[illegible] OF *CASSIA ALATA* ON
[illegible] OF MELON FRUIT ROT

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THE ANTIMICROBIAL ACTIVITIES OF *CASSIA ALATA* ON THE CAUSAL ORGANISMS OF MELON FRUIT ROT

By
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ENDORSEMENT

The project reported entitled **The antimicrobial activities of *Cassia alata* on the causal organisms of melon fruit rot** by **Wan Mahfuzah binti Wan Ibrahim**, Matric No. **UK15927** has been reviewed and corrections have made according to the recommendations by examiners. This report is submitted to the Department of Agrotechnology in partial fulfillment of the requirement of the degree of Bachelor of Science in Agrotechnology (Post Harvest Technology), Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu

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ABSTRACT

The objectives of this study were to observe the antimicrobial activities of *Cassia alata* (Gelenggang) extractions at different concentration and to evaluate the difference between disk diffusion and spore suspension methods. The crude extract of *C.alata* were prepared from its leaves parts. The antimicrobial activities were measured from the inhibition zone of the *Fusarium oxysporum* and *Fusarium solani* which is the causal organism for fruit rot diseases. The results showed that, from six concentrations that were used, the 10 mg/ml showed the best results because its yields higher inhibition zone in both methods and it is followed by 5 mg/ml, 2 mg/ml, 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml. The difference between this two methods were the spore suspension provides early inhibition compare to disk diffusion method but the disk diffusion methods yields better results in terms of area of the inhibition zone. Besides that, this two different strain also gives effect to the inhibition zone where the *F. solani* were re-growth on the inhibition sites after day 4 but for the *F. oxysporum*, the inhibition zone are maintained until day 8 because they are more susceptible compare to *F. solani*. From the analysis by using Tukey test, it showed that there were significance different ($P<0.05$) between the concentration especially for 10 mg/ml of extraction, compare with the control.

ABSTRAK

Objektif kajian ini adalah untuk memerhatikan aktiviti anti-microbial dari ekstrak *Cassia alata* (Gelenggang) pada kepekatan yang berbeza dan juga untuk menilai perbezaan antara teknik sebaran spora dan juga penyerapan cakera. Serbuk ekstrak daun gelenggang ini diperolehi daripada bahagian daunnya. Aktiviti an-timikrobial ini diukur berdasarkan kawasan perencatan yang ditunjukkan oleh *Fusarium oxysporum* dan juga *F.solani* yang merupakan agen penyebab penyakit reput buah. Hasil kajian menunjukkan kepekatan 10 mg/ml merupakan kepekatan yang terbaik apabila berjaya menghasilkan kawasan terencat yang paling luas dan diikuti pula dengan kepekatan 5 mg/ml, 2 mg/ml, 1 mg/ml, 0.1 mg/ml dan 0.01 mg/ml. Terdapat perbezaan antara kedua-dua teknik yang digunakan iaitu teknik sebaran spora dapat menghasilkan kawasan terencat dengan lebih cepat berbanding dengan teknik penyerapan cakera. Walaubagaimanapun, dari segi keluasan kawasan terencat, teknik penyerapan cakera menunjukkan kesan yang lebih baik. Selain itu, penggunaan dua spesies kulat berbeza juga memberi kesan apabila *F. solani* menunjukkan berlaku pertumbuhan semula kulat pada kawasan terencat manakala *F.oxysporum* menunjukkan kadar yang tidak berubah bermula hari keenam. Hasil daripada ujian Tukey telah menunjukkan terdapat nilai yang signifikan ($P<0.05$) antara kepekatan ekstrak yang digunakan terutama bagi perbandingan kepekatan ekstrak 10 mg/ml dan kawalan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The microorganisms such as bacteria, fungi and viruses are very harmful to human, animals and plants. Today, many research on the microbes have been done especially in antimicrobe properties. In earlier research, there was many chemical produce synthetic substance to treat microorganism such as benzoic acid, propionic acid and sorbic acid (Nguyen *et al.*, 1981). However, after the use of AF-2, [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide] is prohibit because it contains carcinogenicity that can cause cancer among human being, consumer are more aware to the food additive that was use in their food (Nguyen *et al.*, 1981). People are then tending to choose nature base substance as the additive especially to be use in their food. Even before 1950, there is more than 200 plant species are screened to investigate the antimicrobial compound in it that can be used or to be commercialize in daily life (Nguyen *et al.*, 1981). In this study, *Cassia alata* was used to define the antimicrobial activities from their leaves extraction.

Cassia alata or "gelenggang" is one of the natural source that is believe and proven to have antifungal components (Ibrahim & Osman, 1994). This herb can be easily found from wide region which is from America to India and Malaysia too. The antimicrobe compounds are basically extracted from its leave. Therefore, for this

study, the leaves extraction from 'gelenggang' were obtained to observe the antimicrobial activities towards *Fusarium oxysporum* and *Fusarium solani* which is the causal organism for fruit rot diseases. However, other parts of the plant also contain antimicrobial substance and a study shown that the most effective site of its extract is comes from its flower (Khan *et al.*, 2000). This species is a type of shrubs that contain lots of leaves and only small portion of its flower. In traditional medicine practice, *C. alata* has been used to treat several man diseases such as ringworm infection, herpes, gastrointestinal problems, liver disease and several skin diseases such as eczema and itching.

This study are conducted to see the effect of the extract from *C.alata* leaves towards plant pathogens which is *Fusarium* spp. that is the causal organism for the fruit and root rot in cucurbits. The fruit and root disease is one of the post harvest disease that can cause the loss of the yields up to 25-50% from the crop yields (Wilson *et al.*, 1990)

1.2 Problem Statement

The crops that affected by diseases will loss its yield and post harvest quality. The disease can cause by many factors and it is important to treat the harvested fruits, vegetables to ensure fewer diseases during storage. Many inorganic pesticides and fungicides are introduced but the chemical residue can harm human health. Nowadays, people are more concern about what they eat and the trend are changing to organic foods that are more natural and safe.

The problems is when farmers are growing their crop organically, the crop are still susceptible to the same diseases. So, how do they control the diseases without using inorganic chemicals is quite challenging. Thus, the quests of natural antimicrobial starts to become important.

1.3 Significance of Study

The trends of people nowadays has shift from chemical to nature because they are so concern about the chemical that has many side effect to them and environment. *Cassia alata* then already use for a long time ago in a traditionally medication especially to treat skin disease that cause by fungi. So, it can be utilize to create a new natural medication that is proved in scientific ways to convince people about the goodness of natural source which is *C. alata*.

This study is going to examine the effectiveness of the antimicrobial activities from *C. alata* to the fungi that is chosen which is *Fusarium oxysporum*. *F. oxysporum* is a type of microorganism that causing the Fusarium wilt disease that occur when this fungi colonizing the xylem of the plant that will cause the blockage and breakdown of the transport system of the plant that will resulting in leaf wilting and yellowing and also can lead to the death of plant.

If the *C. alata* can give inhibition to *F. oxysporum*, it might help to prevent fruit from the fruit rot diseases. So, we can use it commercially to help farmers to prevent this disease and they could yield better plant for human consumption.

1.4 Objectives of study

1) To observe the *Fusarium* spp. inhibition zone on different concentration of *Cassia alata* extractions.

2) To evaluate the differences between disk diffusion method and spore suspension method.

CHAPTER 2

LITERATURE REVIEW

2.1 Antimicrobial activities

Antimicrobial activities can be defined as the activities of the compound to fight the microbial infection. The source of antimicrobial agent can be use as the food preservatives since it can prolong the shelf life of the food by inhibit the microbial invasion that can destroy food and give food borne illness to the consumer. For example, the lactic acid bacteria that can produce peptides which known as bacteriocins that can give bactericidal action against a limited range of bacteria that is usually closely related to its producer organism. The best known of these bacteriocins is the lanthionine-containing nisin from *Lactococcus luctis* (Roller, 1995). Nisin has been known for over 50 years and is used effectively in processed cheese, cheese spreads, dairy desserts and canned foods worldwide (Delves-Broughton, 1990). The antimicrobial are important in food and agriculture industry since the microbial infection are one of the factor that causing deterioration in food and plant. Thus, the using of inorganic antimicrobial were introduced to fight the microbial invasion

effectively. However, since the human are more conscious about the inorganic material in their foods nowadays, the organic farming are start to take place in consumer demands (Roller, 1995). The organic farming means that there are no inorganic material or substance are using throughout the process from planting to harvest. So, many natural antimicrobial agent are introduced to replace the usage of fungicide during the plantation such as grape pomace extract (Ozkan *et al.,* 2004) and olive oil (Tassou & Hychas, 1995).

Besides the exploration of new substance as the antimicrobial agent, there are also development on the techniques or method to prevent the microbial invasion in produce. The biological control has proposed in a studied by Wilson *et al.* (1990) where they suggested the antagonistic microorganism as the control to plant pathogen. In 1983, there are a research that shows that application of *Trichoderma* species were able to reduce the Botrytis disease on the strawberry (Tong-Kwee & Rohrback, 1980). Then, in 1984, Colver and Mount has prove that the treatment of seed by using *Pseudomonas putida* by dip the seed in the suspension of the antagonistic bacterium before planting can help to reduce 15% of the occurrence of this disease. However, most research about antagonistic microorganisms to control diseases only shows successful result in laboratory but fail to shows the same symptoms in field (Wilson *et al.,* 1990).

2.2 Candle Bush, (*Cassia alata)*

Cassia alata is an attractive shrub that named for its flower buds which grow in a column and look like fat yellow candles and each of them complete with a flame.

The leaves fold together at night. It is native to Central America and is mainly encountered in the Caribbean area (Hennebelle *et al.*, 2009) but then it was introduced to other tropical areas from the America to India (Kirtikar and Basu, 1975), Fiji and also Malaysia and is now widely considered a weed. It is been use traditionally to treat various skin diseases such as Herpes ulcer, eczema, and also liver and gastrointestinal diseases (Ibrahim & Osman, 1994). Last time, it is known to contain active ingredients that can bring antimicrobial activities. In Philippines, it is one of the components that is added to the soap, shampoo and other detergent agent (Hennebelle *et al.*, 2009). While in Africa, its leaves are boiled to treat high blood pressure. The usage of *C. alata* is different in other region. Hennebelle *et al*,. (2009) had discussed about the ethnopharmacological of *C. alata* throughout a few region as in the Table 2.1. Etnopharmacological is a research on how people according to its tribe or ethnic utilize the herbs in terms to providing health benefits. There, we can see that the patterns of using the *C. alata* are different according to the functions and their mode of consumption. The people at Guatemala are the major user of *C. alata* where they use it for digestive, dermatologic, anti-infectious, and anti-diabetic. It involves the consumption of its leaves, decoction and maceration of *C. alata* during the treatment process. Asia people are the minimum user of the *C. alata* and in Sukajadi, West Java, Indonesia. The people there use the pounded leaves to treat dermatitis problem such as herpes and others.

Table 2.1 : Reported uses of *Cassia alata* in ethnopharmacological surveys.

(Hennebelle *et al.*, 2009)

Type of use		**Geographic zone or *population* (country)**	**Part used, method, mode of consumption**
Digestive	Constipation	Livingston (Guatemala)	Leaves, decoction, oral
		Trinidad	n.r.
		Martinique (France)	Leaves, decoction, oral
	Stomach pains	Livingston (Guatemala)	Leaves, infusion, oral
	Liver disease	Martinique (France)	Leaves, decoction, oral
	Pre-hepatic jaundice	Sango Bay area (Uganda)	Leaves, infusion, oral
Dermatologic	Skin diseases in general	Livingston (Guatemala)	Leaves, maceration, bath
		Akwa Ibom state (Nigeria)	Leaves, powder, external
			Leaves, decoction, oral
	Dermatitis	Sukajadi, West Java (Indonesia)	Leaves, pounded, topical
	Skin rash	Martinique (France)	Leaves, crushed, bath
	Athlete's foot	Martinique (France)	Leaves, crushed, external
	Herpes zoster	Akwapim-North district (Ghana)*	Leaves, juice/infusion, topical
	Eczema		
	Mycosis		
Antiinfectious	Malaria	Livingston (Guatemala)	Leaves, decoction, oral
	Flu	*Quilombola de Olho D'água dos Pires*, Esperantina, state of Piauí (Brazil)	Flowers, "tea", oral
	"Infectious diseases"	Guinea*	Fruit, decoction, oral
Antidiabetic		Livingston (Guatemala)	Leaves, decoction, oral
		Lagos, Ogun, Oyo, Osun states (Nigeria)*	Leaves, maceration, oral
Miscellaneous	Inflammation	Martinique (France)	Leaves, decoction, oral
	Thoracic pain	Martinique (France)	Flower buds, decoction, oral

This plant is belonging to the Caesalpinaceae Family (Hannabelle *et al.*, 2009). It is an annual herb with leathery compounded leaves. It can grow up to 6' tall and has erected waxy yellow spikes that resemble fat candles before the individual blossoms open. The large leaves are bilateral and symmetrical opposed and are fold together at night (Figure 2.1).

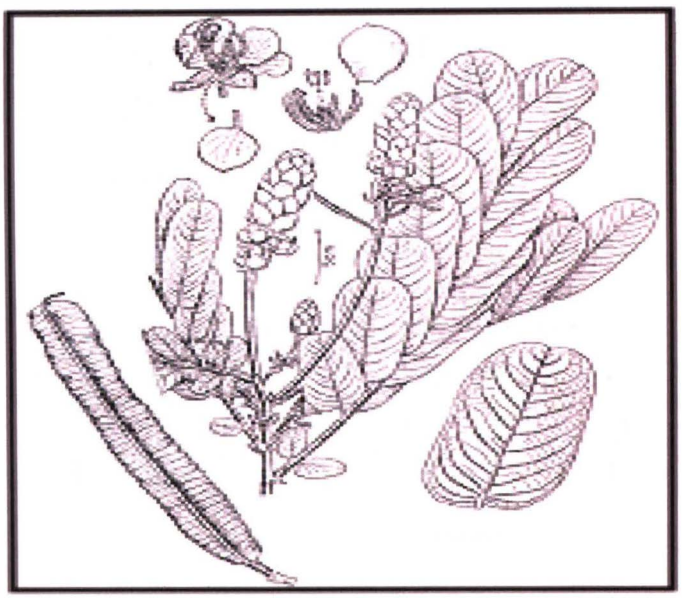

Figure 2.1 : The botanical features of *C.alata*

The fruit is in a pod, while the seeds are small and square in shape. *Cassia alata* is indigenous to Suriname and it is can be found in secondary vegetation or along riverbanks or moist and even wet spots. It is also a host plant to many species of Lepidoptera such as sulphur caterpillars, included the orange barred sulphure (Hannabelle *et al.*, 2009). The other common name for *C. alata* is Guajava, Fleur palmiste, fleur dartre, candlestick senna, wild senna, ringworm cassia, guajava, ketepeng badak, flor del Secreto, Tarantana, candle bush, akapulko, man-slabriki, akapulco, and gelenggang (Khan *et al.*, 2000). It had been reported that it contains

1,5,7-trihydroxy-3-methylanthraquinone which is known also as alatinone. It is a type of anthraquinone which is a substance that can inhibit the fungus and gives antiviral effect (Kirtikar & Basu, 1975). It also contain saponin that can functions as laxatives to expel intestinal parasites (Khan *et al.*, 2000). Previous studies had shows that all parts of *C.alata* which is root bark, stem, leaves and flowers are containing the antimicrobial agent when it is tested in-vitro and the flowers part are showing the most efficient compare to the other parts (Khan *et al.*, 2000). The sap or its extract from leaves are reported to have laxative effect and can be used against ringworm infection, scabies, ulsers and other skin diseases (Seaforth, 1960). The flowers, bark and wood can be boil to treat skin diseases also such as eczema, itching and asthma and bronchitis (Kirtikar & Basu, 1975). However, previous studies also shows that the *C. alata* extract can works effectively towards dermatophyte fungi and less efficient to the non-dermatophyte fungi that we use in this studies (Ibrahim & Osman, 1994).

2.3 Fusarium spesies causing fruit rot of melon

The fruit rot diseases are an example of the post harvest diseases that always attack the crops. The diseases can cause the lost of yield. In some crops, it is the major problem. For example, in the strawberry industry, the fruit rot diseases are causing losses up to 15% that valued about 100 million dollar in production of strawberry at Florida (Legard, *et al.*, 1997). The disease are enhanced by warm temperature and frequent rain during harvest (Wedges *et al.*,2006). There are several causal fungi that can causing this diseases such as *Colletotrichum* spp., *Botrytis* spp. and *Fusarium* spp.

The distribution of the fruits all over the world makes the diseases can spread more widely (Snowdon, 1990).

There are wide range of pathogen that can be the causal agents of post harvest diseases that often causing the fruit root symptoms such as *Phfyctuena vagabunda*, *Penicillium expansum* and *Botrytis cinerea* (Zhou et al., 2008). In the apples production, the fungi that frequently effecting apples during the storage stage are *Phlyctuena vagabunda* that causing the 'lenticel rot' that shows symptoms like dry and sunken discolored lesions surrounding the fruits.

One of the causal agents of fruit rots in melons are the *Fusarium* spp (Soriano-Martin *et al.*, 2006). This type of fungi is the soil-borne pathogens that can be found at the soil. It is common diseases to the cultivation of melon because melon is a type of crops that grows by its vine (Suavez-Estrella *et al.*, 2004). Melons are from the Family Cucurbitaceae that also including large numbers of cucurbits like gourds, squash, cucumber and luffas . Most cucurbits are growth on annual vines with large,, yellow or white flowers but there are also woody, shrubs or trees in this Family. The stems of Cucurbitaceae are hairy and its leaves are simple palmate lobed or compound. The reproductions of Cucurbits can be dioecious or monoecious (Yong-Hong *et al.*, 2008).

The pre-harvest diseases that cause by *Fusarium* spp to the melon crops are the Fusarium Wilt which is a very dangerous disease that contributes to 90% of the crop losses in melon cultivations (Soriano-Martin *et al.*, 2006). Besides that, it also can cause the post harvest diseases such as the fruit rots because the melon fruits are attach to the soil and have direct contact to the soil that may contaminated by the Fusarium spp (Soriano-Martin *et al.*, 2006). Most cases of fruit rot in melon are cause

by the improper post harvest handling of the melon fruits where the pathogens such as *Fusarium* spp are not fully eliminate from the fruits. So, the diseases are still can occur during the storage (Wedges *et al.*,2006). The fruit rots in the melon can causing the symptoms like lesion on the skins that can reduced the quality of the fruits and lower the value of the fruits at the markets (Snowdon, 1990).

CHAPTER 3

MATERIALS AND METHODS

3.1 Methods

There are two stages in this project which is the extract preparation and in-vitro screening. For the in-vitro screening, there are two method used because we want to see the differences between this two method. It is disk diffusion method and spore suspension method.

3.2 Extract Preparation

The sample of *C. alata* leaves were taken from Bukit Payung, Marang. The leaves were clean and it dried in the oven at 40°C for 48 hours. The leaves were then grinded into powder form by using the blender. The *C. alata* powders was then soaked into ethanol and put it on orbital shaker for 24hours to make sure it soaked thoroughly. Then, the mixture was evaporated using rotary evaporator to eliminate the ethanol and

produced a crude extract of *C. alata* that were going to be used in the experiment. The extract was diluted by distilled water to get six concentrations that were used which were 0.01mg/ml, 0.1mg/ml, 1.0 mg/ml, 2.0 mg/ml, 5.0 mg/ml and 10.0 mg/ml.

3.3 In-Vitro Screening

This is a method to evaluate the antimicrobial activities of the *C. alata* extraction towards the growth of *F. oxysporum* and *F. solani* that are used in this paper. The culture of fungi were comes from the culture stock that were isolated from the fruit rot diseases of melon. The inhibition zone of this fungi were observed every two days until day 8.

3.3.1 Disk Diffusion Method

The Potato Dextrose Agar (PDA) plates were prepared. The micro disks were soaked into the extraction that were prepared previously. Then, transfer the disk onto the PDA plate (Figure 3.1). The inoculums of fungi was transfer into the center of the plate. Seal the plate with parafilm and observe the plate every two days for eight days.

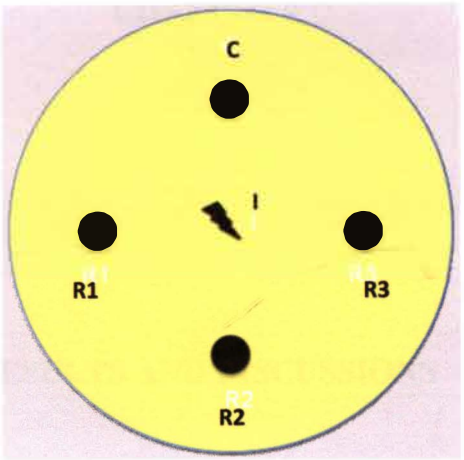

Figure 3.1 : PDA plate with I: Fungi inoculums, C: Control , R1,R2 & R3 : Replicates

3.3.2 Spore Suspension Method

Streaks of fungi culture were added to the unsolidified warm Potato Dextrose Agar. Then, pour the PDA into the petri dish and let it solidified. The micro disks are then soaked into those six concentrations and put it onto the center of the plate. Seal the plate with parafilm and observe the plate every two days for eight days.

3.4 Data Analysis

The data that are obtained in this experiment was analyzed by using the SPSS software to get the mean and standard deviation. Then, Tukey Test was used to define the significance of the data to compare between this two methods.

CHAPTER 4

RESULTS AND DISCUSSIONS

From the results that obtained from the experiment, there are several things that we need to take note to conclude whether the objectives are achieved or not.

4.1 Spore Suspension Method

This method are conducted for two strain of *Fusarium* which is *F. oxysporum* and *F. solani*. There are six concentrations of extractions that were used which are 1mg/ml, 0.1mg/ml, 0.01mg/ml, 2mg/ml, 5mg/ml and 10 mg/l. We use high concentration like 2mg/ml, 5 mg/ml and 10 mg /ml were used because from previous study, it shows that the extraction from *C. alata* are showing high activity in inhibition of dermatophyte pathogens such as *Cladosporium wernecki* and *Penicillium* sp. (Ibrahim & Osman, 1995) compare to non-dermatophyte pathogen that affecting the fruits. The figure below shows the inhibition zone of *F. oxysporum* and *F. solani* towards the days.

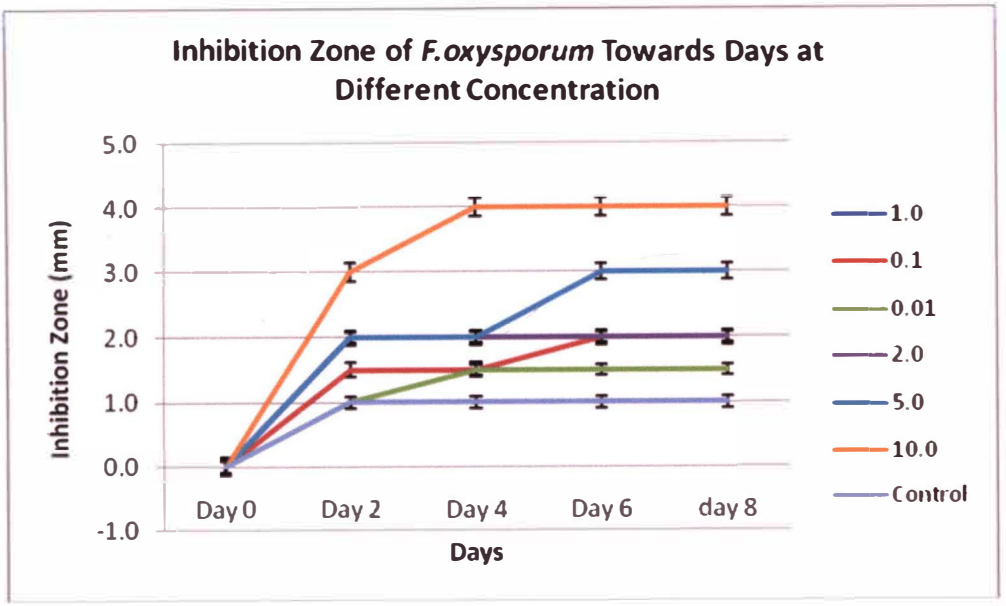

Figure 4.1 : Inhibition Zone of *F. oxysporum* towards days at different concentration in spore suspension method.

Table 4.1 : The inhibition zone of *F. oxysporum* towards day in spore suspension method from day 0 to day 8

Treatment	Strain	Concentration (mg/ml)	Day				
			0	2	4	6	8
Spore Suspension	*F. oxysporum*	1	-	2.5 ± 0.5^{cd}	2.0 ± 0.2^{b}	2.0 ± 0.2^{b}	2.0 ± 0.3^{ab}
		0.1	-	1.5 ± 0.3^{ab}	1.5 ± 0.2^{ab}	2.0 ± 0.4^{b}	2.0 ± 0.4^{ab}
		0.01	-	1.0 ± 0.3^{a}	1.5 ± 0.1^{ab}	1.5 ± 0.3^{ab}	1.5 ± 0.6^{a}
		2	-	2.0 ± 0.2^{bc}	2.0 ± 0.3^{b}	2.0 ± 0.4^{b}	2.0 ± 0.3^{ab}
		5	-	2.0 ± 0.2^{bc}	2.0 ± 0.4^{b}	3.0 ± 0.4^{c}	3.0 ± 0.4^{bc}
		10	-	3.0 ± 0.5^{d}	4.0 ± 0.5^{c}	4.0 ± 0.4^{d}	4.0 ± 0.3^{c}
		Control	-	1.0 ± 0.2^{a}	1.0 ± 0.5^{a}	1.0 ± 0.3^{a}	1.0 ± 0.1^{a}

Data shown are the means of three replicates ±standard deviation

* Means separations followed by the same letter within the same column are not significantly differently according to Tukey test

From the Figure 4.1 and Table 4.1, it shows the inhibition zone of *F. oxysporum* towards concentration of *C. alata* extract by using the spore suspension method. From observation, the inhibition zone started on day 2 for F. oxysporum on Potato Dextrose Agar (Figure 4.1 and Table 4.1) . Previous research that has been done by Ibrahim and Osman (1995), it shows that the extraction of *C.alata* are very effective when the concentrations is higher. In this study, 10 mg/ml of *C. alata* extraction produced larger inhibition zone compared to others where on the day 4, the inhibition zone for this concentration are 4 mm and its retain until day 8. It is followed by 5 mg/ml, 2 mg/, 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml of *C. alata* extraction and lastly the control that only shows 1mm of inhibition zone. There was no changes from day 6 to day 8 for all those six *C. alata* extractions concentration. This is because the C. alata extraction had inhibit the *F. oxysporum* and it cannot groth any mycellium. So, for the spore suspension method on *F. oxysporum*, the concentration of 10.0 mg/ml produce better results compare to others and 0.01 mg/ml are showing the least inhibition.

From the Tukey's test that conducted to observe the significance different between each concentration that were used. Table 4.2 showed the results from the Tukey tests. At day 8, concentration 1 mg/ml and 0.1 mg/ml, there were no significantly difference ($P>0.05$) between this two concentration but comparison of the 10 mg/ml and the control, there are highly significance ($P<0.05$) different between this two concentration where the control shows almost no inhibition zone at day 8 but for concentration 10 mg/ml , the inhibition zone are 4.0 mm (Table 4.1).

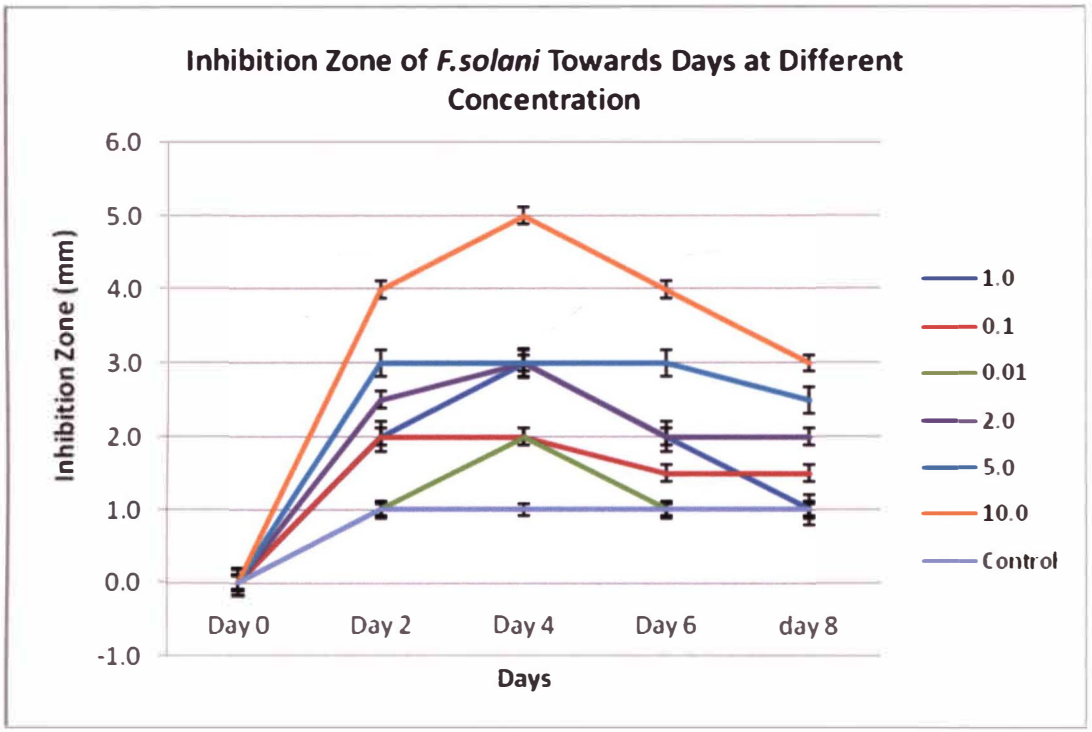

Figure 4.2 : Inhibition Zone of *F. solani* towards days at different concentration in spore suspension method

Table 4.2 : The inhibition zone of *F.solani* towards day in spore suspension method from day 0 to day 8

Treatment	Strain	Concentration (mg/ml)	Day				
			0	2	4	6	8
Spore	*F.solani*	1	-	2.0 ± 0.7^{ab}	3.0 ± 0.7^{b}	2.0 ± 0.2^{b}	1.0 ± 0.2^{a}
Suspension		0.1	-	2.0 ± 0.3^{ab}	2.0 ± 0.4^{ab}	1.5 ± 0.2^{ab}	1.5 ± 0.1^{ab}
		0.01	-	1.0 ± 0.4^{a}	2.0 ± 0.2^{ab}	1.0 ± 0.3^{b}	1.0 ± 0.1^{a}
		2	-	2.5 ± 0.2^{b}	3.0 ± 0.4^{b}	2.0 ± 0.4^{b}	2.0 ± 0.3^{bc}
		5	-	3.0 ± 0.6^{bc}	3.0 ± 0.2^{b}	3.0 ± 0.2^{c}	2.5 ± 0.3^{cd}
		10	-	4.0 ± 0.4^{c}	5.0 ± 0.5^{c}	4.0 ± 0.3^{d}	3.0 ± 0.4^{d}
		Control	-	1.0 ± 0.3^{a}	1.0 ± 0.2^{a}	1.0 ± 0.5^{a}	1.0 ± 0.4^{a}

Data shown are the means of three replicates ±standard deviation

* Means separations followed by the same letter within the same column are not significantly differently according to Tukey test

For the *F. solani* strain, the antimicrobial activities at the day 0 to day 4 are showing the same symptoms with the treatment to *F. oxysporum* which is the increasing of the inhibition zone starting from day 2 after the growth of fungi colonies are become visible. However, starting from day 4 to day 8, the patterns of inhibitions zone are starting to decline for all concentration. It is because the fungi were start to re-growth of its mycelium on the inhibition zone that makes the inhibition zones disappear. A concentration that can inhibit the most are the 10.0 mg/ml because at day 4, it reach 5 mm of inhibition zone and although the inhibition zone were declining to 3.0 mm at day 8, the inhibition zone at day 8 were still the higher among other concentration compare to other concentration. The re-growth shows that the *F.solani* are not completely inhibit and fungi can growth robustly back at the site of inhibition because *F.solani* is the stronger strain (Suarez-estrella, 2004). The Tukey test are showing that there were significance value between each concentration and it is highly significance ($P<0.05$) for the 10 mg/ml extraction and the control plate. For the concentration 1.0 mg/ml, 0.1 mg/ml and 0.01 mg/ml of extraction, there are no significant difference ($P>0.05$) between this three concentrations.

So, in the spores suspension method, the higher concentrated *C.alata* extraction which is 2 mg/ml, 5 mg/ml and 10 mg/ml were showing significance value ($P<0.05$) towards the inhibition zone of both *Fusarium* species. However, for the *F. solani*, the mycelium was growth again at the inhibition site after day 6.

4.2 Disk Diffusion Method

The disk diffusion method are the second method that applied by placing the disk that dipped in the extraction and place them on the Potato Dextrose

Agar and the inoculums were placed at the center of the plates. This method was applied to both *F. oxysporum* and *F. solani* by using six different concentrations which is 10 mg/ml, 5mg/ml, 2 mg/ml, 1 mg/ml, 0.1 mg/ml, and 0.01 mg/ml. The results are shown on Figure 4.3 and 4.5

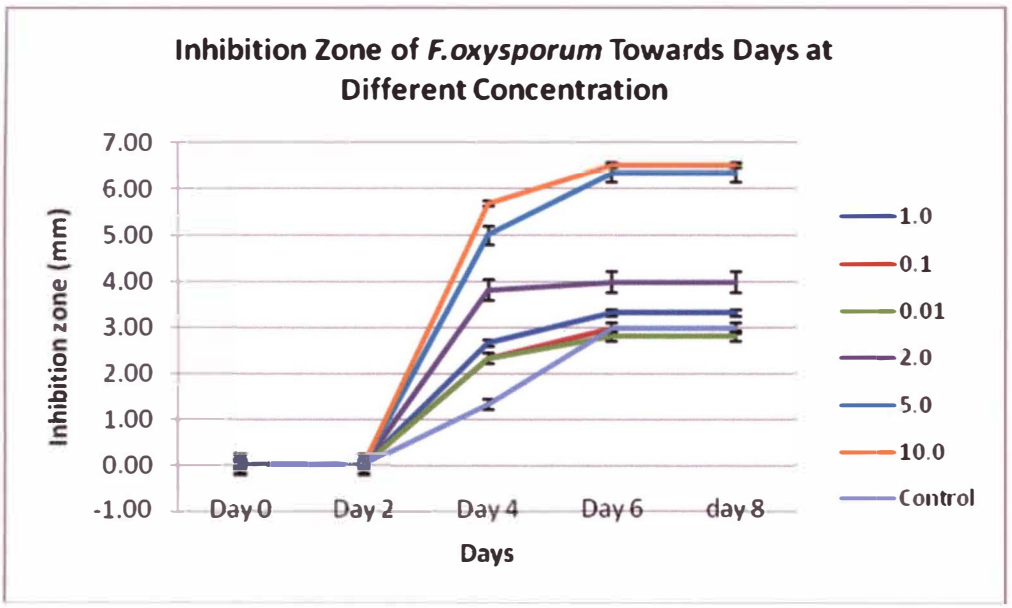

Figure 4.3 : Inhibition Zone of *F. oxysporum* towards days at different concentration in disk diffusion method

Table 4.3 : The inhibition zone of *F. oxysporum* towards day in disk diffusion method from day 0 to day 8

Treatment	Strain	Concentration (mg/ml)	Day				
			0	2	4	6	8
Disk Diffusion	*F.oxyspo rum*	1	-	-	2.7 ± 0.3^{b}	3.3 ± 0.2^{a}	3.3 ± 0.1^{a}
		0.1	-	-	2.3 ± 0.4^{b}	3.0 ± 0.3^{a}	3.0 ± 0.2^{a}
		0.01	-	-	2.3 ± 0.1^{b}	2.8 ± 0.3^{a}	2.8 ± 0.6^{a}
		2	-	-	3.8 ± 0.3^{c}	4.0 ± 0.8^{a}	4.0 ± 0.8^{a}
		5	-	-	5.0 ± 0.7^{d}	6.3 ± 0.3^{b}	6.3 ± 0.2^{a}
		10	-	-	5.7 ± 0.2^{d}	6.5 ± 0.1^{b}	6.5 ± 0.05^{a}
		Control	-	-	1.3 ± 0.2^{a}	3.0 ± 0.7^{a}	2.7 ± 0.4^{a}

Data shown are the means of three replicates ±standard deviation

* Means separations followed by the same letter within the same column are not significantly differently according to Tukey test

From Figure 4.5, it shows that the inhibition zone are only can be observe starts from day 4 because its take longer time for the fungi to growth until it reach the disk. Extraction at 10 mg/ml are showing the highest inhibition zone where at day 6 and day 8, the inhibition zone are reached 6.5 mm which is the highest among other concentration. It is followed by the 5 mg/ml, 2 mg/ml, 1 mg/ml, 0.1 mg/ml and lastly 0.01 mg/ml. As well as in the spores suspension method, the actions of *C.alata* extraction towards *F .oxysporum* are showing the same pattern which it's remain constant from day 6 and day 8. As for the concentration 0.01 mg/ml, there are no significance different ($P>0.05$) with the control at the end of the experiment because of the low concentration of active ingredients from the extraction of *C. alata*. The

inhibition activities for *F .oxysporum* are similar to the spore suspension method where the zone are increasing from day 4 to day 6 and it is remain constant until day 8. Based on the Tukey's Test (Table 4.6), there are significance difference ($P<0.05$) between 10 mg/ml extraction and control starting from the day 4 (Figure 4.4).

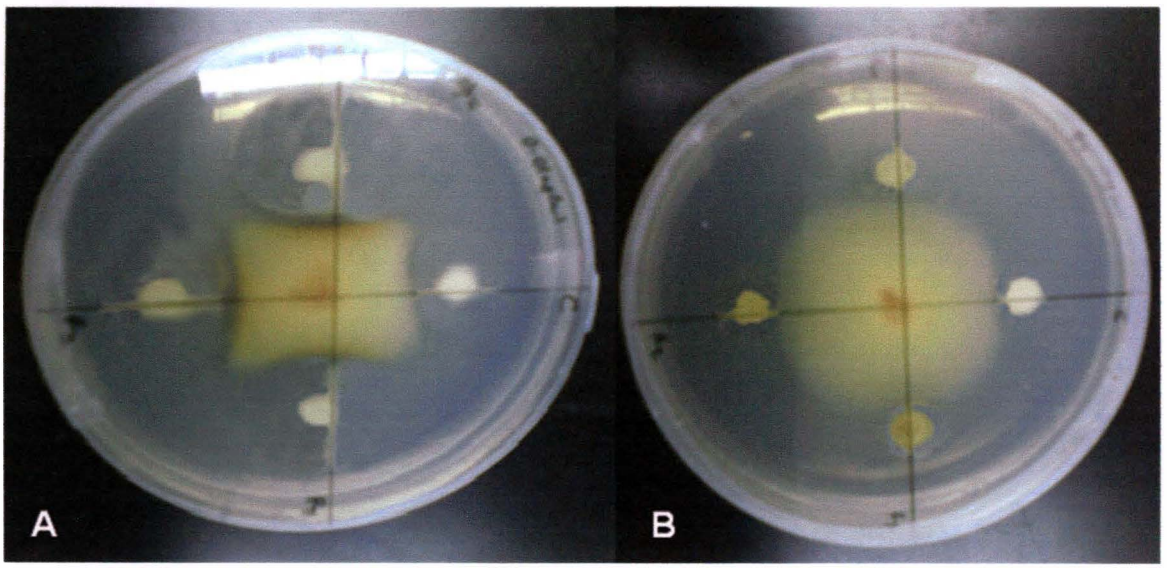

Figure 4.4: Inhibition zone of *F. oxysporum* using disk diffusion method at Day 8

A : 10mg/ml of *C. alata* extraction
B : Control

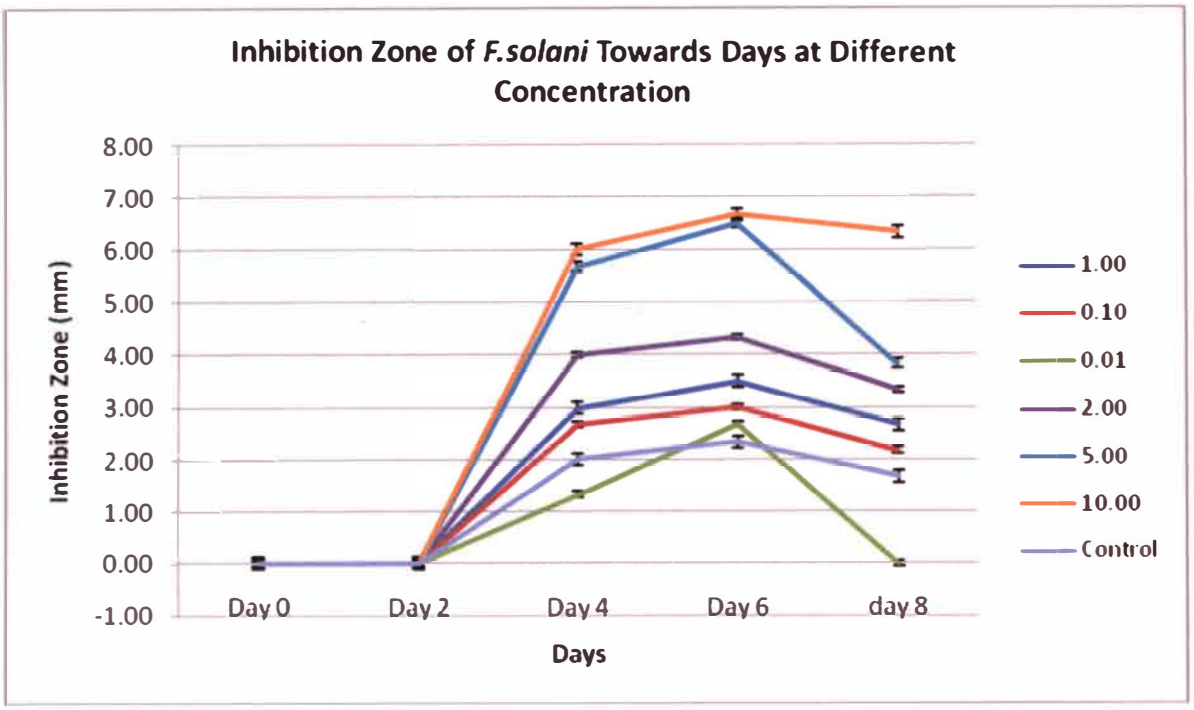

Figure 4.5 : Inhibition Zone of *F. solani* towards days at different concentration in disk diffusion method

Table 4.4: The inhibition zone of *F. solani* towards day in disk diffusion method from day 0 to day 8

Treatment	Strain	Concentration (mg/ml)	Day				
			Day 0	Day 2	Day 4	Day 6	Day 8
Disk Diffusion	*F.solani*	1	-	-	3.0 ± 0.3^{bc}	3.5 ± 0.1^{c}	2.7 ± 0.4^{cd}
		0.1	-	-	2.7 ± 0.1^{abc}	3.0 ± 0.2^{bc}	2.2 ± 0.2^{bc}
		0.01	-	-	1.3 ± 0.2^{a}	2.7 ± 0.1^{ab}	-
		2	-	-	4.0 ± 0.2^{c}	4.3 ± 0.2^{d}	3.3 ± 0.1^{de}
		5	-	-	5.7 ± 1.2^{d}	6.5 ± 0.2^{e}	3.8 ± 0.3^{e}
		10	-	-	6.0 ± 0.3^{d}	6.7 ± 0.4^{e}	6.3 ± 0.2^{f}
		Control	-	-	2.0 ± 0.3^{ab}	2.3 ± 0.2^{a}	1.7 ± 0.4^{b}

Data shown are the means of three replicates ±standard deviation
* Means separations followed by the same letter within the same column are not significantly differently according to Tukey test.

For the antimicrobial activities of *C. alata* towards *F .solani*, (Figure 4.5 & Table 4.4) the inhibition zones of *F. oxysporum* were only visible on the day 4 due to the times that it takes for the fungi to growth. The extraction that giving the higher inhibition were also 10 mg/ml and it was followed by 5 mg/ml, 2 mg/ml, 1 mg/ml, 0.1 mg/ml and lastly 0.01 mg/ml.. The inhibition zones of *F.solani* in disk diffusion method were also shows the degradation after day 6. This is because, the *F.solani* strains are more resistance compare to *F.oxysporum* that enable *F.solani* to re-growth its mycelium on the inhibition site (Suarez-estrella,2004). For the control, at day 8, the inhibition zone was completely vanished by the fungi because there was no active compound that presents at control that allowed the mycelium to growth robustly on the inhibition site.

4.3 Comparison Between Disk Diffusion Method and Spore Suspension Method

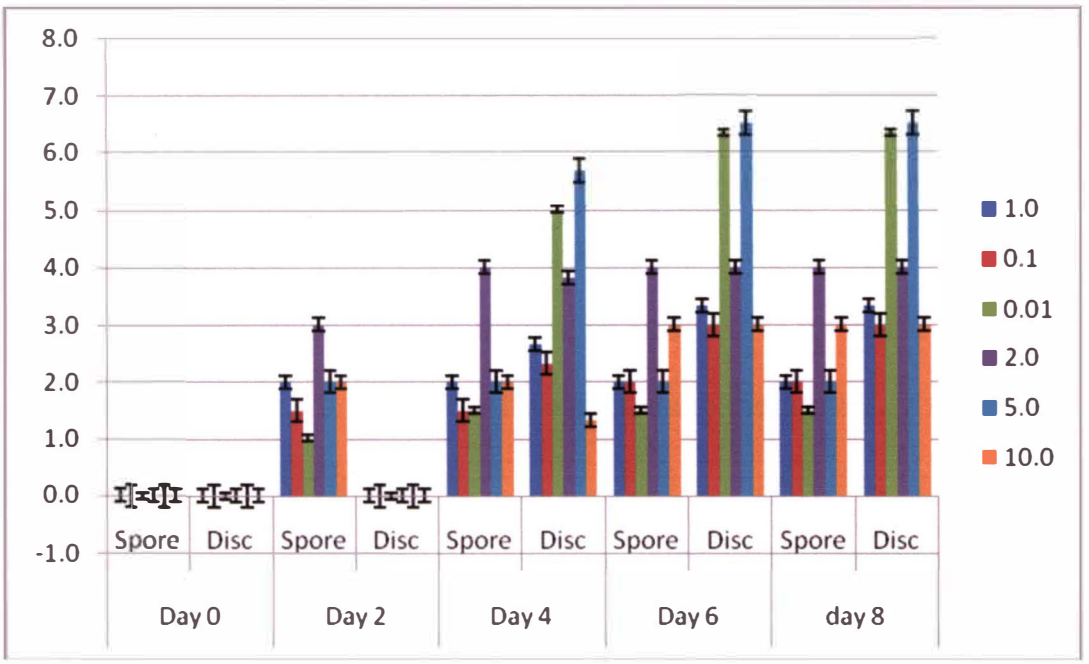

Figure 4.6 : The inhibition zone of *F. oxysporum* towards day in spore suspension method and disk diffusion method from day 0 to day 8

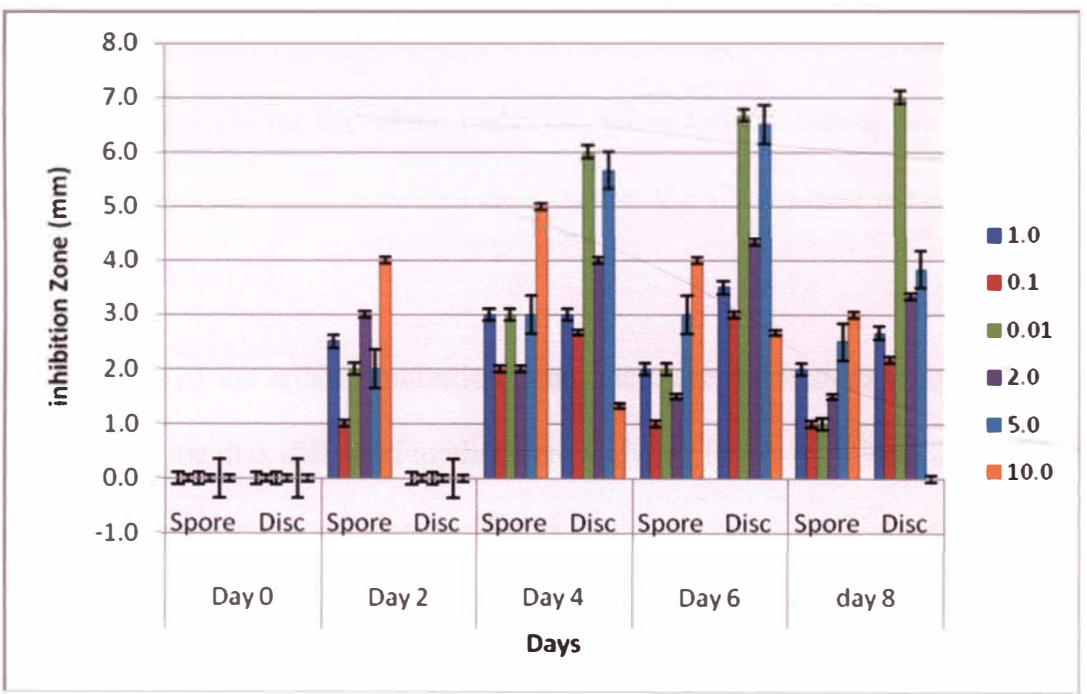

Figure 4.7 : The inhibition zone of *F. solani* towards day in spore suspension method and disk diffusion method from day 0 to day 8

Inhibition zones were occured in all concentrations by using both methods for *F. oxysporum* and *F. solani* (Figure 4.6 & 4.7) . For the *F. oxyporum*, the zone area are increased from day 0 until day 6, but from day 6 to day 8, the zone are remain unchanged for both spore suspension and disk diffusion. This happened because the fungi were not growth any mycelium towards its reactions to the active compound in *C. alata* extractions. As for the disk diffusion method, the inhibition zone are only appeared on the day 4 compare to the spore suspension method that already showing the inhibition much earlier which is started from day 2 for both reactions towards *F. oxysporum* and *F. solani* (Figure 4.6 & 4.7). So, in terms of time of inhibition, the spore suspension methods were showing faster effect from the antimicrobial activities from extraction. This is because the length between the fungi inoculums and the extraction disks will require time for the fungi to growth and reach the area that filled by the extraction from the disks compare to the spore suspension methods, the fungi inoculums are already on the whole plate. So, when the extractions are absorbed by the media, the antimicrobial activities can occur at the site without need to wait until the fungi growth.

In terms of the area of inhibition zone, there are differences between this two methods where the disk diffusion methods are showing larger inhibition zone compare to spore suspension methods. For the disk diffusion method, the highest point are on day 6, the 10 mg/ml concentration produced 6.5mm and 6.7mm of inhibition zone for *F. oxysporum* and *F. solani* respectively. Meanwhile, for the spore suspension method, the highest point for *F. oxysporum* are only 4.0mm and 5.0mm for *F. solani*. So, the disk diffusion can yields better results from the spore suspension techniques in terms of inhibition area because the larger the inhibition zone shows that the

antimicrobial activities are more efficient. Previous studies had shows that disk diffusion method are a basic methods that use in microbiology fields that has been use for a long time (Langvard, 1999).

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

For the spore suspension methods, the concentration that given the best results are the 10mg/ml and it is followed by 5 mg/ml, 2 mg/ml, 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml. The highest value on *F. oxysporum* is 4 mm at day 8 for 10 mg/ml and the highest value for *F. solani* are 5 mm on the day 4. As for the disk diffusion method, the highest value for *F. oxysporum* is 6.5 and 6.7 for *F. solani*. There are several comparison that occur from the results. First, the difference between these two fungi that we used in this study. Both of the fungi are showing the same behavior of antimicrobial activities in both two method that we used where the inhibition zone of *F.oxysporum* are increasing at the early stage and become constant after day 6. However, for *F.solani*, the inhibition zone will start to decline after day 4 when the fungi are starts to re-growth on the inhibition site.

Then, there are also differences between the times for the inhibition to occur. For the spore suspension method, the inhibition zone can be observed on day 2 but for the disk diffusion method, the inhibition zone only can be observed on day 4. This is

influence by the length of fungi to the media that had absorbed the extraction from the disk. That is why in disk diffusion method, we need to wait for the fungi to growth into the disks area. However, although the spore suspension can yield observation faster than disk diffusion method, there are difference between the areas of inhibition where the disk diffusion yield higher inhibition zone when 10 mg/ml of *C .alata* extraction are able to inhibit 6.7 mm of inhibition zone for the *F. solani* on day 4 compare to the same concentration for the spore suspension that only reach 5.0 mm.

So, the objectives of this paper are successfully achieved when the extraction of *C.alata* are show the inhibition to the *Fusarium* spp that can cause post harvest diseases in fruits such as melons. As for the second objectives to evaluate between these two methods the spore suspensions method can yield faster result but the inhibition zone of the fungi are higher in the disk diffusion methods. So, the disk diffusion methods are the better methods.

5.2 Recommendations

For the further research, there are a few recommendations that can be considered. First, to conduct the microbiology experiments, one must have knowledge on how to deals with microbes to avoid contamination to the samples. Besides that, for handling of microbial, we actually needs a special rooms that are sterile and closed for public because the contamination can come from many sources when people are going in and out at the laboratory. For the improvement in the results for the disk diffusion

method, the length between the fungi inoculums and extraction disk can be shorten. Thus, the inhibition zone can presence earlier.

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APPENDICES

Appendix A : Table of the average of inhibition zone of *F. oxysporum* by using spore suspension method

Concentration (mg/ml)	Day 0	Day 2	Day 4	Day 6	Day 8
1.0	0.0	2.0	2.0	2.0	2.0
0.1	0.0	1.5	1.5	2.0	2.0
0.01	0.0	1.0	1.5	1.5	1.5
2.0	0.0	2.0	2.0	2.0	2.0
5.0	0.0	2.0	2.0	3.0	3.0
10.0	0.0	3.0	4.0	4.0	4.0
Control	0.0	1.0	1.0	1.0	1.0

Appendix B : Table of the average of inhibition zone of *F. solani* by using spore suspension method

Concentration (mg/ml)	Day 0	Day 2	Day 4	Day 6	Day 8
1.0	0.0	2.0	3.0	2.0	1.0
0.1	0.0	2.0	2.0	1.5	1.5
0.01	0.0	1.0	2.0	1.0	1.0
2.0	0.0	2.5	3.0	2.0	2.0
5.0	0.0	3.0	3.0	3.0	2.5
10.0	0.0	4.0	5.0	4.0	3.0
Control	0.0	1.0	1.0	1.0	1.0

Appendix C : Table of the average of inhibition zone of *F. oxysporum* by using disk diffusion method

Concentration	Day 0	Day 2	Day 4	Day 6	day 8
1.0	0.00	0.00	2.67	3.33	3.33
0.1	0.00	0.00	2.33	3.00	3.00
0.01	0.00	0.00	2.33	2.83	2.83
2.0	0.00	0.00	3.83	4.00	4.00
5.0	0.00	0.00	5.00	6.33	6.33
10.0	0.00	0.00	5.67	6.50	6.50
Control	0.00	0.00	1.33	3.00	3.00

Appendix D : Table of the average of inhibition zone of *F. solani* by using disk diffusion method

Concentration	Day 0	Day 2	Day 4	Day 6	day 8
1.00	0.00	0.00	3.00	3.50	2.67
0.10	0.00	0.00	2.67	3.00	2.17
0.01	0.00	0.00	1.33	2.67	0.00
2.00	0.00	0.00	4.00	4.33	3.33
5.00	0.00	0.00	5.67	6.50	3.83
10.00	0.00	0.00	6.00	6.67	6.33
Control	0.00	0.00	2.00	2.33	1.67

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THE ANTIMICROBIAL ACTIVITIES OF CASSIA ALATA ON THE CAUSAL ORGANISMS OF MELON FRUIT ROT - WAN MAHFUZAH BINTI WAN IBRAHIM