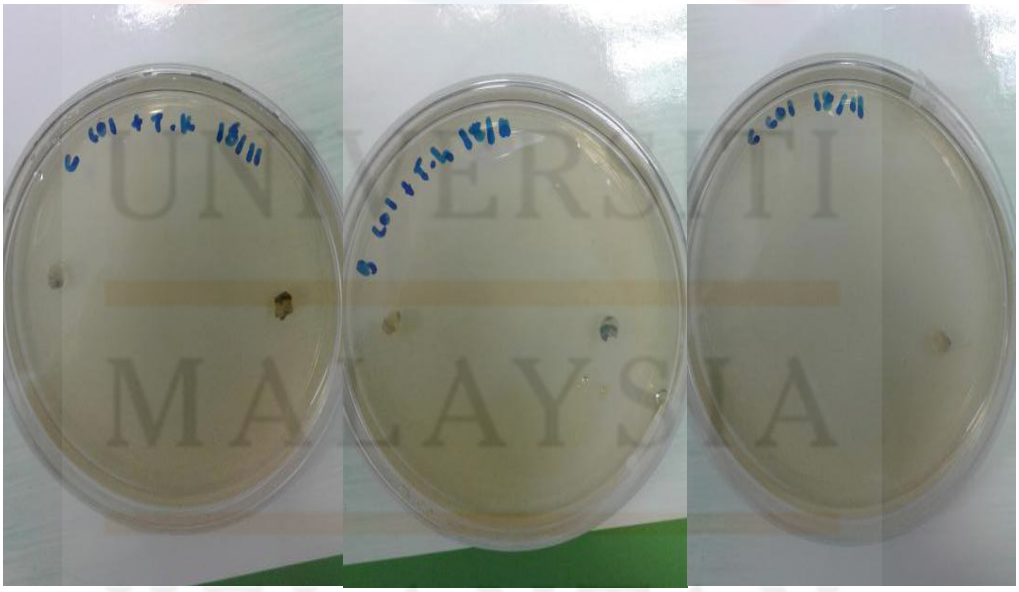

Figure 2: Day 1:Dual culture method of Antagonistic experiment

Figure 3: Day 4 of antagonistic experiment

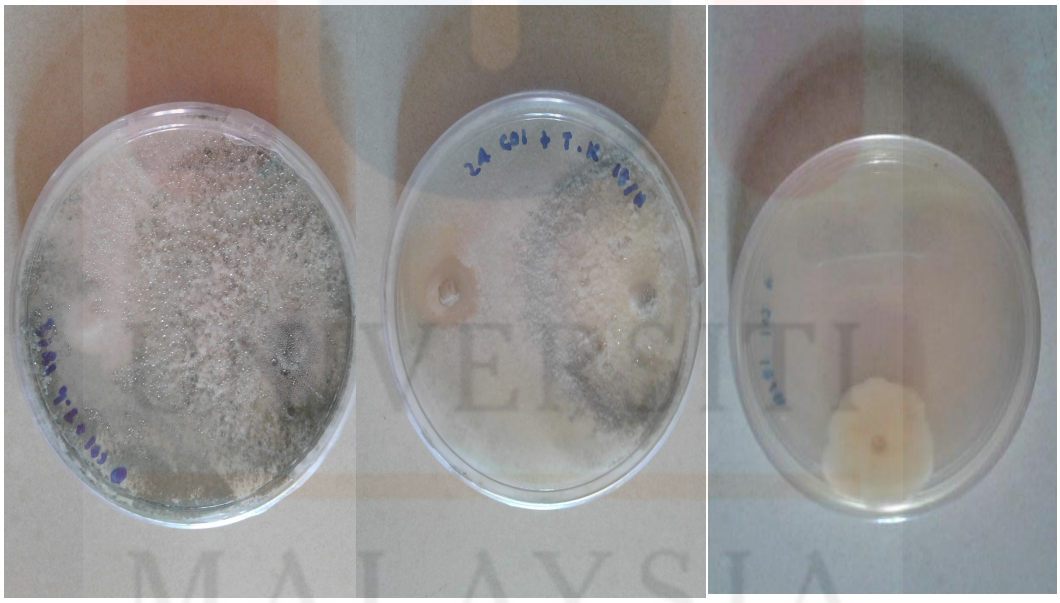

Figure 4: Day 7 of antagonistic experiment

Figure 5: Measuring the growth of *Colletotrichum* sp. for antagonisitic.

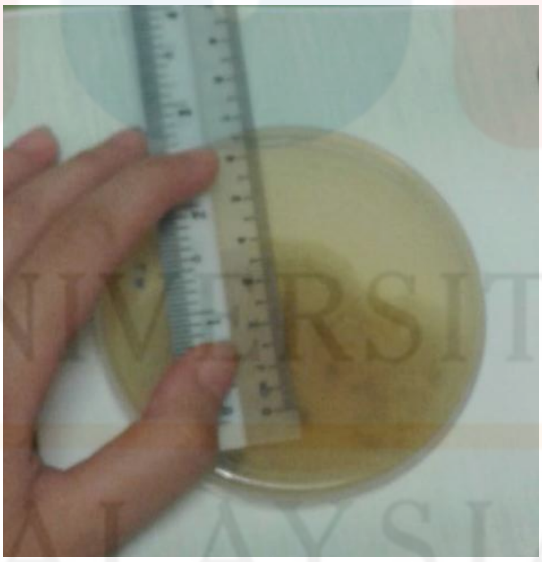

Figure 6: Measuring the *Colletotrichum* sp. for control sample.