



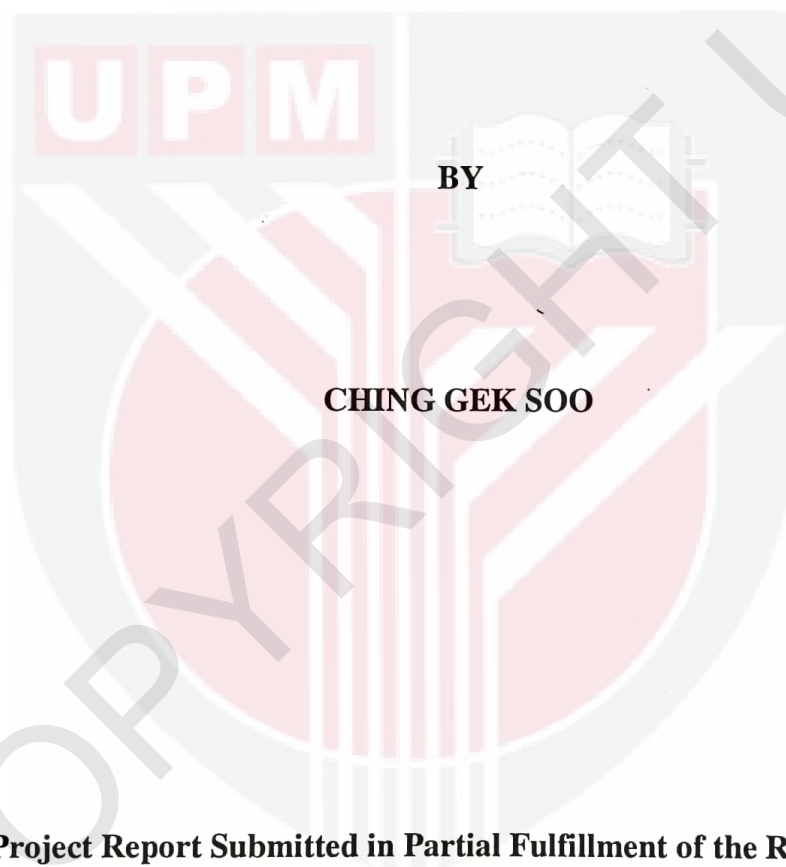
UNIVERSITI PUTRA MALAYSIA

***POTENTIAL OF EXSEROHILUM ROSTRATUM AS
BIOHERBICIDE FOR CONTROLLING DIGITARIA CILIARIS***

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**POTENTIAL OF *Exserohilum rostratum* AS BIOHERBICIDE FOR
CONTROLLING *Digitaria ciliaris***



BY

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A Project Report Submitted in Partial Fulfillment of the Requirement

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2007

The most graceful

*Especially dedicated to my beloved parents,
Dad, Ching Chin Koi,
Mum, Yu Booï Keen.*

*And other family member:
Brother, Steven Ching Teik Soo
And my cute sister, Ching Soo Jan.*

Thank you for your enormous care and support

ABSTRACT

The potential of *Exserohilum rostratum* (Drechsler) Leonard & Suggs as a bioherbicide for controlling southern crabgrass (*Digitaria ciliaris*) was evaluated in this study. The mycelial growth and sporulation was affected artificially by different light periods. The reductions of light periods significantly increased sporulation of *E. rostratum*. Meanwhile, the effect of surfactants on mycelial growth and sporulation varied with surfactant concentration (0, 0.01, 0.05, 0.1, 0.5 and 1 %). Both surfactants (Tween 20 and Act-Fast) increased the mycelial growth under lower surfactants concentration. Based on the results of pathogenicity tests, *E. rostratum* was highly pathogenic to *D. ciliaris*. The initial disease symptoms appeared with tiny spots and water soaking reactions which rapidly developed into elongated dark brown necrotic streaks. As the disease progressed, the inoculated plants were severely infected and turn to light brown; it finally died with dries up. The disease development of *E. rostratum* on different growth stages of *D. ciliaris* was expressed by the AUDPC and the steepness of slope (r_L). Two leaf-stage plants were most susceptible to *E. rostratum* ($r_L = 1.01$ logit / day; AUDPC = 610.75). From the greenhouse trial, *E. rostratum* was pathogenic to 2 weeks old crops seedlings (*Zea mays* and *Oryza sativa*). However, *Zoysia matrella* was resistant to the disease and *Ophiopogon japonicus* was immune to the disease. Additional studies are needed to develop the appropriate formulations and delivery method to improve the efficacy of bioherbicide. From this study, *E. rostratum* had demonstrated the potential as a bioherbicide for controlling *D. ciliaris*.

ABSTRAK

Potensi *Exserohilum rostratum* (Drechsler) Leonard & Suggs sebagai bioherbisid untuk pengawalan rumpai *Digitaria ciliaris* telah dikaji dalam penyelidikan ini. Pertumbuhan miselium dan penghasilan spora adalah dipengaruhi oleh tempoh cahaya yang berlainan. Pengurangan tempoh cahaya mengakibatkan peningkatan secara bererti terhadap penghasilan spora *E. rostratum*. Sementara itu, kesan surfaktan ke atas pertumbuhan miselium dan penghasilan spora adalah pelbagai dengan kepekatan surfaktan yang berbeza (0, 0.01, 0.05, 0.1, 0.5 dan 1 %). Kedua-dua surfaktan (Tween 20 dan Act-Fast) meningkatkan pertumbuhan miselium pada kepekatan surfaktan yang rendah. Berdasarkan keputusan ujian patogenisiti, *E. rostratum* didapati sangat patogenik terhadap *D. ciliaris*. Simptom awal yang muncul adalah bintik-bintik yang kecil dan lecuran, di mana ia berkembang dengan cepat menjadi tanda nekrotik yang memanjang berwarna coklat kehitaman. Dalam proses perkembangan penyakit ini, tumbuhan yang diinokulasi akan mengalami jangkitan yang serius dan bertukar kepada warna coklat cerah; akhirnya mati kekeringan. Perkembangan penyakit akibat *E. rostratum* pada peringkat pertumbuhan *D. ciliaris* yang pelbagai telah dijelaskan oleh AUDPC dan kecerunan (r_L). Peringkat dua daun rumpai adalah paling mudah dijangkiti oleh *E. rostratum* ($r_L = 1.01$ logit / hari; AUDPC = 610.75). Dari kajian rumah hijau, *E. rostratum* didapati patogenik kepada anak pokok tanaman (*Zea mays* dan *Oryza sativa*) yang berumur 2 minggu. Akan tetapi, *Zoysia matrella* didapati resistan terhadap penyakit dan *Ophiopogon japonicus* adalah kalis terhadap penyakit. Kajian lanjutan diperlukan untuk memperkembangkan formulasi dan kaedah pembawaan yang sesuai

untuk meningkatkan keberkesanan bioherbisid. Daripada kajian ini, *E. rostratum* telah menunjukkan potensi sebagai bioherbisid bagi pengawalan *D. ciliaris*.



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I certify that this research project report entitled “**Potential of *Exserohilum rostratum* as a Bioherbicide for Controlling *Digitaria ciliaris***” has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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TABLE OF CONTENTS

| | Page |
|---|-------------|
| DEDICATION | ii |
| ABSTRACT | iii |
| ABSTRAK | iv |
| ACKNOWLEDGEMENTS | vi |
| APPROVAL | vii |
| TABLE OF CONTENTS | viii |
| LIST OF TABLES | x |
| LIST OF FIGURES | xi |
| LIST OF ABBREVIATIONS | xii |
| CHAPTERS | |
| 1 INTRODUCTION | 1 |
| 1.1 General | 1 |
| 1.2 Background of study | 2 |
| 1.3 Importance of study | 2 |
| 1.4 Objectives of study | 4 |
| 2 LITERATURE REVIEW | 5 |
| 2.1 Morphology and Biology of <i>Digitaria ciliaris</i> | 5 |
| 2.2 Economic Importance of <i>Digitaria ciliaris</i> | 8 |
| 2.3 Management of <i>Digitaria ciliaris</i> | 8 |
| 2.3.1 Physical Control | 8 |
| 2.3.2 Chemical Control | 9 |
| 2.3.3 Biological Control | 11 |
| 2.3.3.1 Classical Strategy | 11 |
| 2.3.3.2 Inundative Strategy | 12 |
| 2.3.4 Integrated Weed Management | 13 |
| 2.4 Bioherbicide | 14 |
| 2.5 <i>Exserohilum rostratum</i> | 15 |
| 3 MATERIAL AND METHODS | 17 |
| 3.1 Samples Collection | 17 |
| 3.2 Media Preparation | 17 |
| 3.3 Characterization of Fungal Colony | 18 |
| 3.4 Effect of Light Period on Mycelial Growth and Sporulation | 18 |
| 3.5 Effect of Surfactants on Mycelial Growth and Sporulation | 19 |
| 3.6 Plants Production | 20 |
| 3.7 Inoculum Production | 20 |
| 3.8 Plant inoculation | 21 |

| | | |
|------|---|----|
| 3.9 | Disease Assessment | 21 |
| 3.10 | Host-Range Determination | 22 |
| 3.11 | Data Analysis | 23 |
| 4 | RESULTS | 24 |
| 4.1 | Characterization of Fungal Colony | 24 |
| 4.2 | Effect of Light Period on Mycelial Growth and Sporulation | 26 |
| 4.3 | Effect of Surfactants on Mycelial Growth and Sporulation | 28 |
| 4.4 | Pathogenicity Testing | 32 |
| 4.5 | Effect of <i>Exserohilum rostratum</i> on Disease Development of Different Growth Stages of <i>Digitaria ciliaris</i> | 34 |
| 4.6 | Host-Range Determination | 38 |
| 5 | DISCUSSION | 40 |
| 6 | CONCLUSION | 44 |
| | REFERENCES | 46 |
| | APPENDICES | 51 |
| | PUBLICATION OF THE PROJECT UNDERTAKING | 65 |

LIST OF TABLE

| Table | | Page |
|-------|---|------|
| 1 | Statistical parameters of spore morphology of <i>Exserohilum rostratum</i> | 25 |
| 2 | Mycelial growth and sporulation of <i>Exserohilum rostratum</i> in response to different light period after 5 days of incubation | 27 |
| 3 | Mycelial growth and sporulation of <i>Exserohilum rostratum</i> in response to different concentrations of Tween 20 after 6 days of incubation | 30 |
| 4 | Mycelial growth and sporulation of <i>Exserohilum rostratum</i> in response to different concentrations of Act-Fast after 6 days of incubation | 31 |
| 5 | The disease development (AUDPC) and infection rate of <i>Exserohilum rostratum</i> on the difference growth stages of <i>Digitaria ciliaris</i> | 37 |
| 6 | Host-range test and assessment of virulence of <i>Exserohilum rostratum</i> on economic importance plants | 39 |

LIST OF FIGURES

| Figure | | Page |
|--------|--|------|
| 1 | Conidia of <i>Exserohilum rostratum</i> under magnification power of 100 x (A) and 400 x (B) | 25 |
| 2 | Colony of <i>Exserohilum rostratum</i> (D) and an 8 mm agar disc on PDA (C) | 26 |
| 3 | Effect of light period on mycelial growth of <i>Exserohilum rostratum</i> throughout 5 days of incubation | 28 |
| 4 | Effect of Tween 20 on mycelial growth of <i>Exserohilum rostratum</i> throughout 6 days of incubation | 30 |
| 5 | Effect of Act-Fast on mycelial growth of <i>Exserohilum rostratum</i> throughout 6 days of incubation | 31 |
| 6 | Effect of <i>Exserohilum rostratum</i> on different growth stages of <i>Digitaria ciliaris</i> at fifth day after inoculation: 2 leaf-stage non-inoculated (A) and inoculated plants (B); 4 leaf-stage non-inoculated (C) and inoculated plants (D); 6 leaf-stage non-inoculated (E) and inoculated plants (F) | 33 |
| 7 | Disease progress on difference growth stages of <i>Digitaria ciliaris</i> inoculated with 1.32×10^6 conidia/ml of <i>Exserohilum rostratum</i> | 35 |
| 8 | Transformed disease progress using logistic model $\ln[y/(1-y)]$ on different growth stages of <i>Digitaria ciliaris</i> inoculated with 1.32×10^6 conidia/ml <i>Exserohilum rostratum</i> | 36 |

LIST OF ABBREVIATIONS

| | | |
|-------|---|--|
| IWM | = | Integrated Weed Management |
| MADA | = | Muda Agricultural Development Authority |
| PDA | = | Potato Dextrose Agar |
| LCB | = | Lactophenol Cotton Blue |
| UPMKB | = | Universiti Putra Malaysia Bintulu Campus |
| SAS | = | Standard Analysis System |
| CRD | = | Completely Randomized Design |
| RCBD | = | Randomized Complete Block Design |
| ANOVA | = | Analysis of Variance |
| DNMRT | = | Duncan New Multiple Range Test |
| P | = | Probability value |
| s^2 | = | Variance |
| s | = | Standard deviation |
| CV | = | Coefficient variation |
| AUDPC | = | Area Under Disease Progress Curve |
| DI | = | Disease Index |
| LAD | = | Leaf Area Damage |
| NA | = | Not Applicable |
| R^2 | = | Square of the Multiple Correlations |
| r_L | = | Infection rate |
| USDA | = | United States Department of Agriculture |

CHAPTER 1

INTRODUCTION

1.1 General

Weeds are the plants that interfere with the growth of desirable plants and that are unusually persistent and pernicious (Ross and Lembi, 1999). Weeds have great influence on the crop growth and reduce the yield of the required crops through competition for light, moisture and soil minerals. The effect of weed competition is greatest when the crop is young; thus, crop yields are much more likely to be reduced by early-season weed competition than by late-season competition. The persistence and ubiquity of weeds are due primarily to its ability to produce numerous, long-lived, and easily transportable seeds (Ross and Lembi, 1999).

Weeds can be described in several different ways: by habitat, life cycle, morphology or structure and physiology. Recently, weeds bring a lot of problems to farmers which can cause high losses in terms of yield and management cost. Meanwhile, it also creates fire hazards and unsightly areas; contribute to unsanitary conditions; and interfere with recreational activities, transportation and the delivery of public utilities (Ross and Lembi, 1999). Weeds cause an aggregate reduction in yield of the major world crops, representing huge loss of food. The lack of good weed control is considered as one of the dominant constraints to increase crop yield.

1.2 Background of Study

Digitaria ciliaris or southern crabgrass is a monocot weed which belongs to Family Poaceae. It is an economically important weed in temperate and tropical crops (Webster and Coble, 1997). Southern crabgrass is normally annual, but can exhibit perennial growth in some climates. The weed can root at nodes and form mats in moist soils. It is considered to be one of the serious weeds in some crops such as vegetable, soybean, cotton and peanut. This is a major weed found in roadsides, orchards, waste grounds and other agriculture areas. Southern crabgrass can highly reproduce by seeds in the soil when moisture and light are present.

This weed are tolerate to hot, dry and compacted soils. Once established, it can spread aggressively to crowd out desirable grasses. Southern crabgrass has to compete with crops for sunlight, water, space and nutrients. Thus, chemical herbicides are used to control southern crabgrass. Since chemical herbicides has been associated with development of resistant plants and cause toxicity to the environment. Therefore, an alternative control method is required to control this weed. In this study, *Exserohilum rostratum* was evaluated as bioherbicide to control southern crabgrass.

1.3 Importance of Study

This study was carried out to evaluate the use of bioherbicide to cause sufficient infection of the target weed. The scientific approach to weed control involves the following major components: (1) a thorough understanding of the overall objectives of weed management, (2) a step-by-step procedure for dealing with an individual weed

problem and (3) a realistic appraisal of the limitations and potentials of individual available technologies (Ross and Lembi, 1999).

There are many weed control methods which are usually implemented to reduce weed population. In actual practice, most of the emphasis and effort spent on controlling weeds is on physical and chemical methods. Physical method is still the major method of control in undeveloped countries. However, mechanical control is laborious, straining and time consuming operation due to strong extensive rooting system of weeds.

Chemical herbicides are dominated weed management strategies in developing countries especially in Malaysia. The chemical herbicides are able to kill weeds rapidly and may reduce the weed population. However, use of chemical herbicides leads to environmental pollution besides causing its own inherent ill effects (Barreto *et al.*, 2000). The continued use of some herbicides has resulted in the occurrence of herbicide resistance in some weed species. Nevertheless, the right usage of herbicide is needed to ensure that the recommended rate of herbicides do not exceeded.

Biological weed control is seen as a method to alleviate the problems arising out of the use of chemical herbicides (Charudattan, 2001; Mohan Babu *et al.*, 2003a). Biological weed control is an approach utilizing living organisms to control or reduce the population of weeds. Nowadays, people have strong self-conscious on using chemicals and practice healthy lifestyle. There was considerable current interest in the use of

plant pathogenic microbes as agents for the biological control of weeds. One potential approach to control weeds is to use phytotoxins or its derivatives for direct application to the noxious plant (Mahan Babu *et al.*, 2002b). The development of a low cost method for mass-producing stable bioherbicidal propagules is a critical step in the commercialization of these products (Bower, 1982). In an environmentally conscious world, the effective implementation of biological control might be essential for future food self-sufficiency.

1.4 Objectives of Study

The objectives of the present study are:

- a) To investigate the potential of *Exserohilum rostratum* as a bioherbicide in controlling *Digitaria ciliaris*.
- b) To evaluate the formulations to improve performance and standardization of *Exserohilum rostratum*.

CHAPTER 2

LITERATURE REVIEW

2.1 Morphology and Biology of *Digitaria ciliaris*

Digitaria ciliaris are commonly known as southern crabgrass and belongs to family Poaceae (Gramineae). It was a widely distributed tropical and subtropical weed. It was first described from Tropical South America but is said to be a native of Taiwan (Barnes and Chan, 1990). It is a common weed found in dryland field crops, vegetable crops, gardens, plantations, waste grounds and roadsides. This weed is also thrives in moist areas. *D. ciliaris* competes aggressively with other plants by spreading and crowding.

D. ciliaris is an annual grass which ascending up to about 60 cm high and rooting from nodes. Leaves sheath is bluish green and glabrous except for a few stiff bulbous-based hairs on the margin. Leaf-blade is soft tapering to both end, broad and margins cartilaginous. Ligules are very prominent, membranous and clasping the stem, junction of blade and leaf sheath has long white hairs. Inflorescence usually consists of several divergent spikes and digitately to subdigitately arrangement (Gilliland, 1971). A group of inflorescences can form a terminal whorl and the others are set singly below or in another false whorl. The spikelets are in pair, one long-stalked and the others short-stalked, elliptical, rarely tinged purple and single spikelet with sparse hairs along edges of glumes (Barnes and Chan, 1990).

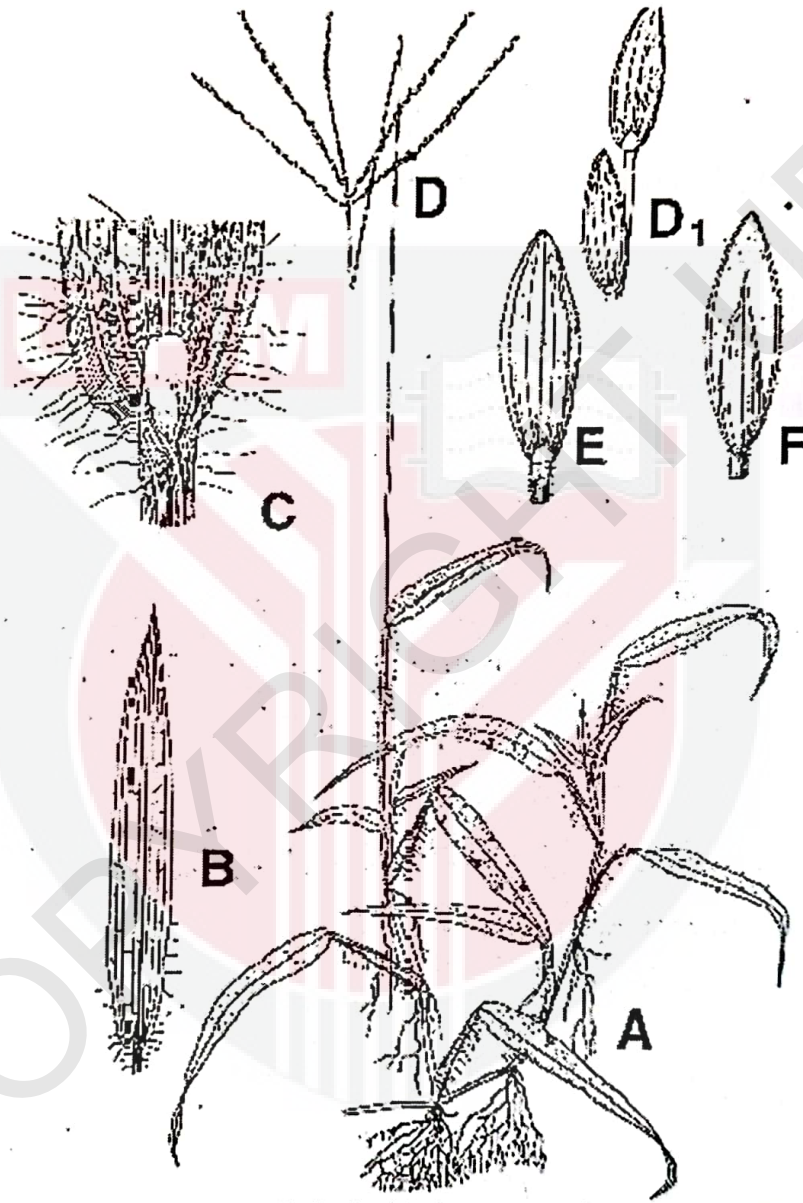
Longevity of *D. ciliaris* seeds in the soil has reported to be relatively short around two or three years (Noguchi and Morita, 1997). Germination of *D. ciliaris* seeds is stimulated by wide diurnal fluctuations in temperature and by light through a phytochrome-mediated system (Kobayashi *et al.*, 2003). Furthermore, weed seedlings emergence from deep layers of the soil is relatively slight even if seeds also exist in the deep layers (Kobayashi *et al.*, 2002).

Generally, weed seed germination that requires light is recognized to contribute to formation of a seed bank through seed burial (Wesson and Wareing, 1969). Weeds have good rooting system which can absorb nutrient and water aggressively. This becomes the important factor that makes weeds as highly competitive to other crops or plants.

Scientific name : *Digitaria ciliaris* (Retz.) Koel.

Common name : Southern crabgrass, known as “rumput jelambaran” in Malaysia

Family : Gramineae (Poaceae)



Digitaria ciliaris (Retz.) Koel. (Barnes and Chan, 1990)

- A) Creeping or ascending stem rooting from nodes B) Leaf
C) Ligule D) Inflorescence D1) Spikelets
E-F) Spikelet with sparse hairs along the edges of glume

2.2 Economic Importance of *Digitaria ciliaris*

Digitaria ciliaris can be used to produce forage, hay and silage for animal feed. It has a high nutritive value as fodder and herbivore animals can find it palatable. This species can also be used as ground covers and sand-binders.

However, *D. ciliaris* was considered as a serious weed of vegetables in Malaysia, tea in Taiwan and cotton in Australia. It is an alternate host of viruses causing stripe disease and streaked dwarf of rice (Barnes and Chan, 1990). *D. ciliaris* often becomes dominant weed in untilled soybean fields in north-east Japan (Kobayashi *et al.*, 2004). In United States, a single weed produced 700 tillers and 150,000 seeds and almost 2000 seeds were counted on a single weed in Philippines (Holm *et al.*, 1977). Fifty-six countries reported that southern crabgrass as weed in 33 crops. This weed has highly seeds production and extensive rooting system make it highly competitive to other crops.

2.3 Management of *Digitaria ciliaris*

There are 4 major methods of weed control which is physical control, chemical control, biological control and Integrated Pest Management.

2.3.1 Physical Control

The most commonly used physical methods are hand hoeing and pulling, tillage and mowing. Manipulation of the environment by physical methods includes mulching, flooding, fire and heat. Hand pulling and hoeing are the most primitive physical weed

control method. However, this method is time consuming and expensive when conducted on large areas with heavy infestations of weeds (Ross and Lemb, 1999).

Tillage with modern equipment can efficiently control the weed populations. Tillage destroys weeds by breaking it apart, cutting or tearing it loose from soil and causing it to dry out. However, tillage are timely operations, can damage soil structure and cause soil erosion. Mowing is another method that can effectively control weeds by removing tops prior to seed production. Species with growing points near the soil surface are not controlled effectively by mowing (Ross and Lemb, 1999).

Mulching is the method that control weeds by excluding light. This method is appropriate on small areas and on high value crop. It is best suited to emerged, established or transplanted crops (Ross and Lemb, 1999). However, this method is costly and limits the number of usable alternative weeding techniques.

2.3.2 Chemical Control

Chemical control can be administered to the pre-plant, pre-emergence and post-emergence. Pre-emergence and post-emergence chemical herbicides are usually used to control *Digitaria ciliaris*. Pre-emergence herbicides are applied according to the date of emergence for each target species. This effective chemical control are requires proper rate, accurate timing and uniformity of application. Pre-emergence herbicides are used to control *D. ciliaris* with preventing the seedlings become established in the target

areas. However, post-emergence chemical herbicide was reported easily absorbed throughout the plant. It is most effective used to control the young seedlings.

Chemical control is the most efficient, cheap and easy method to control weeds (Ismail, 1995). Chemical herbicides have been highly accepted due to the unparalleled success associated with its use and many contributions to crops production systems (Ross and Lemb, 1999). Weeds that are not economically controlled by other methods frequently can be controlled effectively and low cost with herbicide.

However, chemical herbicides leave behind residual and toxic chemical which have been reported to be detrimental to human, an animals and plants. The continued used of some herbicides has resulted in the occurrence of herbicide resistance in some weed species. Decomposition of herbicides in streams has resulted oxygen depletion and killed large amount of aquatic plants. Chemical herbicides are very difficult to degrade in the soil which has less organic material. Runoff will occur during rainy day and pollute water beneath the soil (Ross and Lemb, 1999).

There are urgent needs to discover and develop new weed control technologies and improve existing weed control technologies which are economical and environmentally sustainable.

2.3.3 Biological Control

Biological weed control is the intentional use of living organisms to reduce the vigor, reproductive capacity, density, impact of weeds (Quimby and Birdsall, 1995). Biological control agent must possess certain characteristics other than an ability to reduce a weed population to non-destructive levels. There is considerable current interest in the use of plant pathogenic microbes as agents for the biological control of weed (Jutsum, 1988). An endemic pathogen might be completely destructive to its weed host by applying a massive dose of inoculum at a particular vulnerable stage of weed growth. Biological weed control is seen as a method to alleviate the problems arising out of use of chemical herbicides (Charudattan, 2001; Mohan Babu *et al.*, 2003a). The strategies of biological weed control can be classified into two broad categories: 1) classical or inoculative and 2) inundative (Harley and Forno, 1992).

2.3.3.1 Classical Strategy

The concept of classical approach is to discover effective and highly host-specific agents from weed's native geographic range, confirm its safety and effectiveness by rigorous experimental evaluation, and introduce it into regions where the weed has been newly introduced and requires control (Charudattan and Dinoor, 2000). The major concern for biotic agents is host-specificity, are released into weed-infested sites and allowed to adapt and flourish in its new habitat eventually establishing a self-perpetuating regulation of weed infestation at acceptable levels (Kremer, 2002). Classical biological control requires a time period of one to several years to achieve adequate control. One of the most successful examples of classical biological weed

control is the introduction of a rust fungus, *Puccinia chondrilla* to control rush skeleton weed in Australia.

2.3.3.2 Inundative Strategy

The inundative strategy attempts to overwhelm a weed infestation with massive numbers of a biotic agent in order to reduce weed populations. In contrast to classical biological control, inundation involves timing of agent release to coincide with weed susceptibility to the agent and formulation of the agent to provide rapid attack of the weed host (Kremer, 2002). Biological control agents are concentrated, packaged and inundated to the target weed when the condition is favorable for infection. This manipulated agent must be reapplied to obtain continuing control.

A development of the inundation strategy is the bioherbicide approach, which involves application of weed pathogens in manner similar to herbicide applications. Fungal pathogens have the greatest potential as biological control agents because they can gain entry into the host plant without the assistance of a vector. Fungal pathogens are usually referred to as mycoherbicides. The use of pathogens for weed management could offer specificity in control, environmental safety, avoidance of herbicide resistance and less cost compared to chemical herbicides (Charudattan, 1982).

2.3.4 Integrated Weed Management

Integrated Weed Management (IWM) is a system of sustainable weed management that combines various control methods such as biological control, chemical control and others; in order to reduce the impact of the weeds to an economically acceptable level. An expanded and long-term approach to weed control is IWM in which all available strategies including tillage, cultural practices, herbicides, allelopathy and biological control are used to reduce the weed seedbank, prevent weed emergence and minimize competition from weeds growing with desired plants (Aldrich and Kremer, 1997).

Smith (1994) point out that combination of chemical and biological control to the weed will be more effective and decrease the chemical application. Similar to chemical herbicides, bioherbicides may be most effective as a component in an overall management program rather than as a single tactic approach. When considered as a three-part system, weed management offers several opportunities for integration of bioherbicides at the critical stages during weed development: as seeds in soil, as growing and competitive plants and during seed production (Aldrich and Kremer, 1997).

In Malaysia, IWM started on 1989 in paddy field Muda Agricultural Development Authority (MADA) and it showed the increased of yield by reducing herbicide application (Ho, 1994). IWM in rice emphasizes the simultaneous combination of various methods to mitigate the weeds problems.

2.4 Bioherbicide

Bioherbicide is defined as a plant pathogen which is formulated and applied in a manner analogous to chemical herbicide in an effort to control specific weed. The original bioherbicide concept was based on mass artificial culture of organism to obtain large quantities of inoculum for inundative application to the weed host to achieve rapid epidemic buildup and high levels of disease (Charudattan, 1991). Bioherbicide has been proven as a feasible, successful and environmentally sustainable method. The use of bioherbicide is based on the fundamental epidemiological principles of plant pathology. The preferred characteristics of a potential bioherbicide pathogen include: 1) growth and sporulation on artificial media, 2) highly virulent, 3) genetic stability, 4) restricted host range, 5) broad tolerance range, 6) prolific propagules production, 7) capacity to damage its host plant, and 8) innocuous in ecological effects (Templeton *et al.*, 1979).

The growth habit, growth rate and other biological parameters which determine susceptibility and subsequent disease development are critical than selection of particular weed species. Prevention of seed germination and seedling emergence is fundamental to maximally effective long-term weed management. Thus, bioherbicide can play a significant role in reducing weed infestations by attacking seeds and seedlings before it become competitive with crops plants (Kremer, 2002). Several approaches for managing the seed bank and seedling emergence have been described including direct application of biotic agents to soil or crop residues, or to crop seeds to prevent emergence of weeds in the crop seed-germinating zone, and in combination with solarization for enhancing seed deterioration in soil (Kremer, 1993).

Currently, five fungi and one bacterium are registered as bioherbicides in Canada, Japan, South Africa and United States (Charudattan, 2000). However, uses of bioherbicide for weeds control are not well recognized in Malaysia. These may due to the poorly resourced programs. At the same time, there is not much concern at both public and government levels, regarding the pollution for our environment with herbicides.

2.5 Exserohilum rostratum

Exserohilum rostratum is the most common species listed in current Florida and USDA disease indices (Alfieri *et al.*, 1993). Spores are produced in moist environments on the surface of the diseased leaves and spread by wind splashing water. A prolonged period of moisture on susceptible foliage provides an excellent environment for *E. rostratum* to infect and cause disease (Chase, 1997).

Colonies of *E. rostratum* are grey to blackish-brown, suede-like to floccose in texture and have an olivaceous black reverse. Conidia are straight, curved or slightly bent, ellipsoidal to fusiform and are formed apically through a pore on a sympodially elongating conidiophore. Terminal septa are particularly dark and thickened. Pigmented conidia are quite variable with approximately 200 x 8 μm and rostrate shaped with 6 to 16 septa. Conidia have a strongly protruding, truncate hilum and the septum above the hilum is usually thickened and dark. The end cells are often paler than the other cells and the walls are often finely roughened. Conidia germination is bipolar.

Exserohilum leaf spots are first observed as small, circular, translucent or water-soaked areas which rapidly develop into elongated dark necrotic streaks. These streaks expand and may coalesce, eventually blighting infected foliage (Chase, 1997). Foliar lesions (1-2 x 2-5 mm) are straw colored with brown margin. Lesions are often brown at first and then become light brown. Spores produced in leaf lesions have a tendency to wash into the centrally located, vase-shaped cup where extensive spotting can occur. Severe infections in the area usually lead to terminal decline of the host plant.

Previous study done by Kundat (2003), demonstrated that *E. rostratum* had potential as a bioherbicide for controlling Barnyard grass.

CHAPTER 3

MATERIALS AND METHODS

3.1 Samples Collection

The culture of *Exserohilum rostratum* was originally obtained from Associate Professor Dr. Jugah Kadir in Universiti Putra Malaysia Serdang. The stock culture was kept on half strength PDA (Potato Dextrose Agar) in the slant tubes and placed in the refrigerator at 8 °C. The culture was maintained by sub-culturing on fresh Potato Dextrose Agar (PDA) media for spore production which was used as inoculums in this study.

3.2 Media Preparation

Potato Dextrose Agar (PDA) was prepared with suspended hydrated PDA powder by the rate of 39 g per litre distilled water in the scope bottle. The solution was heated and stirred with magnetic stirrer on the stirring hotplate until the PDA powder was completely dissolved. Then, the solution was sterilized in autoclave at 121 °C (1.05 kg / cm²) for 15-20 minutes. After autoclaving, the solution was let to cool down for 10-15 minute until moderate warm. The solution was poured into plastic petri dishes and let it harden at room temperature (28 ± 2 °C). All these process was carried out inside the laminar flow chamber to avoid contamination.

3.3 Characterization of Fungal Colony

Cultures of *Exserohilum rostratum* were produced in Water Agar (WA) for this study. After 5 days of incubation, the conidia and conidiophores were scraped from WA with a scalpel and transferred directly to glass slides. The conidia was observed in term of growth pattern, shape, colour, number of cells per conidium and other external characteristics by using light compound microscope. Lactophenol Cotton Blue (LCB) was used during slides preparation in order to simplify the observation of conidial morphology and growth characteristics. Size of spores was one of the important characteristic to determine the species of fungi. The length and width of 80 conidia were measured through light compound microscope with a calibrated ocular micrometer.

3.4 Effect of Light Period on Mycelial Growth and Sporulation

Light period was tested at 0, 8, 12 and 24 hours with 5 replicates for each treatment. For each replication, an 8 mm diameter agar disc was taken from the edge of a 5 days-old PDA culture of *Exserohilum rostratum* and aseptically transferred to the center of a 9 cm diameter plastic petri dish of fresh PDA. These plates were incubated for 5 days at room temperature (28 ± 2 °C). Radial mycelial growth was determined at interval of 24 hours for 5 days as the mean of two perpendicular diameters minus the diameter of the inoculum plug (8 mm). The radial mycelial growth rate was expressed as mm/24 hours.

For light period study, spore counting was carried out after 5 days of incubation. The agar disc was removed from each petri dish before spore harvesting. Then, conidia were

harvested by flooding each plate with 10 ml of distilled water and scraping the agar surface with an “L” shape glass rod. The conidial suspension was filtered through a layer of cheesecloth and the final conidial concentration was determined with the aid of a hemacytometer.

3.5 Effect of Surfactants on Mycelial Growth and Sporulation

Two types of surfactants were selected for this study, namely Tween 20 (polyoxyethylenesorbitan monolaurate) and Act-Fast. The effect of surfactants on mycelial growth was evaluated by incorporating surfactants into PDA media at concentrations of 0, 0.01, 0.05, 0.1, 0.5 and 1 % (v/v). This experiment was conducted with 4 replicates for each treatment. An 8 mm diameter agar disc was taken from the edge of a 5 days-old PDA culture of *Exserohilum rostratum* and aseptically transferred to the center of a 9 cm diameter plastic petri dish of fresh PDA. These plates were incubated for 6 days at 30 °C. Radial mycelial growth was determined at interval of 24 hours for 6 days as the mean of two perpendicular diameters minus the diameter of the inoculum plug (8 mm). The radial mycelial growth rate was express as mm / 24 hours.

The agar disc was removed from petri dish before spore harvesting. Then, conidia were harvested by flooding each plate with 10 ml of distilled water and scraping the agar surface with an “L” shape glass rod. The conidial suspension was filtered through a layer of cheesecloth and final conidial concentration was determined with the aid of hemacytometer on the sixth day after incubation.

3.6 Plants Production

Seeds of the southern crabgrass (*Digitaria ciliaris*) were collected from Ladang Kongsi, UPM Bintulu Campus. Seeds of the southern crabgrass were transplanted to plastic pots and placed in greenhouse. Mineral soils of which the southern crabgrass were found was used as the growing media. This experiment was carried out with 3 different leaf-stages which include 2 leaf-stage, 4 leaf-stage and 6 leaf-stage. Each replicate consist of five seedlings were planted in a 15 cm diameter plastic pot.

The southern crabgrass seedlings were watered twice a day until soil saturation. Seedlings of the 4 leaf-stage had four fully expanded true leaves with the fifth leaf beginning to expand and the sixth leaf starting to open (14 days after planting for some species), as described by Mintz *et al.*(1992).

3.7 Inoculum Production

For inoculum production, a small piece of agar disc was taken from the stock culture of the isolate and aseptically transferred to a fresh PDA plate. The plate was sealed with Parafilm and incubated at room temperature (28 ± 2 °C) for 7 days. Conidia were harvested 7 days after incubation by flooding each plate with 10 ml of distilled water and scraping the agar surface with “L” shape glass rod. The conidial suspensions were adjusted to desire concentration (10^6 conidia per ml) with the aid of a hemacytometer.

3.8 Plant Inoculation

Southern crabgrass seedlings of different growth stages (2 leaf-stage, 4 leaf-stage and 6 leaf-stage) were used in this study. Conidial suspensions containing isolate of *Exserohilum rostratum* within the range of 10^6 conidia/ml were amended with 0.05 % (v/v) Tween 20 and 5 % (v/v) sunflower oil. The conidial suspensions with oil-surfactant mixture was then stirred using a magnetic stirrer. Then, the conidial suspensions were sprayed on the grass seedlings until runoff with approximately 10 ml per pot by using a hand-held plastic sprayer. Control seedlings with different leaf stages were sprayed with distilled water amended with 0.05 % (v/v) Tween 20 and 5 % (v/v) sunflower oil.

Then, all the inoculated and control seedlings were covered with polyethylene bags for 24 hours to maintain the relative humidity. These plastic pots were arranged in a Randomized Complete Block Design (RCBD) with 4 replicates per treatment. Disease incidence and disease severity was assessed daily at the interval of 24 hours after inoculation for 7 days. The susceptibility of the plants was based on the development of foliar necrotic spots or lesions.

3.9 Disease Assessment

Host reaction to *Exserohilum rostratum* was determined from the level of disease development on the inoculated plant seedlings. Disease incidence was expressed as the percentage of diseased plants based on the number of plants affected among the total plants inoculated (Horsfall and Cowling, 1978; Kranz, 1988). Plant reactions to disease

were based on disease index (levels of susceptibility of the plants and virulence of the pathogen) and disease severity [proportion of plant tissue area diseased (Kranz, 1988)].

The disease index scale (Appendix B) were based on the Leaf Area Damage (LAD) where 0 = no visible reaction, 1 = < 25 % LAD, 2 = 26-50 % LAD, 3 = 51-75 % LAD, 4 = 76-100 %. Disease severity was assessed on all plant seedlings in each pot by estimating the proportion of leaves with lesions and necrotic or dying leaves per seedling, using the disease rating scale developed by Kadir (1997). The rating scale consisted of 11 class values representing the percentage of disease severity (Appendix A). Disease severity was assessed daily after inoculation for 7 days.

3.10 Host-Range Determination

Host-range study was conducted to ensure the safety of the bioherbicide towards non-target plants. Plants closely related to the target weed were most rigorously tested while plant species in more distant taxonomic groups were less tested (Wapshere, 1974). In this study, the plant species were selected based on taxonomically close related plants, plants of economic important and pathological relevance. Medium 3:2:1 (top soil: sand: organic matter) were used as planting medium for host-range study.

There were 4 types of selected plants used in this study which was bario rice (*Oryza sativa*), corn (*Zea mays*), Zoysia grass (*Zoysia matrella*) and *Ophiopogon japonicus*. All these plants were grown in pots at greenhouse and were maintained by regular watering. Each pot consists of 2 seedlings of the selected plants. This experiment was conducted

with 4 replications. Conidial suspensions containing isolate of *Exserohilum rostratum* within the range of 10^6 conidia/ml were amended with 0.01 % (v/v) Tween 20 and 5 % (v/v) sunflower oil. After that, the conidial suspensions were sprayed on plant seedlings until runoff with approximately 10 ml per pot by using a hand-held plastic sprayer. Control plant seedlings were sprayed with distilled water amended with 0.05 % (v/v) Tween 20 and 5 % (v/v) sunflower oil. Both inoculated and control seedlings were covered with polyethylene bags for 24 hours to maintain the relative humidity.

Disease incidence and disease severity was assessed daily at the interval of 24 hours after inoculation for 7 days. The susceptibility of the plants was based on the development of foliar necrotic spots or lesions.

3.11 Data Analysis

The Statistical Analysis System (SAS) was used for the Analysis of Variance (ANOVA). The data were analyzed by using Analysis of Variance (ANOVA) and the treatment means were statistically compared by Duncan New Multiple Range Test (DNMRT) at the 5 % level of significance. The control efficacy was analyzed by plotting the Disease Progress Curve. Area Under Disease Progress Curve (AUDPC) and slope analysis were subjected to ANOVA procedure of SAS and the treatment mean were separated by using DNMRT at the 5 % level of significance.

CHAPTER 4

RESULTS

4.1 Characterization of Fungal Colony

The characterization of fungal colony was done by measuring the conidia length, width and counting the number of septa per conidium. The fungal spores were isolated and identified by Associate Professor Dr. Jugah Kadir as *Exserohilum rostratum* (Drechsler) Leonard and Suggs. The conidia were straight, curved or slightly bent, ellipsoidal to fusiform and were formed apically through a pore on a sympodially elongating conidiophore.

The pigmented conidia were quite variable in size and number of septa per conidium with the range of 33.53–102.81 μm length, 8.94–13.41 μm width and 5-12 septa per conidium (Table 1). The mean conidia length, width and number of septa per conidium were 72.60 μm , 11.51 μm and 9 septa respectively (Table 1). The conidia had a strong protruding truncate hilum and the septum above hilum was usually thickened and dark. The hilum constitutes the attachment point between the conidium (asexual spore) and the conidiophore from which the spore was produced. The end cells were often paler than the other cells and the walls were often finely roughened. The conidia were rostrate shaped and germination of conidia was bipolar.

Colonies of *E. rostratum* were grey to blackish-brown, suede-like to floccose in texture and had an olivaceous black reverse.

Table 1: Statistical parameters of spore morphology of *Exserohilum rostratum*

| Statistical parameters | Spore morphology | | |
|---------------------------|--------------------------|-------------------------|---------------------------|
| | Length (μm) | Width (μm) | No. of septa per conidium |
| Range | 33.53 – 102.81 | 8.94 – 13.41 | 5 – 12 |
| Mean | 72.60 | 11.51 | 9 |
| Variance (s^2) | 141.11 | 0.74 | 2.38 |
| Standard deviation (s) | 11.88 | 0.86 | 1.54 |
| Coefficient variation (%) | 16.36 | 7.47 | 7.47 |



Figure 1: Conidia of *Exserohilum rostratum* under magnification power of 100 x (A) and 400 x (B)

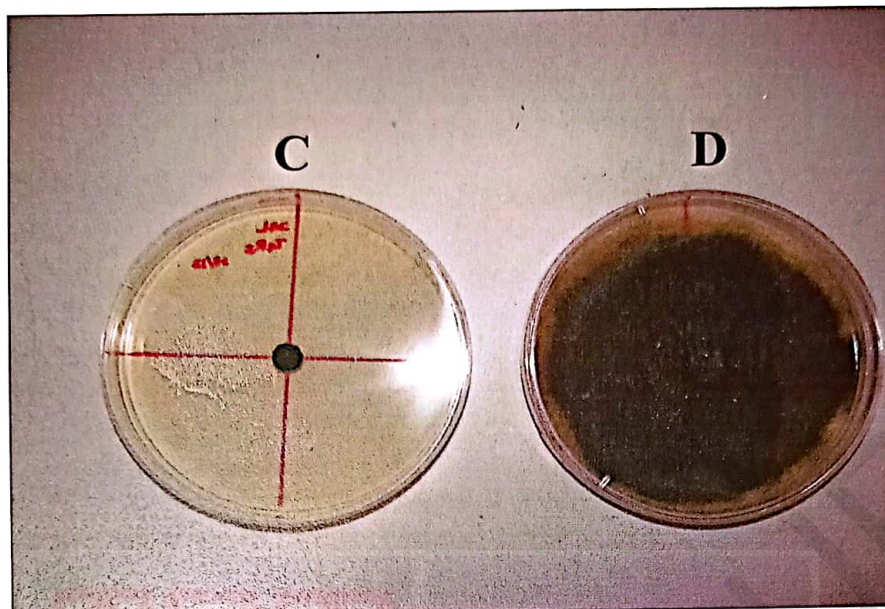


Figure 2: Colony of *Exserohilum rostratum* (D) and an 8 mm agar disc on PDA (C)

4.2 Effect of Light Period on Mycelial Growth and Sporulation

In this study, the effect of light period on mycelial growth and sporulation was evaluated by means of artificial light. Results showed that visible light exposure significantly affects mycelial growth, spore germination and reproduction after 5 days of incubation (Appendix E and F). The effect of light period on daily mycelial growth of *E. rostratum* throughout 5 days of incubation was presented in Figure 3.

The highest rate of mycelial growth was achieved in the 24 hour light period with 14.45 mm, followed by 0, 12 and 8 hour light period respectively (Table 2). However, the highest sporulation per area was achieved in the 0 hour light period with value of 1.553×10^3 spores per mm^2 , 1.093×10^3 , 0.607×10^3 and 0.107×10^3 spores per mm^2 for 8, 12 and 24 hour light period respectively (Table 2). The results demonstrated that the sporulation decreased with the increment of light period. Meanwhile, the highest value

in colony diameter of 5 days-old-culture was also achieved in the 24 hour light period with value of 72.25 mm; 65.95, 63.10 and 59.90 mm for 0, 12 and 8 hour light period respectively (Table 2). From this study, it shows that the duration of light exposure can cause inhibition of spore production.

Table 2: Mycelial growth and sporulation of *Exserohilum rostratum* in response to different light period after 5 day of incubation

| Light period (hour) | Mean radial growth (mm) | Mean colony diameter of 5 days-old-cultures (mm) | Mean sporulation (No. of spores/mm ²) |
|---------------------|-------------------------|--|---|
| 0 | 13.19 b | 65.95 b | 1.553 x 10 ³ a |
| 8 | 11.98 c | 59.90 c | 1.093 x 10 ³ b |
| 12 | 12.62 bc | 63.10 bc | 0.670 x 10 ³ c |
| 24 | 14.45 a | 72.25 a | 0.107 x 10 ³ d |

Note: Means followed by similar letters in the same column are not significantly different at P = 0.05 by Duncan New Multiple Range Test.

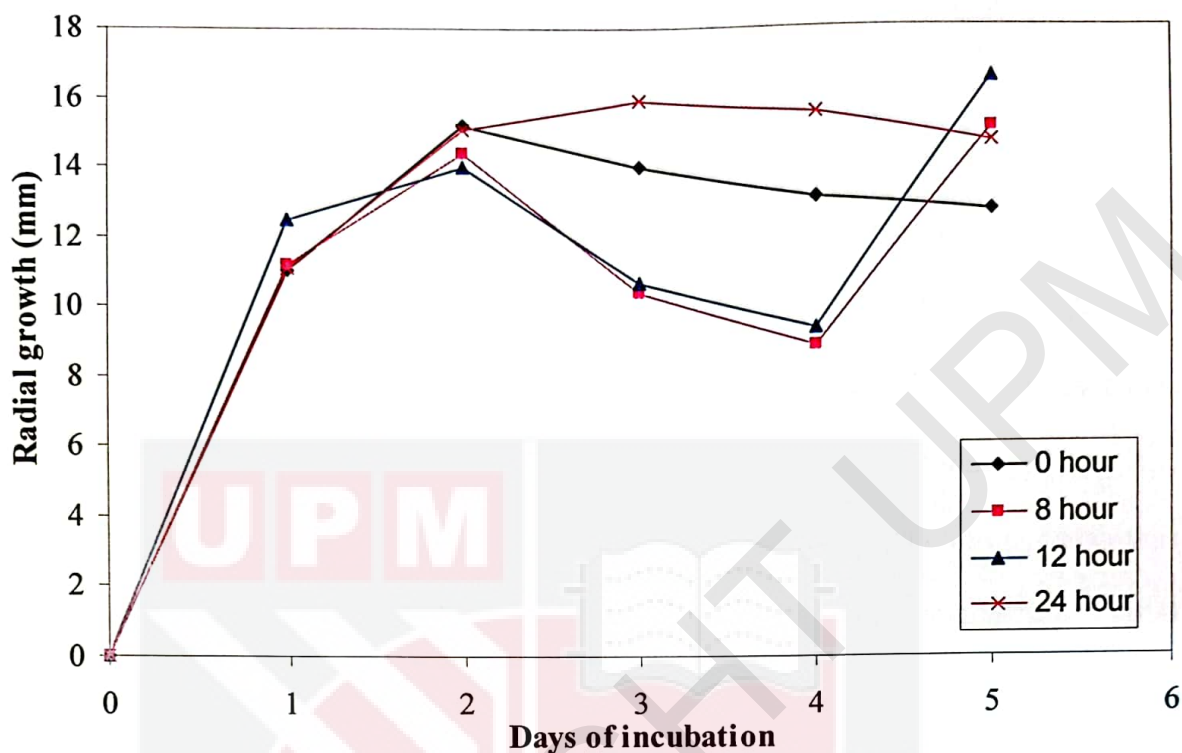


Figure 3: Effect of light period on mycelial growth of *Exserohilum rostratum* throughout 5 days of incubation

4.3 Effect of Surfactants on Mycelial Growth and Sporulation

Daily mycelial growth of *Exserohilum rostratum* in response to different concentrations of Tween 20 and Act-Fast throughout 6 days of incubation was presented in Figure 4 and 5 respectively. From the results obtained, Tween 20 significantly increased mycelial growth and colony diameter of a 6 days-old-culture at low concentrations (Appendix H and J). It was noted that Tween 20 stimulated mycelial growth of *E. rostratum* at lower surfactant concentrations, but inhibited mycelial growth at higher surfactant concentrations (Table 3). In contrast, Tween 20 did not show any significant differences between the concentrations on sporulation of *E. rostratum* (Appendix I). The highest sporulation per area was achieved in 0 % concentration of Tween 20 with value of 1.344×10^3 spores/mm²; 1.148×10^3 , 1.009×10^3 , 0.897×10^3 , 0.892×10^3 and

0.887×10^3 spores/mm² for 0.05, 0.1, 1, 0.01 and 0.5 % surfactant concentrations respectively (Table 3).

Meanwhile, Act-Fast gave better mycelial growth and increased colony diameter of a 6 days-old-culture at the lower concentrations (Appendix K and M). However, Act-Fast inhibited mycelial growth at all concentrations when compared to Tween 20. Act-Fast also showed the significant differences in sporulation of *E. rostratum* among the different surfactant concentrations (Appendix L). The highest sporulation per area was achieved in 1 % concentration of Act-Fast with value of 5.966×10^3 spores/mm²; 3.490×10^3 , 2.414×10^3 , 2.328×10^3 , 2.192×10^3 and 1.344×10^3 for 0.5, 0.01, 0.1, 0.05 and 0 % concentrations respectively (Table 4). This shows that Act-Fast does not inhibit sporulation of *E. rostratum*. The results indicate that Act-Fast increased total spores count of cultures under lower surfactant concentrations.

Table 3: Mycelial growth and sporulation of *Exserohilum rostratum* in response to different concentrations of Tween 20 after 6 days of incubation

| Concentration (v/v) | Mean radial growth (mm) | Mean colony diameter of 6 days-old-cultures (mm) | Mean sporulation (No. of spores/mm ²) |
|---------------------|-------------------------|--|---|
| 0% | 11.396 a | 68.375 a | 1.344 x 10 ³ a |
| 0.01% | 10.146 b | 60.875 b | 0.892 x 10 ³ b |
| 0.05% | 9.208 c | 55.250 c | 1.148 x 10 ³ ab |
| 0.1% | 8.834 cd | 53.000 cd | 1.009 x 10 ³ ab |
| 0.5% | 8.833 cd | 53.000 cd | 0.887 x 10 ³ b |
| 1% | 8.531 c | 51.188 c | 0.897 x 10 ³ b |

Note: Means followed by similar letters in the same column are not significantly different at P = 0.05 by Duncan New Multiple Range Test.

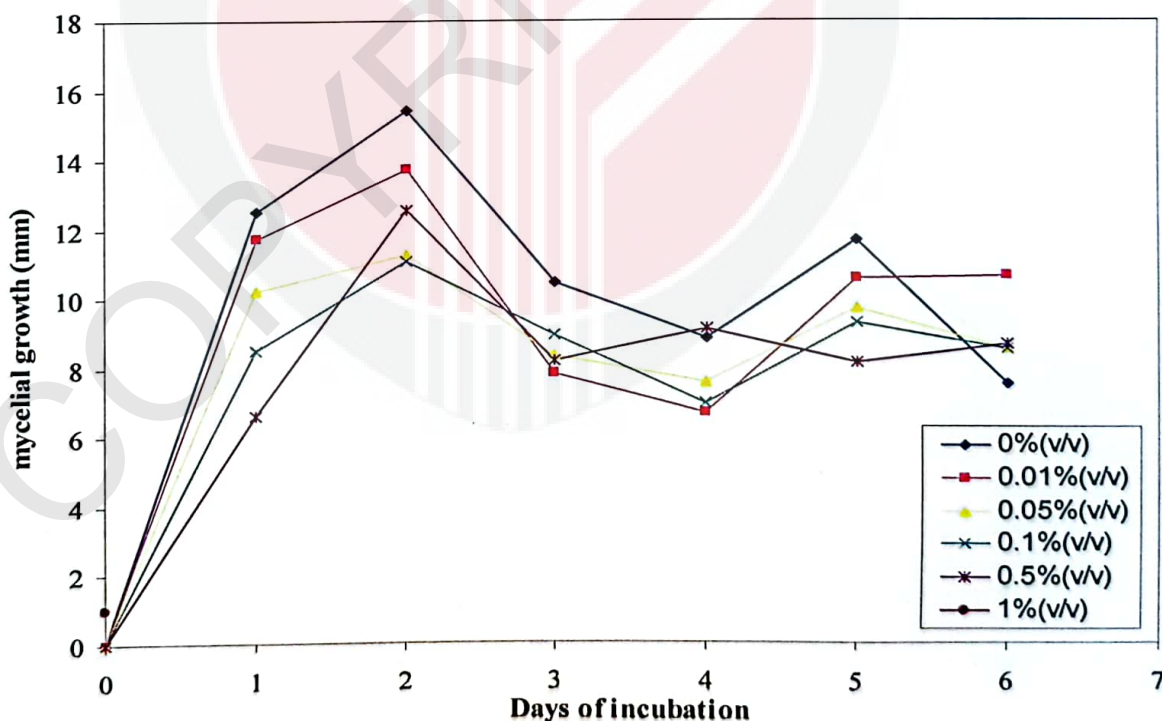


Figure 4: Effect of Tween 20 on mycelial growth of *Exserohilum rostratum* throughout 6 days of incubation

Table 4: Mycelial growth and sporulation of *Exserohilum rostratum* in response to different of concentrations of Act-Fast after 6 days of incubation

| Concentration (v/v) | Mean radial growth (mm) | Mean colony diameter of 6 days-old-cultures (mm) | Mean sporulation (No. of spores/mm ²) |
|---------------------|-------------------------|--|---|
| 0% | 11.396 a | 68.375 a | 1.344 x 10 ³ c |
| 0.01% | 6.917 b | 41.500 b | 2.414 x 10 ³ bc |
| 0.05% | 6.198 c | 37.188 c | 2.192 x 10 ³ bc |
| 0.1% | 4.938 d | 29.625 d | 2.328 x 10 ³ bc |
| 0.5% | 2.677 e | 16.063 e | 3.490 x 10 ³ b |
| 1% | 1.740 f | 10.438 f | 5.966 x 10 ³ a |

Note: Means followed by similar letters in the same column are not significantly different at P = 0.05 by Duncan New Multiple Range Test.

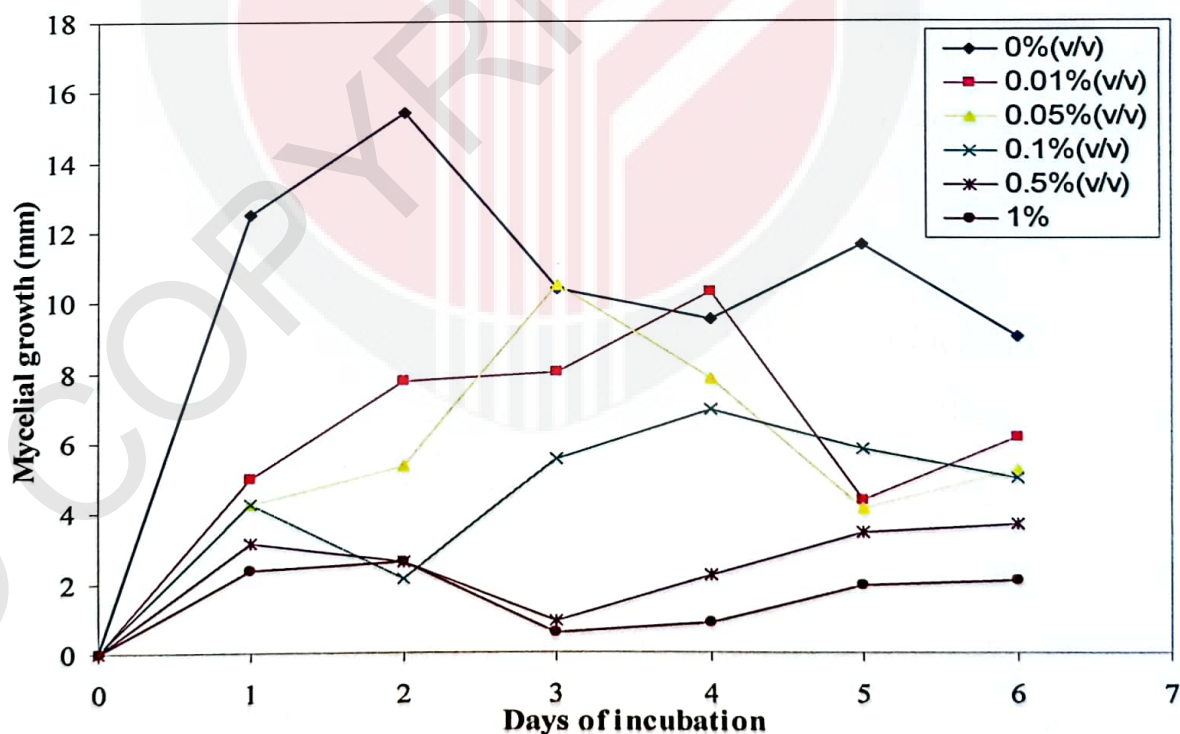


Figure 5: Effect of Act-Fast on mycelial growth of *Exserohilum rostratum* throughout 6 days of incubation

4.4 Pathogenicity Testing

The suspension of *Exserohilum rostratum* consisting of conidia and mycelia was highly pathogenic to *Digitaria ciliaris*. The initial symptoms were observed 24 hours after the plants were inoculated. The first symptoms on the inoculated plants appeared as tiny circular spots, with translucent or water soaked areas which rapidly developed into elongated dark brown necrotic streaks. These streaks expand and coalesce, eventually blighting infected foliage. Blighting often occurred on leaf tips and margins. Spores produced in leaf lesions had a tendency to wash into centrally located regions where extensive spotting could occur (Leahy, 1999). The inoculated plants were severely infected and turned to light brown; and finally died with dries up. Most of the leaves were dead 7 days after inoculation. However, control plants that were sprayed with 5 % (v/v) of sunflower oil and 0.05 % Tween 20 did not show any damage on the leaves and remained healthy throughout the study. The re-isolation of the pathogen from the diseased areas confirmed that the conidium had similar characteristics with the conidium used for inoculation, thus satisfying Koch's Postulate.

The infection progress of the leaf blight caused by *E. rostratum* on *D. ciliaris* was assessed visually according to the disease rating scale (Appendix A). The infection had a short incubation period, and it progressed slowly at the initial stage of disease development. However, the disease progress accelerated drastically to reach a disease severity level of ≥ 80 % after 6 days of inoculation. After this period, nearly 100 % of the inoculated plants were heavily infected with ≥ 85 % disease severity. The conidium was unable to cause secondary infection under greenhouse conditions. Meanwhile, there

was 0 % severity on the adjacent non-inoculated control plants. The disease progress can be described more precisely with the logistic model.

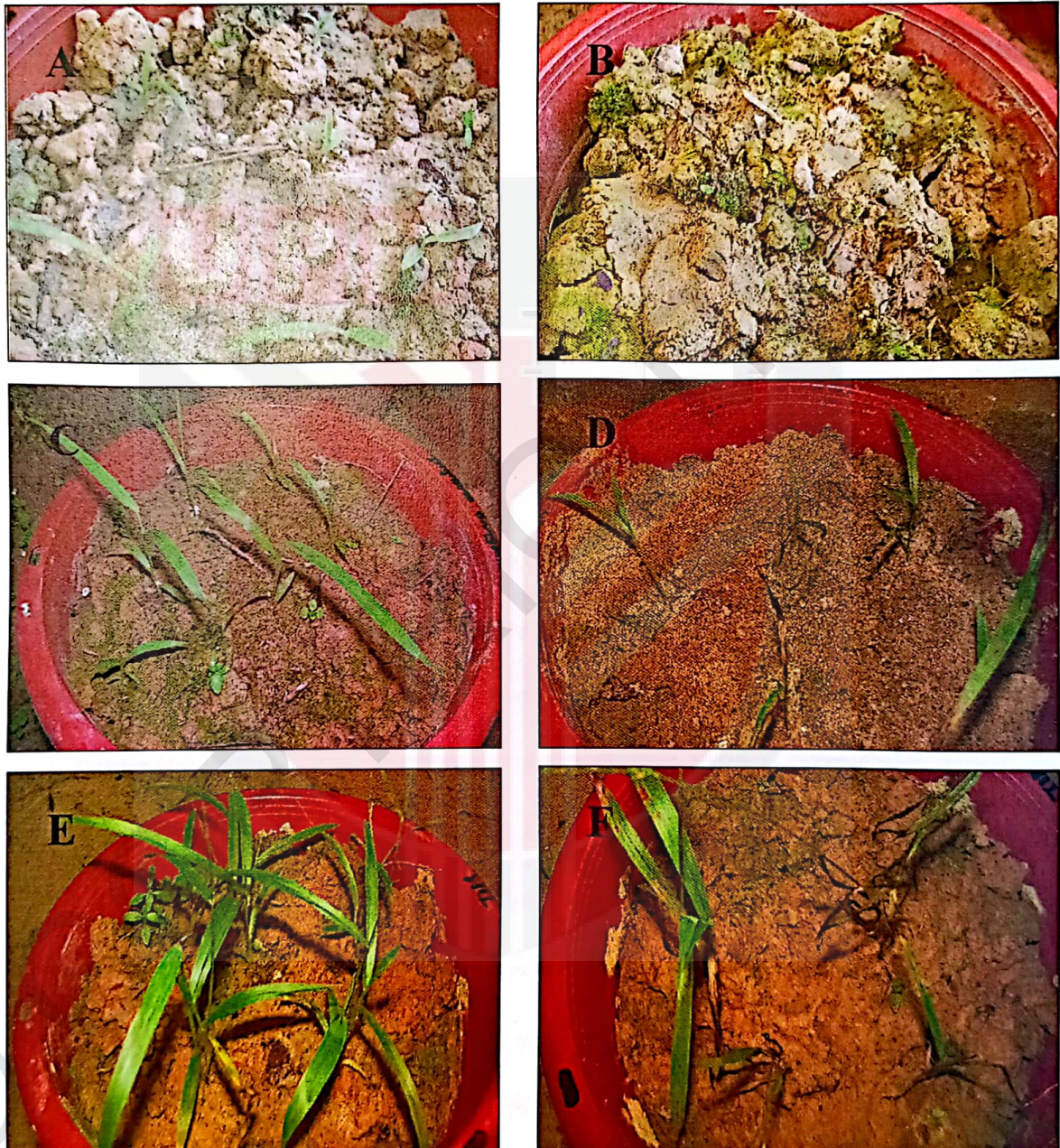


Figure 6: Effect of *Exserohilum rostratum* on different growth stages of *Digitaria ciliaris* at fifth day after inoculation: 2 leaf-stage non-inoculated (A) and inoculated plants (B); 4 leaf-stage non-inoculated (C) and inoculated plants (D); 6 leaf-stage non-inoculated (E) and inoculated plants (F)

4.5 Effect of *Exserohilum rostratum* on Disease Development of Different Growth Stages of *Digitaria ciliaris*

Disease development was evaluated on *D. ciliaris* at different growth stages; 2, 4 and 6 leaf-stage plants. The different growth stages of plants that were spray-inoculated with 1.32×10^6 conidia/ml developed severe disease and died. However, non-inoculated control plants did not demonstrate any disease symptoms.

The effect of *E. rostratum* on disease development for *D. ciliaris* can be depicted by the Area Under Disease Progress Curve (AUDPC) and the slope (Infection rate). The results showed that *E. rostratum* caused significant reduced on disease development with increasing leaf stage (Appendix N and O). The AUDPC of all different growth stages including control plants showed significant differences between each other (Appendix N). Meanwhile, the highest disease severity was achieved on 2 leaf-stage plants; 4 leaf-stage, 6 leaf-stage and untreated control plants 7 days after inoculation respectively (Figure 7). The disease developed was faster on 2 leaf-stage plants as indicated by the highly apparent infection rate ($r_L = 1.01$ logit/day). The infection rate at 2 leaf-stage was significantly higher compared to the 4 leaf-stage and 6 leaf-stage on disease development (Table 5). While, the infection rates of 4 leaf-stage and 6 leaf-stage plants were not significantly different among each other. The rate of disease development decreased with increasing age of the plants. This indicates that *E. rostratum* provided better control in younger plant seedlings. A linear relationship was obtained by transforming disease severity values using the logistic model ($\ln [y/(1-y)]$) (Figure 8). Meanwhile, there was no disease development on non-inoculated control plants.

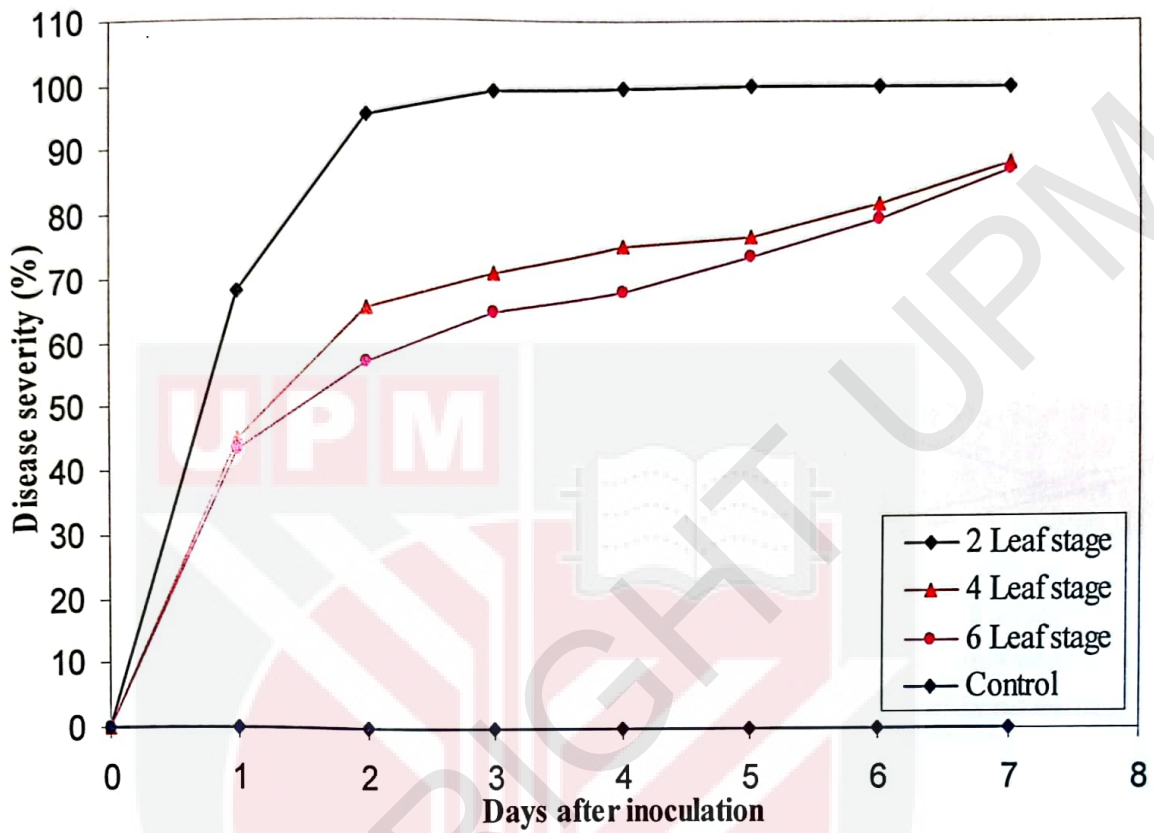


Figure 7: Disease progress on difference growth stages of *Digitaria ciliaris* inoculated with 1.32×10^6 conidia/ml of *Exserohilum rostratum*

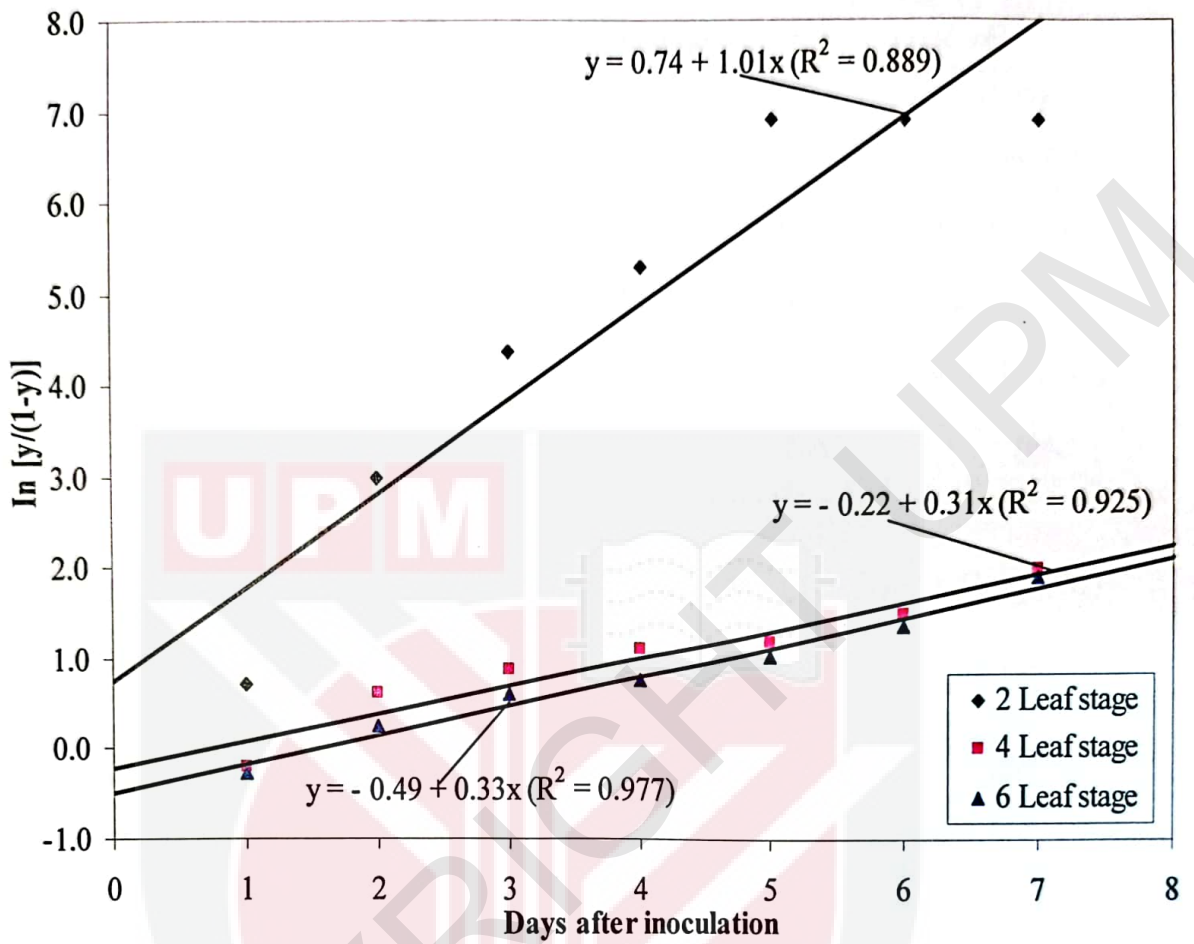


Figure 8: Transformed disease progress using logistic model $\ln[y/(1-y)]$ on different growth stages of *Digitaria ciliaris* inoculated with 1.32×10^6 conidia/ml *Exserohilum rostratum*

Table 5: The disease development (AUDPC) and infection rate of *Exserohilum rostratum* on the difference growth stages of *Digitaria ciliaris*

| Treatment | AUDPC ^A | Infection rate, r_L (logit / day) ^B | R ² | Regression equation |
|--------------|--------------------|---|----------------|---------------------|
| 2 leaf stage | 610.75 a | 1.01 a | 0.889 | $y = 0.74 + 1.01x$ |
| 4 leaf stage | 458.75 b | 0.31 b | 0.925 | $y = -0.22 + 0.31x$ |
| 6 leaf stage | 429.75 c | 0.33 b | 0.977 | $y = -0.49 + 0.33x$ |
| Control | 0 d | NA* | NA* | NA* |

^A Area Under Disease Progress Curve (AUDPC) = $\sum_1^{n-1} \frac{(Y_i + Y_{i+1})}{2} \times (X_{i+1} - X_i)$

^B Infection rate determined by using the regression of the transformed disease severity values on days

Note: Means followed by similar letters in the same column are not significantly different at P = 0.05 by Duncan New Multiple Range Test.

NA* = Not Applicable

4.6 Host-Range Determination

The susceptibility of the non-target plants to *Exserohilum rostratum* was assessed by the presence of disease symptoms and the susceptibility of the host plants according to the disease index which was developed by Kadir (1997). There were 4 different types of host plant seedlings (2 weeks old) inoculated with 1.46×10^6 conidia/ml of *E. rostratum*. From the results obtained, *E. rostratum* was pathogenic to the 2 weeks old seedlings of *Zea mays* and *Oryza sativa* with both the disease incidence of 100 % and disease index of 4. The 2 weeks old seedlings of *Zoysia matrella* were resistant to the pathogen as indicated by the disease incidence of 50 % and disease index of 1. However, the 2 weeks old of *Ophiopogon japonicus* were immune to *E. rostratum* as indicated by the disease index of 0 (Table 6).

Meanwhile, the 2 weeks old of control plant seedlings for each host plants remained immune condition throughout the study. No secondary infection was observed although the pots were arranged closely together. It showed that the initial of inoculation was responsible to the mortality of tested seedlings.

The results of the host-range study demonstrated that the younger seedlings of the crop plants were physically weak and can be easily infected by *E. rostratum*. Meanwhile, the mixture of the sunflower oil and Tween 20 might improve the infection rate of *E. rostratum* on crop plants.

Table 6: Host-range test and assessment of virulence of *Exserohilum rostratum* on economic importance plants

| Host | Disease Incidence (%) ^A | | Disease Index ^B | |
|-------------------------------------|------------------------------------|------------|----------------------------|------------|
| | Control | Inoculated | Control | Inoculated |
| Target weed | | | | |
| <i>Digitaria ciliaris</i> | 0 | 100 | 0 | 4 |
| Turf grass | | | | |
| <i>Zoysia matrella</i> | 0 | 50 | 0 | 1 |
| Crops | | | | |
| <i>Zea mays</i> (Thai sweet) | 0 | 100 | 0 | 4 |
| <i>Oryza sativa</i> (Bario variety) | 0 | 100 | 0 | 4 |
| Ornamental plant | | | | |
| <i>Ophiopogon japonicus</i> | 0 | 0 | 0 | 0 |

^A Disease incidence was based on the number of plants affected among the total plants inoculated (percentage of diseased plants)

^B Disease index was based on disease rating scale, where 0 = immune; 1-2 = resistant reaction; 3 = susceptible; and 4 = very susceptible (mortality)

CHAPTER 5

DISCUSSION

Conidia of *Exserohilum rostratum* were straight, ellipsoidal to narrowly rostrate, had a hilum protruding from end of the basal cell, 5-12 cells per conidium, and measured 33.53-102.81 x 8.94-13.41 μm . These features were comparable to those described by Alcorn, 1988. The measurement demonstrated that the conidia were variable in sizes (Table 1). *E. rostratum* grew fast on PDA to fully colonize the 9 cm petri dish in 5-7 days.

Exposure to artificial light was an important factor that affected mycelial growth and spores germination of *E. rostratum*. The highest mycelial growth was achieved at 24 hours light period, but the highest spore production was exhibited in complete darkness condition. The number of spore production was not correlated with the rate of colony growth (Wyss *et al.*, 1999). Meanwhile, it is possible that a medium that supports heavy sporulation may not be the best to assure quality of spores (Wyss *et al.*, 1999). The significant reduction of spores could be attributed by the increment of light period. The lowest spore production was observed at the 24 hours light period which showed that the light could stimulate the mycelial growth but inhibited the sporulation. Complete darkness was the best condition for mass production of *E. rostratum*.

The surfactant Tween 20 stimulated mycelial growth and sporulation of *E. rostratum* at lower concentrations and inhibited it at higher concentrations. This observation was

consistent with another report on *Collectrichum* spp. (Zhang *et al.*, 2002). Grant *et al.* (1990) also observed that Tween 20 stimulated germination of *Collectrichum* spp., but they did not use higher concentrations as in the present study. The reduction of radial growth was more consistent compared to sporulation per unit area. The results showed that lower concentration of Tween 20 was more suitable for inoculation purposes. Tween 20 had been commonly used as one of the primary surfactants for the initial screening of bioherbicide candidates (Boyette *et al.*, 1996). Tween 20 was primary surfactants that reduced surface tension and increased leaf-surface wettability to improve spores deposition and retention on sprayed leaves.

Meanwhile, Act-fast increased the mycelial growth, but reduced the sporulation per unit area at lower concentrations (Table 4). However, mycelial growth in all concentrations of Act-Fast was less when compared to the Tween 20 and control treatment. Act-Fast did not inhibit sporulation at higher concentration. This study showed that Act-Fast was less compatible than Tween 20 in the fungal cultures. The conidia of many fungi might have mechanisms for the self-inhibition of germination and appressoria formation (Kolattukudy *et al.*, 2000). In bioherbicide research and application, inoculums at high densities were generally sprayed onto target weeds (Klein and Auld, 1995), but self inhibition mechanism may reduce the overall effectiveness of biocontrol pathogen by restricting inoculum germination and penetration. The ability of Tween 20 and Act-Fast might reduce the risk of poor germination at high inoculum densities applied in the field. The release of conidia from self-inhibition might be related to surface tension, so that surfactants were considered to adjust the surface tension of foliar to enhance

infection. However, sunflower oil was used as an oil emulsion to stick spores and distribute spores uniformly on the leaves.

Leaf spots caused by *E. rostratum* were similar to those caused by *Bipolaris* sp. Initial spots were pinpoint, water-soaked and dark brown. The shapes were circular to elliptical. Lesions were dark brown at first then become light brown. Lesions appeared on mature plants as straw coloured with light brown margins, elongated spots.

Plant age was considered as a factor affecting disease expression and plant susceptibility. Determination of the plant growth stages at which host was susceptible to a bioherbicide was important for understanding the potential of bioherbicide candidates (Watson and Wymore, 1990). In the greenhouse study, *E. rostratum* was highly pathogenic to the younger plants. The AUDPC and infection rate (r_L) increased with the reduction of leaf stage (Table 5). It showed that the highest infection rate was at 2 leaf stage ($r_L = 1.01$ logit / day). This result was similar to the outcomes of other potential bioherbicides in which younger seedlings had the greatest susceptibility to disease (Boyette and Walker, 1985; Charudattan, 1990; TeBeest *et al.*, 1978; Kadir *et al.*, 2000). This was because younger plants tissues were tender and very susceptible to infection (Azean, 2004). The studies of host-pathogen interaction at microscopic level were needed to confirm the pathological reaction of these crop plants to *Digitaria ciliaris*.

Host-range evaluation was an important step in determination of the safety of fungal pathogens as bioherbicide. Conidial concentration at 10^6 conidia/ml was suggested to inoculate in the younger growth stages to achieve an excellent control of *D. ciliaris*. *E. rostratum* can cause severe infection on 2 weeks old crop plants. In contrast, the 4 leaf-stage crop plants were resistant to the disease (Kundat, 2003). Meanwhile, free moisture and temperature affected spore germination, infection, disease severity and subsequent weed control. These conditions were not provided naturally under field conditions. Lack of moisture for penetration and infection was considered as a major constraint to development of bioherbicides. A prolonged period of moisture on susceptible foliage can provide an excellent environment for fungal infestation. Further investigations are necessary to develop and improve the methods for mass production and application of the fungus as bioherbicide.

Disease developments were affected by other factors such as temperature, light and other epidemiology factors. Further experiments must be carried out in greenhouse to assess disease severity efficiently with other factors. An appropriate formulation of infective propagules, which reduces or eliminates the dew requirement, would greatly improve the potential of a pathogen as a bioherbicide. Inert solid carriers, alginate granules, invert emulsions and oil-in-water emulsion had all been considered (Hanspeter and Genevieve, 1998; Greaves *et al.*, 1998).

CHAPTER 6

CONCLUSION

The fungus *Exserohilum rostratum* had demonstrated the potential as a bioherbicide for controlling *Digitaria ciliaris*. Since chemical herbicides had been associated with development of resistance, toxicity to expose population and environment, active research had been going on certain countries to search for alternative bioherbicide. Meanwhile, Tween 20 stimulated mycelial growth and sporulation of *E. rostratum* at lower concentrations and inhibited it at higher concentrations. However, Act-Fast increased the mycelial growth, but reduced the sporulation per unit area at lower concentrations.

The bioherbicide approaches were suitable to most agriculture condition in Malaysia where specific control of the weeds was required to avoid damage to the non-target plants. This method was suggested since the chemical controls were likely to be non selective on grassy weed and may also caused environmental pollution. In this study, *E. rostratum* caused 100% mortality against all *D. ciliaris* inoculated at 2 leaf-stage.

However, there were also several constraints in bioherbicide development which can be classified as biological, environmental, technological or commercial. Biological constraints were included host variability and resistance which normally could be recognized at an early stage of a project. Meanwhile, environmental constraints were included temperature and humidity which had been recognized as major factors

influencing the efficacy of bioherbicides. Technological constraints were included mass production and formulation which had often blocked bioherbicide development. However, the requirement for extended periods of high humidity or leaf wetness (dew period) for maximum infection by most plant pathogens had restricted commercial bioherbicides to irrigated systems when applied as an aerial spray.

Further studies can be done on field trial to solve these constraints of bioherbicide development. Development of new formulations and delivery method was needed to enhance the potential of *E. rostratum* as bioherbicide. Recently, fungus had been formulated in oil emulsion in order to eliminate its dew-period requirement and still allow it to cause high disease severity. A combination of pathogens in one formulation or the use of a broad host-range pathogen in a novel way also expanded the horizons for bioherbicide markets.

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APPENDICES

Appendix A: Disease Rating Scale (Kadir, 1997)

| Class | Disease Severity (%) |
|-------|----------------------|
| 1 | 0 |
| 2 | 1-5 |
| 3 | 5-10 |
| 4 | 10-15 |
| 5 | 15-30 |
| 6 | 30-55 |
| 7 | 55-65 |
| 8 | 65-75 |
| 9 | 75-85 |
| 10 | 85-95 |
| 11 | 95-100 |

Appendix B: Disease Index (Horsfall and Cowling, 1978)

| Index | Description |
|--------------|--|
| 0 | No visible reaction (immune) |
| 1 | Minute, pinhead-sized spots (Resistant) |
| 2 | Small, brown to dark-brown lesions with no distinguishable centers (Resistant) |
| 3 | Small eyespot-shaped lesion with gray center (Susceptible) |
| 4 | Typical coalescing lesion, elliptical with gray centers (Very Susceptible) |

Appendix C: No. of Cells per Conidium

| No | Cells | No | Cells |
|----|-------|----|-------|
| 1 | 7 | 41 | 8 |
| 2 | 9 | 42 | 10 |
| 3 | 7 | 43 | 10 |
| 4 | 10 | 44 | 10 |
| 5 | 10 | 45 | 8 |
| 6 | 10 | 46 | 11 |
| 7 | 9 | 47 | 8 |
| 8 | 10 | 48 | 8 |
| 9 | 9 | 49 | 12 |
| 10 | 9 | 50 | 9 |
| 11 | 11 | 51 | 9 |
| 12 | 6 | 52 | 8 |
| 13 | 12 | 53 | 6 |
| 14 | 8 | 54 | 8 |
| 15 | 11 | 55 | 9 |
| 16 | 9 | 56 | 8 |
| 17 | 8 | 57 | 12 |
| 18 | 9 | 58 | 10 |
| 19 | 10 | 59 | 6 |
| 20 | 9 | 60 | 10 |
| 21 | 7 | 61 | 12 |
| 22 | 9 | 62 | 8 |
| 23 | 9 | 63 | 10 |
| 24 | 12 | 64 | 10 |
| 25 | 9 | 65 | 11 |
| 26 | 10 | 66 | 9 |
| 27 | 9 | 67 | 10 |
| 28 | 10 | 68 | 8 |
| 29 | 10 | 69 | 10 |
| 30 | 9 | 70 | 9 |
| 31 | 9 | 71 | 9 |
| 32 | 7 | 72 | 10 |
| 33 | 12 | 73 | 12 |
| 34 | 8 | 74 | 8 |
| 35 | 12 | 75 | 9 |
| 36 | 9 | 76 | 8 |
| 37 | 10 | 77 | 10 |
| 38 | 9 | 78 | 10 |
| 39 | 7 | 79 | 5 |
| 40 | 8 | 80 | 8 |

Appendix D: Size of Conidium

| No | Length | Width | No | Length | Width |
|----|---------|--------|----|--------|--------|
| 1 | 55.875 | 13.410 | 41 | 69.285 | 12.293 |
| 2 | 80.460 | 13.410 | 42 | 96.105 | 11.175 |
| 3 | 58.110 | 11.175 | 43 | 82.695 | 13.410 |
| 4 | 69.285 | 11.175 | 44 | 67.050 | 11.175 |
| 5 | 71.520 | 11.175 | 45 | 55.875 | 11.175 |
| 6 | 69.285 | 11.175 | 46 | 84.930 | 11.175 |
| 7 | 71.520 | 11.175 | 47 | 60.345 | 12.293 |
| 8 | 89.400 | 11.175 | 48 | 64.815 | 11.175 |
| 9 | 73.755 | 11.175 | 49 | 75.990 | 11.175 |
| 10 | 78.225 | 11.175 | 50 | 69.285 | 12.293 |
| 11 | 82.695 | 11.175 | 51 | 77.108 | 11.175 |
| 12 | 37.995 | 8.940 | 52 | 64.815 | 10.058 |
| 13 | 102.810 | 11.175 | 53 | 64.815 | 12.293 |
| 14 | 67.050 | 11.175 | 54 | 67.050 | 13.410 |
| 15 | 75.990 | 11.175 | 55 | 67.050 | 13.410 |
| 16 | 82.695 | 11.175 | 56 | 59.228 | 11.175 |
| 17 | 71.520 | 11.175 | 57 | 93.870 | 11.175 |
| 18 | 78.225 | 11.175 | 58 | 78.225 | 11.175 |
| 19 | 71.520 | 11.175 | 59 | 64.815 | 11.175 |
| 20 | 75.990 | 13.410 | 60 | 74.873 | 11.175 |
| 21 | 62.580 | 11.175 | 61 | 93.870 | 11.175 |
| 22 | 65.933 | 11.175 | 62 | 69.285 | 11.175 |
| 23 | 75.990 | 11.175 | 63 | 71.520 | 11.175 |
| 24 | 87.165 | 11.175 | 64 | 74.873 | 11.175 |
| 25 | 69.285 | 11.175 | 65 | 89.400 | 11.175 |
| 26 | 80.460 | 11.175 | 66 | 69.285 | 11.175 |
| 27 | 71.520 | 11.175 | 67 | 89.400 | 12.293 |
| 28 | 71.520 | 11.175 | 68 | 69.285 | 12.293 |
| 29 | 84.930 | 12.293 | 69 | 80.460 | 11.175 |
| 30 | 71.520 | 12.293 | 70 | 71.520 | 11.175 |
| 31 | 78.225 | 10.058 | 71 | 71.520 | 11.175 |
| 32 | 55.875 | 11.175 | 72 | 71.520 | 11.175 |
| 33 | 89.400 | 13.410 | 73 | 89.400 | 13.410 |
| 34 | 60.345 | 11.175 | 74 | 53.640 | 11.175 |
| 35 | 82.695 | 11.175 | 75 | 64.815 | 13.410 |
| 36 | 60.345 | 11.175 | 76 | 58.110 | 11.175 |
| 37 | 78.225 | 11.175 | 77 | 78.225 | 11.175 |
| 38 | 78.225 | 11.175 | 78 | 62.580 | 11.175 |
| 39 | 67.050 | 11.175 | 79 | 33.525 | 11.175 |
| 40 | 84.930 | 13.410 | 80 | 67.050 | 11.175 |

Appendix E: Effect of Light Period on Mycelial Growth

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Treatment | 3 | 16.545000 | 5.515000 | 20.92 | < 0.0001 |
| Error | 16 | 4.218000 | 0.263625 | | |
| Corrected Total | 19 | 20.763000 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|-----------|
| 0.796850 | 3.931426 | 0.513444 | 13.060000 |

Appendix F: Effect of Light Period on Sporulation after 5 Day Inoculation

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Treatment | 3 | 5685799.750 | 1895266.583 | 40.03 | < 0.0001 |
| Error | 16 | 757488.800 | 47343.050 | | |
| Corrected Total | 19 | 6443288.550 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|----------|
| 0.882438 | 25.42321 | 217.5846 | 855.8500 |

Appendix G: Effect of Light Period on Colony Diameter of 5 Days-Old-Cultures

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Treatment | 3 | 413.6250000 | 137.8750000 | 20.92 | < 0.0001 |
| Error | 16 | 105.4500000 | 6.5906250 | | |
| Corrected Total | 19 | 519.0750000 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|----------|
| 0.796850 | 3.931426 | 2.567221 | 65.30000 |

Appendix H: Effect of Tween-20 (Surfactant) on Mycelial Growth

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Treatment | 5 | 23.69435071 | 4.73887014 | 43.57 | < 0.0001 |
| Error | 18 | 1.95767125 | 0.10875951 | | |
| Corrected Total | 23 | 25.65202196 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|----------|
| 0.923684 | 3.474658 | 0.329787 | 9.491208 |

Appendix I: Effect of Tween 20 (surfactant) on Sporulation after 5 Day Inoculation

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|-----------|--------|
| Treatment | 5 | 681593.315 | 136318.663 | 2.32 (ns) | 0.0857 |
| Error | 18 | 1056925.035 | 58718.057 | | |
| Corrected Total | 23 | 1738518.349 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|----------|
| 0.392054 | 23.53545 | 242.3181 | 1029.588 |

Appendix J: Effect of Tween 20 (surfactant) on Colony Diameter of 5 Days-Old-Cultures

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Treatment | 5 | 852.9505208 | 170.5901042 | 43.60 | < 0.0001 |
| Error | 18 | 70.4218750 | 3.9123264 | | |
| Corrected Total | 23 | 923.3723958 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|----------|
| 0.923734 | 3.473279 | 1.977960 | 56.94792 |

Appendix K: Effect of Act-Fast (Surfactant) on Mycelial Growth

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Treatment | 5 | 238.2223507 | 47.6444701 | 407.23 | < 0.0001 |
| Error | 18 | 2.1059213 | 0.1169956 | | |
| Corrected Total | 23 | 240.3282720 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|----------|
| 0.991237 | 6.060128 | 0.342046 | 5.644208 |

Appendix L: Effect of Act-Fast (surfactant) on Sporulation after 5 Day Inoculation

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|--------|
| Model | 5 | 52844806.66 | 10568961.33 | 7.83 | 0.0005 |
| Error | 18 | 24286976.27 | 1349276.46 | | |
| Corrected Total | 23 | 77131782.92 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|----------|
| 0.685124 | 39.30120 | 1161.584 | 2955.593 |

Appendix M: Effect of Act-Fast (Surfactant) on Colony Diameter of 5 Days-Old-Cultures

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Model | 5 | 8576.106771 | 1715.221354 | 407.49 | < 0.0001 |
| Error | 18 | 75.765625 | 4.209201 | | |
| Corrected Total | 23 | 8651.872396 | | | |

| R-Square | CV | Root MSE | Mean |
|----------|----------|----------|----------|
| 0.991243 | 6.058347 | 2.051634 | 33.86458 |

Appendix N: ANOVA and Mean Separation Analysis of AUDPC for Difference Growth Stages of *Digitaria ciliaris* Inoculated with *Exserohilum rostratum*

| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Treatment | 3 | 824858.1875 | 274952.7292 | 9012.79 | < 0.0001 |
| Block | 3 | 81.6875 | 27.2292 | 0.89 | 0.4815 |
| Error | 9 | 274.5625 | 30.5069 | | |
| Corrected Total | 15 | 825214.4375 | | | |

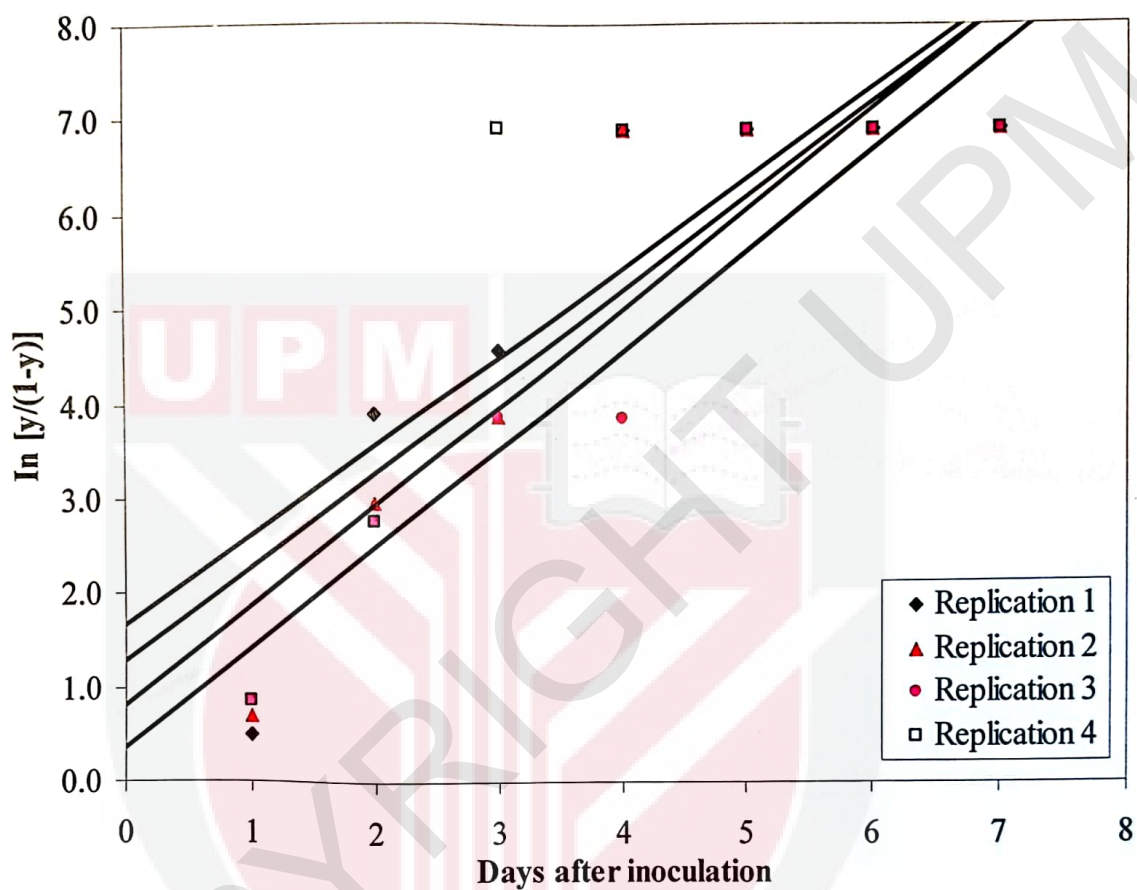
| R-Square | CV | Root MSE | Radial Mean |
|----------|----------|----------|-------------|
| 0.999667 | 1.473619 | 5.523309 | 374.8125 |

Appendix O: ANOVA and Mean Separation Analysis of Slope for Difference Growth Stages of *Digitaria ciliaris* Inoculated with *Exserohilum rostratum*

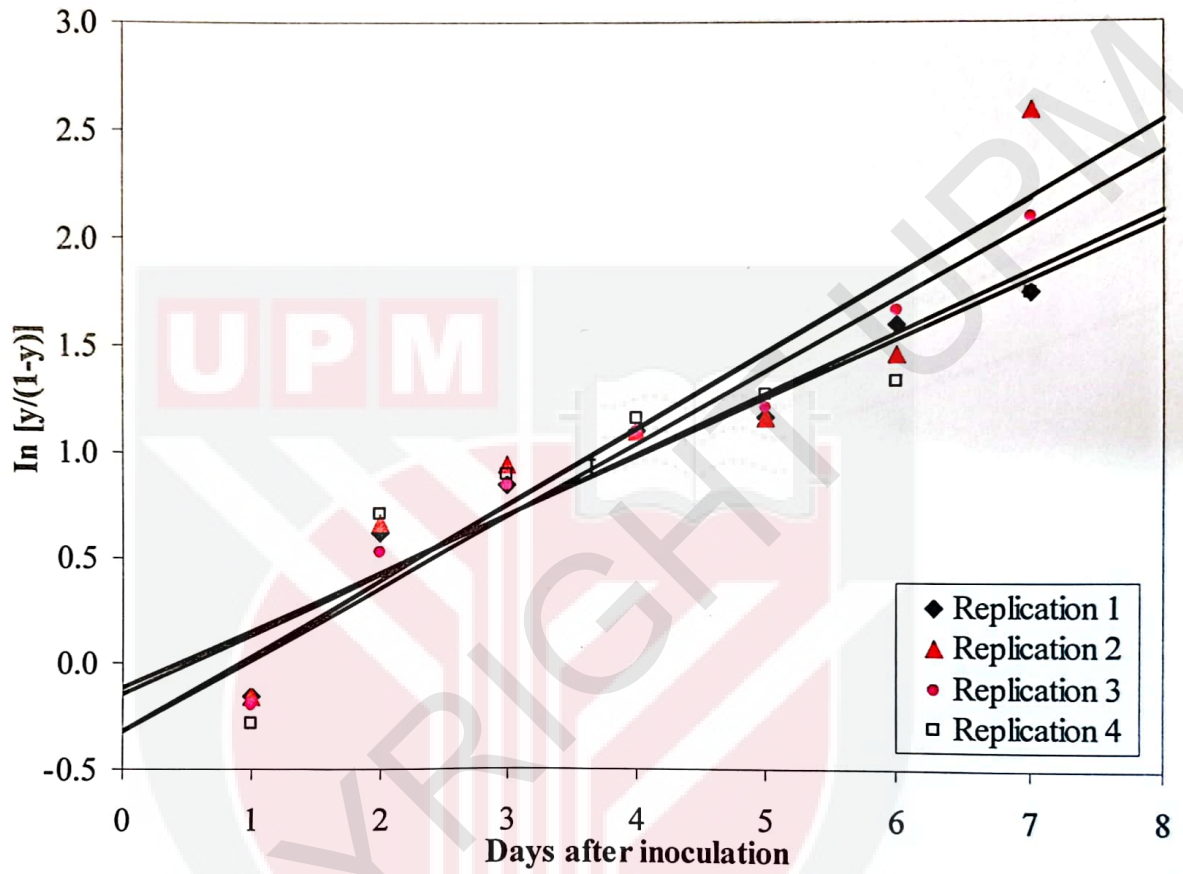
| Source | DF | Sum of Squares | Mean Square | F value | Pr > F |
|-----------------|----|----------------|-------------|---------|----------|
| Treatment | 2 | 1.26026229 | 0.63013115 | 789.06 | < 0.0001 |
| Block | 3 | 0.02318291 | 0.00772764 | 9.68 | 0.0103 |
| Error | 6 | 0.00479152 | 0.00079859 | | |
| Corrected Total | 11 | 1.28823672 | | | |

| R-Square | CV | Root MSE | Radial Mean |
|----------|----------|----------|-------------|
| 0.996281 | 5.121057 | 0.028259 | 0.551825 |

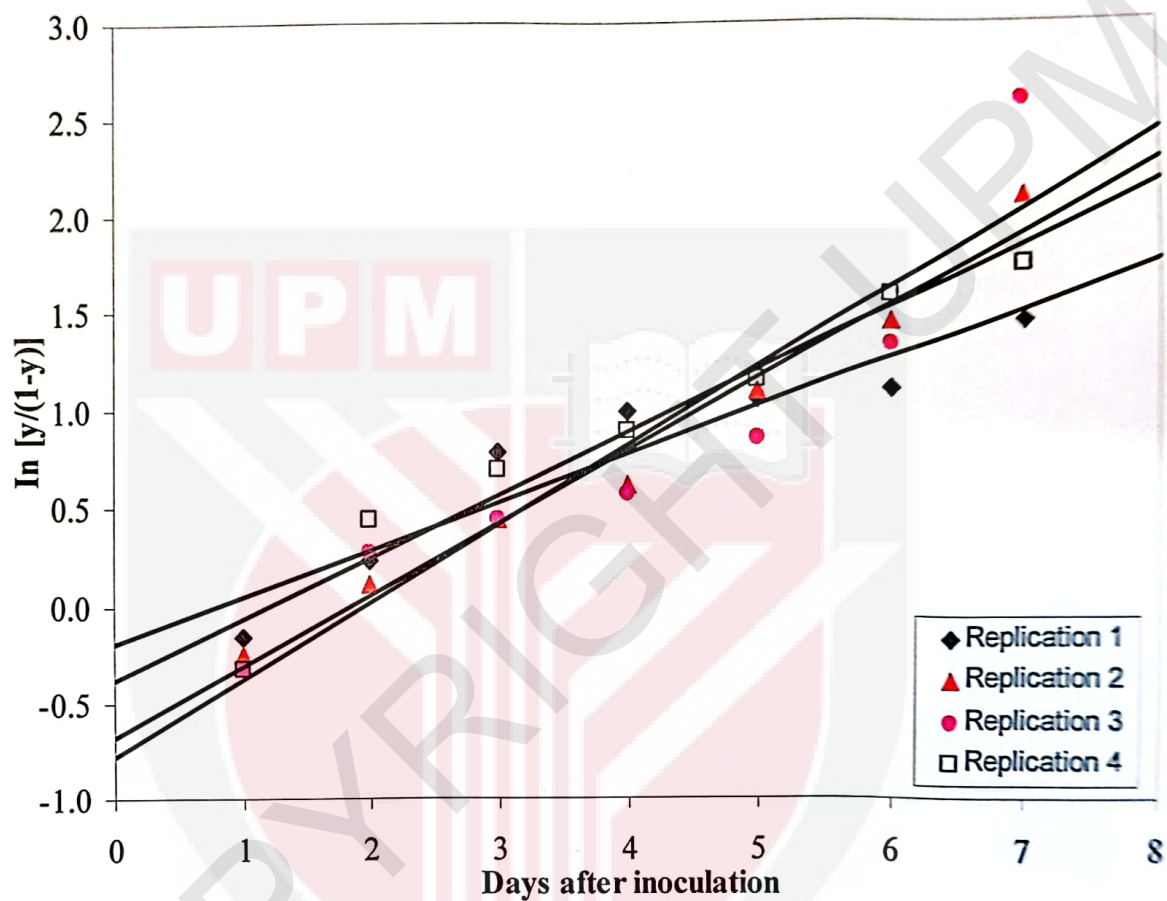
Appendix P: Transformed Disease Progress Using the Logistic Model $\ln [y/(1-y)]$ on 2 Leaf-Stage of *Digitaria ciliaris* Inoculated with *Exserohilum rostratum*



Appendix Q: Transformed Disease Progress Using the Logistic Model $\ln [y/(1-y)]$ on 4 Leaf-Stage of *Digitaria ciliaris* Inoculated with *Exserohilum rostratum*



Appendix R: Transformed Disease Progress Using the Logistic Model $\ln [y/(1-y)]$ on 6 Leaf-Stage of *Digitaria ciliaris* Inoculated with *Exserohilum rostratum*



APPENDIX S: Regression Equations and R-Squared Values for Difference Growth Stages of *Digitaria ciliaris* Inoculated with *Exserohilum rostratum*

| Growth stages | Regression equation | R ² |
|---------------------|---------------------|----------------|
| 2 leaf-stage | | |
| Replication 1 | $y = 1.29 + 0.99x$ | 0.757 |
| Replication 2 | $y = 0.81 + 1.06x$ | 0.811 |
| Replication 3 | $y = 0.37 + 1.05x$ | 0.902 |
| Replication 4 | $y = 1.66 + 0.95x$ | 0.641 |
| 4 leaf-stage | | |
| Replication 1 | $y = -0.15 + 0.28x$ | 0.923 |
| Replication 2 | $y = -0.33 + 0.36x$ | 0.867 |
| Replication 3 | $y = -0.32 + 0.34x$ | 0.959 |
| Replication 4 | $y = -0.12 + 0.27x$ | 0.846 |
| 6 leaf-stage | | |
| Replication 1 | $y = -0.19 + 0.24x$ | 0.894 |
| Replication 2 | $y = -0.68 + 0.37x$ | 0.978 |
| Replication 3 | $y = -0.78 + 0.40x$ | 0.869 |
| Replication 4 | $y = -0.39 + 0.32x$ | 0.952 |

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "**Potential of *Exserohilum rostratum* as Bioherbicide for Controlling *Digitaria ciliaris***" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in form approved by the Faculty.



Ching Gek Soo

Date: 7 Mei 2007