UNIVERSITI PUTRA MALAYSIA

SCREENING OF POTENTIAL PLANTS FOR THE CONTROL OF FUSARIUM WILT DISEASE

TEO ZHI QIANG

SCREENING OF POTENTIAL PLANTS FOR THE CONTROL OF FUSARIUM WILT DISEASE

By

TEO ZHI QIANG

A Project Report Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor of Science Bioindustry in the Faculty of Agriculture and Food Sciences Universiti Putra Malaysia Bintulu Sarawak Campus

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With an immense gratitude, this thesis is dedicated to my supervisor, Dr. Khairulmazmi bin Ahmad and my beloved lecturer, Mr. Philip Lepun, for their endless support and encouragement. I feel deep appreciation for the wise counsel and guidance that I experienced from them who gave generously of themselves in supporting my endeavours. Life blessed me with the opportunity to meet them. It is also dedicated to my parents, who have both imbued in me a tenacity that has enabled me to complete this thesis.

ABSTRACT

Aqueous extracts from *Citrus suhuiensis*, *Eurycoma longifolia*, *Morinda citrifolia*, *Eugenia oleina*, *Cassia alata* and *Nephelium lappaceum* were studied for their antimicrobial activities. The antimicrobial properties were determined to control the pathogenic fungus, namely *Fusarium oxysporum*. *F. oxysporum* was tested using agar diffusing method with 1:10 concentration of the plant extracts. Results of this study showed variable antimicrobial activity in some plant extracts. The most effective leaf extract is *C. suhuiensis* which exhibited the highest inhibitory activity against *F. oxysporum*, while the most effective fruit extract is *E. longifolia*. This study shows the potential of the leaf extract of *C. suhuiensis* and the fruit extract of *E. longifolia* to be used as bio-pesticides.

ABSTRAK

Pengestrakkan akues daripada *Citrus suhuiensis*, *Eurycoma longifolia*, *Morinda citrifolia*, *Eugenia oleina*, *Cassia alata* dan *Nephelium lappaceum* dikaji bagi aktiviti antimikrob. Kesan antimicrob ini ditentukan bagi mengawal kulat pathogen iaitu *Fusarium oxysporum*. *F. oxysporum* diuji dengan menggunakan kaedah penyerapan agar dengan kepekatan 1:10 estrak tumbuhan. Keputusan daripada kajian ini menunjukkan kepelbagaian kesan antimikrob bagi sesetengah estrak tumbuhan. Estrak daun tumbuhan yang paling efektif ialah *C. suhuiensis* yang menunjukkan activiti rencatan yang paling tinggi ke atas *F. oxysporum*, manakala estrak buah tumbuhan yang paling efektif ialah *E. longifolia*. Kajian ini menunjukkan potensi estrak daun *C. suhuiensis* dan estrak buah *E. longifolia* sebagai racun perosak biologi.

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Last, but not least, I thank my parents for giving me life in the first place and for their unconditional support and encouragement at each turn of the road.

This thesis is only the beginning of my journey.

I certify that this research project report entitled " Screening of Potential Plants for the Control of Fusarium Wilt Disease" has been examined and apporved as a partial fullfillment of the requirement for the degree of Bachelor of Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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Dr. Khairulmazmi bin Ahmad
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus
(Supervisor)

_____

Professor Dr. Japar Sidik bin Bujang
Dean
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus

Date:

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CHAPTER 1

INTRODUCTION

1.1 Background

A very large number of fungi such as *Colletotrichum* sp. and *Fusarium* sp. are responsible for producing mycotoxins in various food and feeds (Abarc *et al.*, 1994; Lund *et al.*, 1996; Kedera *et al.*, 1999). These fungi are also known to be responsible for the off-flavour formation and production of allergenic compounds, which often takes place before the growth of fungi is evident. In addition to this, *Colletotrichum* sp. and *Fusarium* sp. also affect many important crops at pre-harvest and post-harvest stages. Thus, the presence of these pathogenic fungi in crops, vegetables, vegetables and fruits has significantly impacted the quality of foods and food related products.

Fungicide application is widely used by farmers to control foliar diseases. Fungicides such as imazil, prochloraz, azoxystrobin and benomyl have been found to be most effective in control of these plant pathogenic diseases. However, over usage of these pesticides would cause negative effect on the other beneficial organisms. In case of benomyl, in order to achieve effective control, it requires weekly spray but sometime it can be extended for several months. This practice is very high cost and it would be difficult for farmers in the developing country especially, to keep up with this very expensive regime. Fungicides are also widely used at post-harvest treatment to control this disease. Overuse of fungicides would be unsafe to the final consumers because of residual effect of the fungicides.

Application of bio-control agents and/or botanical fungicides may offer alternative solution to overcome the diseases. Moreover, this approach is more healthier and environmental friendly. In the past decade, interests on the antimicrobial plants extracts have been growing. Various species and herb extracts have been used for the purpose of food preservation and appetizer promotion as well as medicinal purposes (Zaika, 1988; Cowan, 1999).

In particular, extracts from many oriental spice plants and herbs such as garlic, union, cinnamon, mint and vanilla have been known to possess antimicrobial effects (Shelef *et al.*, 1980; Zaika and Kissinger, 1981; Saleem and Al-Delaimy, 1982; Smith *et al.*, 1998; Nilsen and Rios, 2000; Tassou *et al.*, 2000; Yildirim *et al.*, 2000; Wong and Kitts, 2002 Valero and Salmeroj, 2003).

1.2 Objective of Study

The objective of this study is to determine the potential of selected trees as botanical fungicides against *Fusarium oxysporum in vitro*.

CHAPTER 2

LITERATURE REVIEW

2.1 *Fusarium* sp.

2.1.1 Infection

The fungus, *Fusarium* sp., continues to be a serious problem for crop production worldwide for many years. In the greenhouse, for instance, this pathogenic fungus is able to cause serious damage to the roots, stems or even the leaves of the plants. There are many harmful *Fusarium* species that has been identified and characterized so far, however, there are also many of them that do not cause any problems to the plants and sometime they can be very useful, especially in the formae speciales (sub-species) of *F. oxysporum*. This group of *Fusarium* is known to be a very crop-specific pathogen.

2.1.2 Epidemiology

Various parts of the plants can be attacked by this fungus. Spores usually attack the roots first. After infection and germination, *Fusarium* sp. develops mycelium. This is the vegetative part of a fungus and a microscopic network of one-cell-thick filaments in the substrate.

The mycelium of *Fusarium* sp. grows rapidly in different parts of the plants and hence produces different toxic substances. Because of this, the plants' vascular tissues and surrounding tissues will turn brown and rotten. This will cause problems to their vascular transport systems. Symptoms like yellowing and wilting occur and the plants will eventually die.

The combination of high temperature and high humanity will stimulate the growth of the fungus. Also, plants in bad conditions are more sensitive to be infected by *Fusarium* sp. For example, infection by *Fusarium* sp. may be due to nematode infection or the absence of feeding elements. After infection, the fungus is able to form chlamydospores. These surviving structures can survive for many months in soils, water and dead organic material. When the conditions for spores are optimal, these spores can infect new plant material.

2.1.3 Spread

After infection, the fungus will develop and produce enormous amounts of conidiophores. These spores are easily spread by water, air or human transactions in the field and greenhouse. The fungus infection can spread out rapidly. Therefore, it is very important to remove heavily infected plants carefully out of the field.

2.1.4 *Fusarium* Wilt Disease of Banana (Panama Wilt)

Fusarium wilt is a severe disease of banana plants caused by the fungus *Fusarium oxysporum* f.sp. *cubense* (*Foc*). This disease kills susceptible banana plants and there is no cure. *Fusarium* wilt is the preferred name for what was first called Panama disease because it became prominent in the Central America country early last century. The fungus infects banana plants through the roots and invades the plants' water conducting tissues. Once *Foc* is introduced into the banana gardens, it remains in the soil making it impossible to grow susceptible bananas in the same location for up to several decades.

Foc is thought to have originated in Asia, and then spread during the 20th century to become a major problem throughout most banana production regions in the world. An important exception is South Pacific, where *Fusarium* wilt is a new disease and has not yet become widespread. Moderately damaging strains of *Foc* have been established in some pacific nations close to Southeast Asia and also in Tonga.

As *Foc* will disrupt the plants' water conducting vessels, leaves become yellow (progressing from older to younger leaves) and wilted. This is also a sign of drought stress that is reduced when water supply is good. Distinctive symptoms appear inside the pseudostem: brown, red and yellow lines are visible in the vertical section. These are the infected water conducting vessels. Smaller brown streaks or flecks appear in the corm, at the ground level. Later, all leaves will turn yellow and die and internal rotting become extensive. Splits may also appear in the pseudostem. Infected plants usually do not produce fruit (Richard, 2004).

2.2 Control of Fusarium Wilt Disease

2.2.1 Control Programme: Chemical Control

In general, some effective means of controlling *F. oxysporum* include: disinfestations of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (Jones *et al.*, 1982; Agrios, 1988; Smith *et al.*, 1988). It is much recommended using fungicide concentration under standard manufacturer concentration because it can reduce or very low environmental impact. However, the standard concentration will give the effectiveness effect to the pathogen on the plants.

2.2.2 Alternative Control Measure: Biological Control

Biological control is defined as the action of parasites, predators or pathogens in maintaining another organism's population at a lower average density than would naturally occur (Zimdahl, 1999). Even though the procedure for implementing biological control is relatively complex, yet interest is increasing because biological control is environmental safe and applicable to some otherwise intractable plant problems.

Biological control using antagonistic bacteria has been successful in controlling many postharvest pathogens. *Burkholderia cepacia* and other *Pseudomonas* species are some of the most promising bio-control microorganisms (Cartwright *et al.*, 1995). They are known to produce a wide range of secondary metabolites such as pyrrolnitrin, phenazine, cepabactin and other unidentified volatile or non-volatile compounds (Cartwright *et al.*, 1995).

2.2.2.1 Botanical Fungicide

Plants compounds are being used increasingly since numerous plant chemicals have antifeedant and insecticidal properties (Tripathi and Tripathi, 2000).

According to Samy *et al.*, 2005, there are around 8, 1000 species of plants in Malaysian rainforests, of which 10% of them are reported to have some medicinal values. In addition, the total area of rainforest in Malaysia is around 19.12 million hectares, occupying some 58.1% of the land area in the country. Hence, there is potential for discovering drugs that are beneficial to human health.

Plants, especially medicinal plants, offer a vast resource of novel natural compounds often with exciting activities and biological properties. It has a valuable source of new and biologically active molecules. It is important to have the necessary tools in hand. These include suitable biological assays and chemical screening methods.

In literature, many natural products were reported to have antimicrobial properties which are effective in treatment of infectious diseases such as diarrhea (Mathabe *et al.*, 2006), antibacterial and wound healing (Ming *et al.*, 2006) and treatment of cutaneous condition (Lopez *et al.*, 2001). Antimicrobial of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious disease while simultaneously mitigating many of the side effects that are often associated with synthetic microbial.

According to Nair *et al.* (2005), plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. Subsequently, simple bioassay should be developed for screening plant extracts to detect plant compounds with relevant biological activities which may be used as a guide for fractioning plant extracts.

Allelopathy is defined as the interaction between plants in the ecosystems by chemical exudation into the environment (Rice, 1984). In agriculture practice, allelopathy has been exploited as a tool of weed management (Kohli *et al.*, 1998). Numerous plants are observed to have allelopathic activity. However, only a few among them: *Medicago sativa L.* (alfalfa), *Hordeum vulgare L.* (barley),

Fagopyrum esculentum Moench (buckwheat), *Asparagus officinalis L.* (asparagus), *Oryza sativa L.* (rice), *Zea mays L.* (corn), *Colocasia esculenta Scott* (taro), *Vicia villosa* (hairy vetch), and *Mucuna pruriens* (velvet bean) own strong allelopathic properties (Fujii, 2001; Tsuzuki, 2001; Xuan *et al.*, 2002). *Ageratum conyzoides* was assessed to have a strong invasion capacity in plant communities, significantly reducing natural growth of weeds in its vicinity. The leaves were reportedly to be used by local farmers, as traditional manure in paddy field for soil improvement.

2.2.2.1.1 *Allamanda cathartica*

Allamanda (Apocynaceae) is a genus of climbing shrubs, which comprises ten species distributed in Brazil (Joly, 1975). Some species, including *Allamanda cathartica*, *Allamanda cathartica*, *Allamanda blanchetti* and *Allamanda schottii* have been analyzed with respect to their chemical composition and pharmacological properties. *A. cathartica* has long been used in traditional medicine for different purposes, including, for example, the treatment of liver tumours (Mors *et al.*, 2000). *A. blancetti* and *A. schottii* are economically used as ornamental plants.

Previous work has demonstrated the presence of the bioactive iridoid lactones plumericin, isoplumericin (Abdel-Kader *et al.*, 1997), allamandin (Kupchan *et al.*, 1974), plumieride (Tiwari *et al.*, 2002), plumieride coumarate, and plumieride coumarade glucoside (Coppen *et al.*, 1983) on *A. cathartica*. The compounds plumiericin, isoplumiericin and 5,6-dimethoxycoumarin (uncklin) were previously isolated from *A. blanchetti* (Bhattacharyya and Morais, 1986), while

isoplumericin, plumericin, allamandin, allamacin, scopoletin, scoporone and pinoresinol were obtained from *A. schottii* (Anderson *et al.*, 1988). Plumericin and isoplumericin exhibited cytotoxic activity against Madison lung carcinoma (M109) (Abdel-Kader *et al.*, 1997).

Moreover, fulvoplumierin, another irridoid present in plants of the genus Allamanda, demonstrated antifungal, antileukemic, antimicrobiological and anti-HIV properties (Kardono et al., 1990; Tan *et al.*, 1991). More recently, the anticancer activity of plumieride has been demonstrated (Kupchan *et al.*, 1974).

2.2.2.1.2 *Ageratum conyzoides*

Ageratum conyzoides is an annual herbaceous plant native to Central America and the Caribbean. It is now found in many places in tropical and sub-tropical regions. This species has great morphological variation, and is highly adaptable to different ecological conditions (Sauerborn and Kock, 1988). It has a long history of traditional medicinal uses in several countries. It has insecticidal and nematocidal properties and appears to be a valuable agricultural resource (Sauerborn and Kock, 1988).

A. conyzoides is widely utilized in traditional medicine to treat pneumonia, and to cure wounds and burns (Durodola, 1977). Local communities in India use this species as a bacteriocide, antidysenteric, and antilithic (Borthakur and Baruah, 1987). Aqueous extracts of leaves or whole plants have been used to treat colic, colds, and fevers, diarrhoea, rheumatism, spasms or as a tonic (Oliveira *et al.*, 1993).

Several pharmacological investigations have been conducted to determine its efficacy. Durodola (1977) verified inhibitory activities of ether and chloroform extracts against *in vitro* development of *Staphylococus aureus*.

A. conyzoides has been reported to have potential use in controlling pests. Shabana *et al.* (1991) use aqueous extracts of the whole plant to show reduction of larvae emergence of Meloidogyne incognita. Pu *et al.* (1990) and Liang *et al.* (1994) indicated that this species sheltered predators of the spidermite *Panonychus citri*. Other citrus spidermite populations, *Phyllocoptruta oleivora* and *Brevipalpus phoenicis* were suppressed with maintenance of *A. conyzoides* in the orchards and a reduction of leprosy virus was noted (Gravena *et al.*, 1993).

CHAPTER 3

MATERIALS AND METHODS

3.1 Origin of Pathogenic Fungus

The strain of *Fusarium oxysporum* used in this study was isolated from naturally infected banana tree. Disease tissues were cut into 1 cm pieces, placed in 10 % sodium hypochlorite solution for a while, rinsed in sterile distilled water and dried with sheets of sterile blotted paper. The tissues were then placed on PDA, containing streptomycin sulphate (50mg/L) and were incubated at 22°C for 5 days for assessment period. The fungus was confirmed based on their morphological characteristics (Montesinos *et al.*, 1996).

3.2 Collection of Plant Materials

Six plant species were collected from the vicinity of Universiti Putra Malaysia Bintulu Sarawak Campus to determine their antimicrobial behaviour against the pathogenic fungus by poisoned technique as previously explained by Nene and Thapliyal, 1979. The plant parts were then placed in plastic bags and brought to the Microbiology and Biotechnology Laboratory in the Department of Crop Science, Faculty of Agriculture and Food Sciences for further study. The plant species and their parts used were mentioned in Table 3.1.

Table 3.1: Parts of plant used for extraction process to determine fungicidal activity against plant pathogenic fungus.

Type of Plant	Parts Used
Eurycoma longifolia	Leaf, Fruit
Citrus suhuiensis	Leaf, Fruit
Morinda citrifolia	Fruit
Eugenia oleina	Leaf
Cassia alata	Leaf
Nephelium lappaceum	Leaf

3.3 Extraction and Screening of Plants for Inhibitory Effects

The fresh leaves of healthy plants were collected and washed thoroughly with tap water and air dried. One gram of plant tissue was ground using pestle and mortar by adding 10 mL of distilled water (1:10 w/v). The extract was filtered through muslin cloth. The supernatant was taken as standard plant extraction solution (100%).

Further, the extract was diluted by adding sterilized water to get 10 percents concentration. The plant extracts were subjected to boiling temperature of 50°C in water bath to avoid contamination and then incorporated into PDA media by transferring 10 mL of supernatant into 90 mL of melted warm PDA medium and gently shaken for thorough mixing the extract.

The sterilized leaf extract was dispensed into the Petri dish plate and seeded with 8 mm diameter agar disc of 10 days old culture of the pathogen to the centre of PDA

medium in the Petri dish plate. Three replications were maintained for each treatment. The basal medium (PDA) without any phytoextract was serve as control. All the inoculated Petri dish plates were incubated at 28±2°C. The radial growth of the test fungus in the treated plates was measured in all the treatments when the pathogen growth touched the periphery in the control Petri dish plates. The percent inhibition of fungal growth was estimated by using formula given by Vincent (1927).

$$I = \frac{C-T}{C} \times 100$$

Where,

I = percent inhibition

C = colony diameter in control treatment

T = colony diameter in treatment

3.4 Effects of Dipping Durations on Efficacy of Plant Extracts

One cm agar plugs of *F. oxysporum* were placed into a sterilized glass test tube. Mycelial agar plugs were then dipped into each fungicide suspension and air dried. Treated mycelial plugs were transferred onto Petri dish plates containing PDA medium. Experiment was repeated at least three times for each dipping duration (respectively 10, 20, 40 and 60 minutes). The plates were then incubated at 28±2°C. PDA with only sterilized distilled water was served as control. The observation of radial inhibition of mycelial growth was carried out seven days after incubation (Baldwin and Rathmell, 1988).

3.5 Microscopic Examination Test

In order to investigate the effects of different plant extracts on mycelial growth inhibition and spore formation of *F. oxysporum*, fungal cells were harvested from the culture grown and the morphology of the fungal cells was observed under a light microscope (Nikon Co., Japan) equipped with a digital camera (Nikon E950, Japan).

3.6 Experimental Design

Experimental design used for this study was Completely Randomized Design (CRD). All data were subjected to analysis of variance and treatment means separated by the use of the Least Significant Difference (LSD) at 5% level.

CHAPTER 4

RESULTS

4.1 Screening of Plants Extracts against the Growth of *F. oxysporum*

In this study, five plant extracts were found to have suppressive effect on the growth of *F. oxysporum*. The plant extracts were *E. longifolia, C. suhuiensis, M citrifolia, C. alata and N. lappaceum*. However, only one plant extract, namely *E. oleina*, did not show suppressive effect against *F. oxysporum* since it was not significantly different from the control at 5% level of probability. In this study, two plant parts were tested i.e. leaf and fruit. It was found that both plant parts have the potential to act as botanical fungicides.

The fruit extracts of *E. longifolia* presented the highest inhibitory activity against *F. oxysporum*, followed by *C. suhuiensis* and *M. citrifolia*, with percentage of inhibition of 56%, 55% and 50%, respectively. However, they were not significantly different to each other based on colony diameter (Table 4.1), meaning that they were equally effective to inhibit the growth of *F. oxysporum*.

Plant extracts i.e. fruit and leaf derived from both *E. longifolia* and *C. suhuiensis*, showed significant inhibitory effect against *F. oxysporum*. Based on my results, the best plant extract is *C. suhuiensis*, followed by *C. alata, E. longifolia, M. citrifolia* and, *N. lappaceum*, with percentage of inhibition of 64%, 64%, 56%, 50% and 50%, respectively.

Table 4.1: Inhibitory effects of six plant extracts (leaf and fruit) on mycelial growth of *F. oxysporum* seven days after inoculation.

Treatment	Leaf		Fruit	
	% inhibition	Mean of colony diameter (cm)	% inhibition	Mean of colony diameter (cm)
Control (water)	0.0	5.0^{d}	0.0	5.0^{b}
E. longifolia	56.0	2.2^{b}	56.0	2.2^{a}
C. suhuiensis	64.0	1.8^{a}	55.0	2.25^{a}
M. citrifolia	n. d.	n. d.	50.0	2.5^{a}
E. oleina	7.6	4.62^{d}	n. d.	n. d.
C. alata	64.0	1.8^{a}	n. d.	n. d.
N. lappaceum	50.0	2.5^{bc}	n. d.	n. d.

n. d. = not determined

Values within columns with the same letter(s) are not significantly different at 5% level using the Least Significant Difference test

4.2 Effects of Dipping Durations against the Growth of *F. oxysporum*

Among the leaf extracts of *C. suhuiensis* and *E. Longifolia* tested in this study, *C. suhuiensis* gave the most significant antifungal activity, with percentage of inhibition of 65%, 52%, 60% and 57%, after dipping for 10, 20, 40 and 60 minutes, respectively (Figure 4.1). However, it was found that there was no significant ($p<0.05$) difference in terms of the dipping durations. The effects of antifungal activities on *F. oxysporum* colony development were shown in Figure 4.3.

The percentage inhibition values varied significantly between the fruit extracts of *C. suhuiensis*, *E. longifolia* and *M. citrifolia* (Figure 4.2). There was a significant increase and decrease in the percentage of inhibition of *E. longifolia* and *M. citrifolia*, respectively, at dipping duration of 20 minutes. On the other hand, the percentage of inhibition of *C. suhuiensis* showed no significant changes initially but decreased drastically at dipping duration of 60 minutes.

Growth of *F. oxysporum* treated with *C. suhuiensis* extract was the most inhibited, while *F. oxysporum* treated with *E. oleina* extract showed its highest growth (Figure 4.3). These findings agreed with the previous results on the percentage of inhibition shown for these two extracts, where the leaf extract of *C. suhuiensis* was shown to have the highest antifungal activity and percentage of inhibition against *F. oxysporum*.

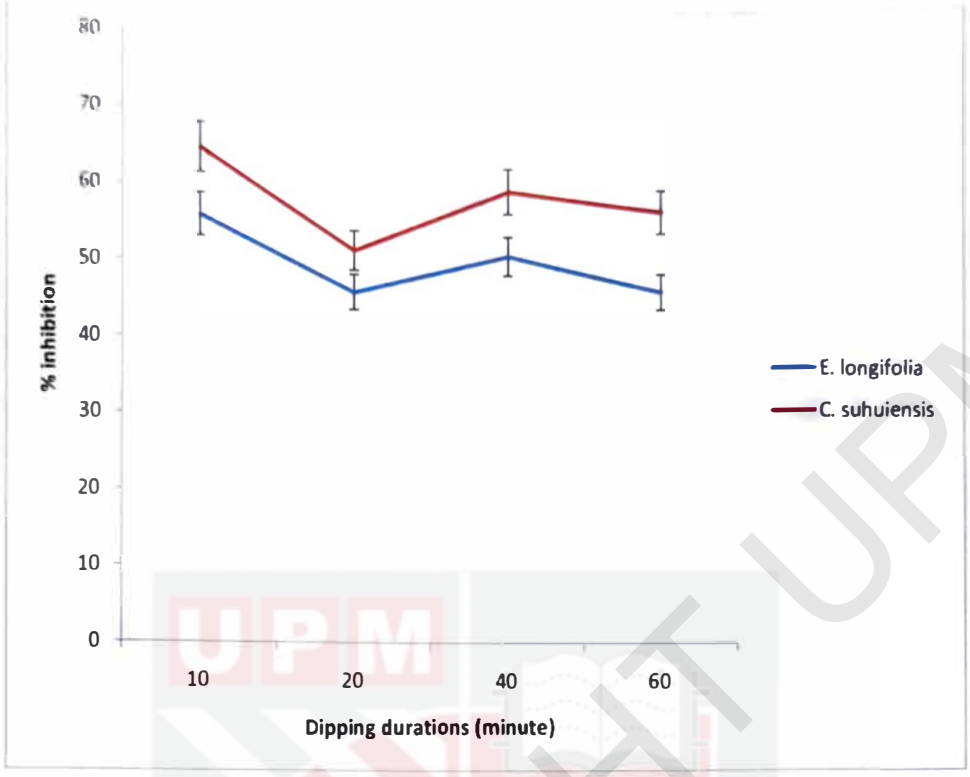

Figure 4.1: Inhibitory effects of leaf extracts of *C. suhuiensis* and *E. longifolia* on mycelial growth of *F. oxysporum* seven days after inoculation.

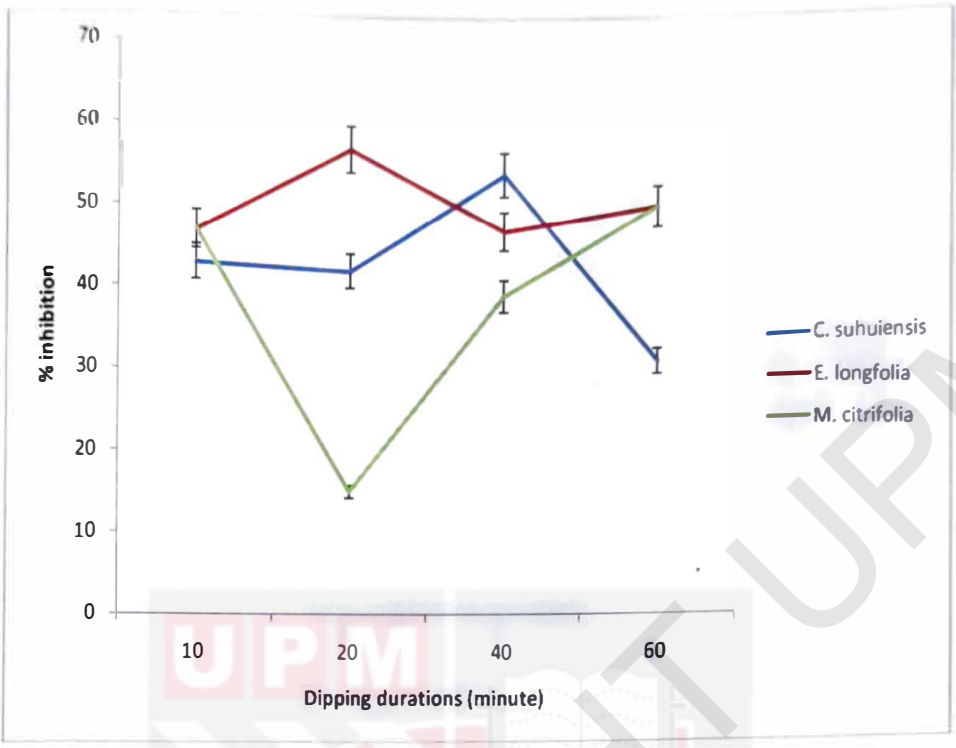

Figure 4.2: Inhibitory effects of fruit extracts of *C. suhuiensis*, *E. longifolia* and *M. citrifolia* on mycelial growth of *F. oxysporum* seven days after inoculation.

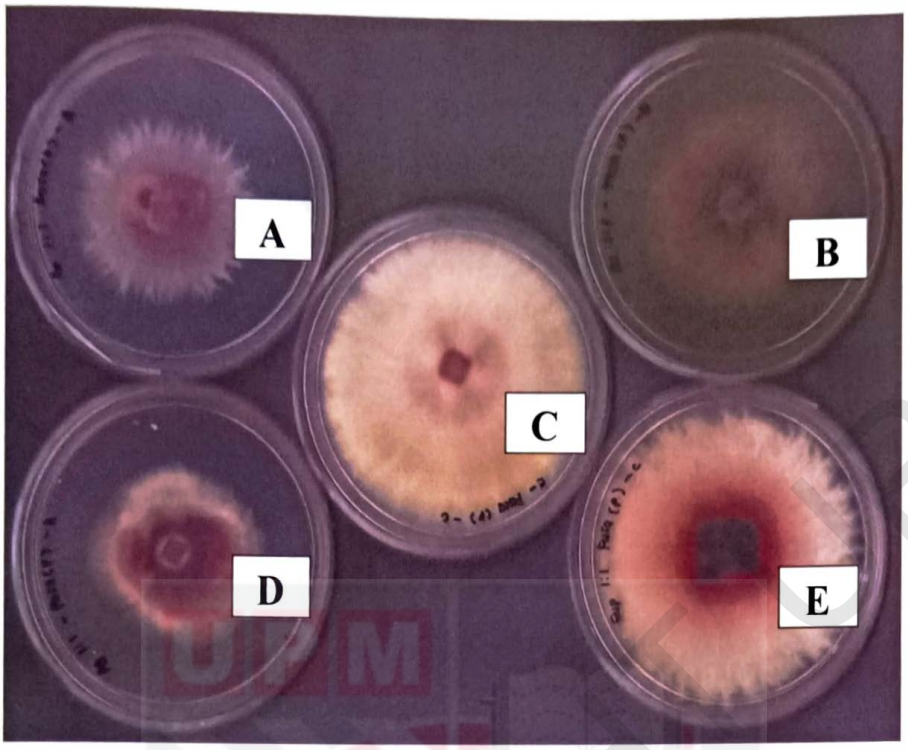

Figure 4.3: Mycelial growth of *F. oxysporum* on PDA supplemented with 1:10 concentration of (A) *M. citrifolia*; (B) *E. longifolia*; (C) Control (water); (D) *C. suhuiensis;* (E) *E. oleina* seven days after inoculation.

4.3 Microscopic Examination Test

In order to investigate the effects of different plant extracts on the growth of spores and mycelia, fungal cells were harvested from the culture grown and the morphology of the fungal cells was observed under a binocular light microscope (Nikon Co., Japan) equipped with a digital camera (Nikon E950, Japan).

The results of the effects of different plant extracts on mycelial growth and spore germination of *F. oxysporum* were presented in figure 4.4. It was observed that the extracts showed significant differences in their efficacy over the control.

The results in Figure 4b showed that lysis occurred in the mycelium of *F. oxysporum* treated with *C. suhuiensis* and fewer spores were developed. Poor mycelial growth and fewer spores were also observed in Figure 4c. It showed that the mycelium of *F. oxysporum* treated with *E. longifolia* had shrunk with lower spore production. On the other hand, the results in Figure 4d indicated that the spore production was not affected by the treatment and had no significant differences between *E. oleina*-treated *F. oxysporum* and the control, but the results in Figure 4e showed that the mycelium of *F. oxysporum* treated with *M. citrifolia* developed normally with high production of spores.

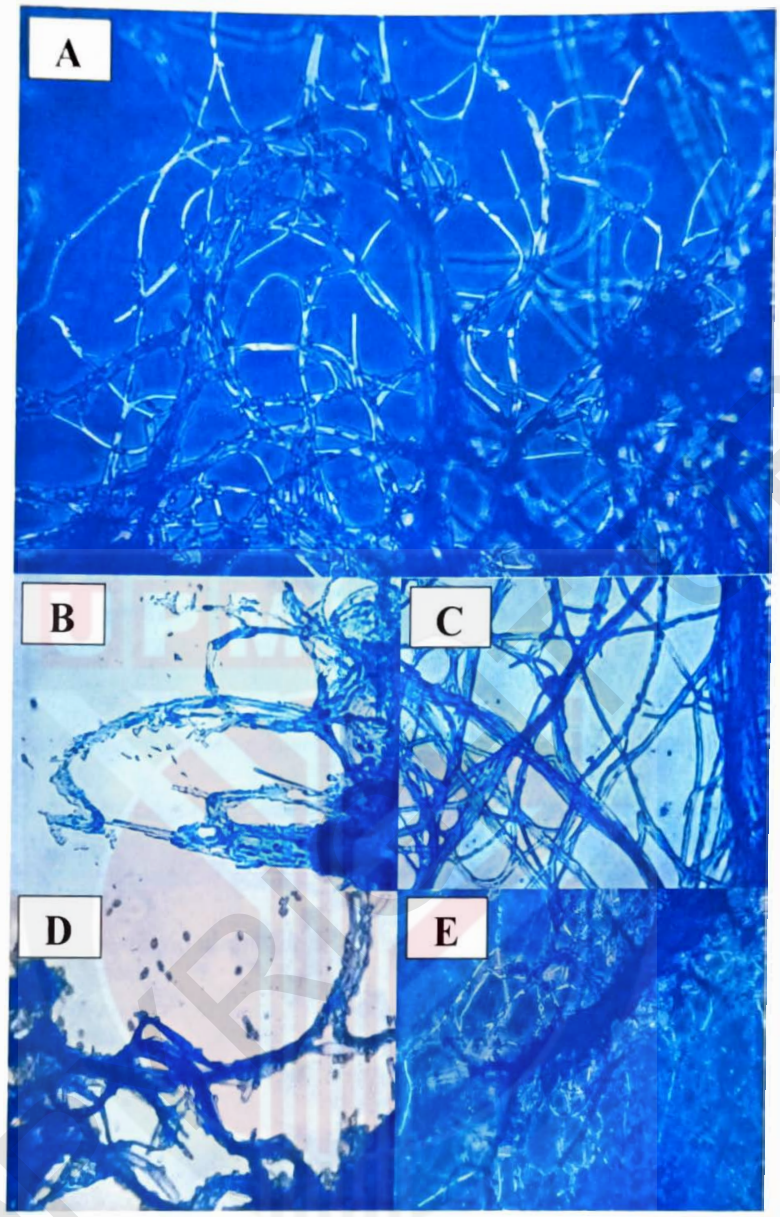

Figure 4.4: Morphology of tested fungus observed by light microscope at a magnification of x400. The fungus was treated with (A) Control (water); (B) *C. suhuiensis*; (C) *E. longifolia*; (D) *E. oleina*; (E) *M. citrifolia* for three days.

CHAPTER 5

DISCUSSION

The presence of antibacterial substances in the higher plants is well established (Srinivasan, 2001). Plants have provided a source of inspiration for novel drug compounds as plant-derived medicines have made a significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Didry *et al.*, 1998).

The fungitoxic effects of aqueous plant extracts in different plant species indicated that the importance of the plant species as natural sources of fungicidal materials that can inhibit the growth of some plant pathogens. From the previous research, many workers have reported antifungal activities of different plant species and stressed the importance of plants as a possible source of natural fungicides (Tewari and Dath, 1984; Lakshmanan, 1990; Tewari, 1995; Philip and Sharma, 1997; Ogbebor and Adekunle, 2005).

A number of factors can influence the antibacterial activity of plant extracts tested, including growth conditions, seasonal variations and extraction methodology. The observed antibacterial effects of the extracts are believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to possess antimicrobial properties (Cowan, 1999; Draughon, 2004). Some workers have also attributed their observed antimicrobial effects of plant extracts to the presence of

these secondary metabolites (Nweze *et al.*, 2004). Some workers have also identified tannins, flavonoids and alkaloids in the extracts of some medicinal plants (Esimone *et al.*, 1998).

Among all the leaf extracts tested, *C. suhuiensis* showed clear inhibitory effect at all dipping durations, thus it was found to be the most effective agent in controlling the mycelial growth of *F. oxysporum*. The results obtained in this study showed that *C. suhuiensis* extract possesses the highest antifungal properties. This is consistent with the finding of Roowi (2008) demonstrated that *C. suhuiensis* extract contains large amounts of flavonoids that present in the leaves of this fruit tree which retards the growth and activity of the pathogen.

Statistical Analysis also revealed that water and *E. oleina* extract had no significant difference in their inhibitory effects at dipping durations of 10, 20 40 and 60 minutes. Thus, we can deduce that *E. oleina* extract possesses some residue activity. This finding showed that the extract did not exhibit either the mycelium or the spore of *F. oxysporum*.

The fruit extracts tested in this study were good growth inhibitors of *F. oxysporum*, but *E. longifolia* extract was more effective against *F. oxysporum* than *C. suhuiensis* and *M. citrifolia* extracts. *E. longifolia* extract has been found to contain quassinoids such as eurycomanol and eurycomanolactone, alkaloids, sterols, sapenins and terpenoids. Several quassinoids isolated from *E. longifolia* extract have shown a variety of pharmacological effects, such as anti-inflammatory activity, antileukaemic activity, and growth inhibitory activity against *F.*

oxysporum. In Malaysia, research has confirmed thatisolated constituents as well as whole plant extract show aphrodisiac, anti-malarial, febrifuge, antihistaminic, anti-tumour, anti-ulcer and antiviral activity.

This dip treatment can be used treatment as recommended by Mesiter (1992) to inhibit *Fusarium* Wilt disease on banana plant.

CHAPTER 6

CONCLUSION

The results of this study clearly indicated that the antifungal activity against *F. oxysporum* varied among plant extracts. This study confirmed that the leaf extract of *C. suhuiensis* and the fruit extract of *E. longifolia* possessed much better *in vitro* antifungal activity than the other leaf and fruit extracts tested, respectively.

The significant effects of plant extracts tested, especially the leaf extract of *C. suhuiensis* and the fruit extract of *E. longifolia,* against *F. oxysporum* suggested that they may possibly be used as bio-fungicides. However, further analysis could be done to isolate the antimicrobial agents present in the extracts and for large scale production.

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APPENDIX

Inhibitory effects of six plant extracts (leaf and fruit) on mycelial growth of *F. oxysporum* seven days after inoculation

Treatment	Colony diameter (cm)			
	10 minutes	20 minutes	40 minutes	60 minutes
Control (water)	5.48	5.48	5.48	5.48
E. longifolia (leaf)	2.40	2.94	2.67	2.93
C. suhuiensis (leaf)	1.92	2.64	2.20	2.35
C. suhuiensis (fruit)	3.12	3.16	2.47	3.77
E. longifolia (fruit)	2.89	2.35	2.85	2.70
M. citrifolia (fruit)	2.88	4.64	3.29	2.69
E. oleina (leaf)	5.08	5.07	5.66	6.35
C. alata (leaf)	2.69	1.96	5.07	5.70
N. lappaceum (leaf)	2.79	3.56	4.19	5.48

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Screening of Potential Plants for the ontrol of *Fusarium* Wilt Disease" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food ciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.

TEO ZHI QIANG

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