UNIVERSITI PUTRA MALAYSIA

POTENTIAL OF EXSEROHILUM ROSTRATUM AS A BIOHERBICIDE FOR CONTROLLING GOOSEGRASS (ELEUSINE INDICA (L.) GAERTN.)

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POTENTIAL OF *Exserohilum rostratum* AS A BIOHERBICIDE FOR CONTROLLING GOOSEGRASS (*Eleusine indica* (L.) Gaertn.)

BY

PRAMESWARI GOPAL

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A Project Report Submitted in 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

2007

ABSTRACT

Exserohilum rostratum (Drecshler) is a fungal pathogen, which was identified by Leonard and Suggs as a leaf blight causing agent in *Echinochloa* spp. *Exserohilum rostratum* was studied for its potential to suppress the growth of goosegrass (*Eleusine indica*). When applied as a postemergence foliar spray at one to four leaf stage, with a 24 hour dew period, *E. rostratum* caused 100% weed mortality in *Eleusine indica* at all four leaf stages tested. *Exserohilum rostratum* does not show any pathogenic activity on economically important crop species tested includeing rice varieties MR219, MR220, ligo corn and sweet corn, as indicated by leaf severity index. The level of disease severity on goosegrass was directly proportional to the conidial concentration of *Exserohilum rostratum.* Concentrations at 10^6 conidia/ml provided excellent control of *Eleusine indica.* At initial infection, *Eleusine indica* showed blight like reaction one day after inoculation followed by rapid necrosis of affected tissue. The symptoms showed by *Exserohilum rostratum* indicated that phytotoxins may be operative in pathogenesis. From the results of this study, proven that *Exserohilum rostratum* has potential as a bioherbicide to control goosegrass.

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ABSTRAK

Exserohilum rostratum (Drecshler) merupakan kulat patogen, yang dikenalpasti oleh Leonard dan Suggs sebagai agen penyebab lecur daun pada *Echinochloa* spp. *Exserohilum rostratum* turut dikenalpasti mempunyai potensi untuk mengawal rumput sambau. Apabila disembur sebagai posmuncul pada satu hingga empat peringkat daun, dengan syarat dibekalkan dengan kelembapan selama 24 jam, *Exserohilum rostratum* menyebabkan 100% kematian *Eleusine indica* pada kesemua empat peringkat daun yang diuji. *Exserohilum rostratum* tidak menunjukkan sebarang kesan pengaruh terhadap tanaman penting ekonomi yang diuji termasuk varieti padi MR219, MR22O, jagung ligo serta jagung manis sepertimana yang ditunjukkan oleh indeks jangkitan daun. Kadar kesan penyakit pada rumput sambau berkadar langsung dengan kepekatan konidia *Exserohilum rostratum*. Kepekatan konidia pada 10^6 konidia/ml memberi kawalan terbaik terhadap *Eleusine indica*. Pada peringkat awal inokulasi dengan *Exserohilum rostratum* menunjukkan reaksi melecur sehari selepas inokulasi diikuti dengan nekrosis pada tisu yang dijangkiti. Kesan yang ditunjukkan oleh *Exserohilum rostratum* menyatakan bahawa terhasilnya phytotoxins bertindak dalam patogenesis. Daripada keputusan kajian ini, terbukti bahawa *Exserohilum rostratum* berpotensi sebagai bioherbisid untuk mengawal rumput sambau.

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APPROVAL SHEET

I certify that this research project report entitled "Potential of *Exserohilum rostratum* as a fungal bioherbicide for controlling goosegrass (*Eleusine indica* (L.) Gaertn)" 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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LIST OF GLOSSARY OF TERMS

	Terms	Meanings
1.	ae	active ingredient
2.	ANOVA	Analysis of variance
3.	GRAM	Graminous based media
4.	hrs	Hours
5.	IPM	Intergrated Pest Management
6.	LIGO	Ligo corn
7.	MR219	Paddy variety MR219
8.	MR220	Paddy variety MR220
9.	PDA	Potato Dextrose Agar
10.	SAS	Statistical Analysis System
11.	SWEET	Sweet orn
12.	V8	V8 juice agar
13.	WA	Water agar

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CHAPTER 1

INTRODUCTION

1.1 General

A weed is defined as a plant growing where it is not desired (Buchholtz, 1967). The Oxford English Dictionary (1973) defined weed as an herbaceous plant not valued for use or beauty, growing wild and rank, and regarded as cumbering the ground or hindering the growth of superior vegetation.

Weeds are one of the major constraints to agricultural production. Crop losses caused by weeds in the agriculture of developing countries are estimated to be above 30% (Labrada, 1994). According to some estimates these losses can be up to 125 million tons of food in developing countries (Parker and Fryer, 1975). We all know that, weeds are able to cause two types of crop losses. The more important one is the direct lost resulting from competition. Second is the indirect loss from reduced crop quality. At the same time, weeds also increase the cost of operations such as harvesting, drying and cleaning. Weeds are also capable of increasing pest infestation.

Eleusine indica (L.) Gaertn. commonly known as goosegrass, is one of the most serious weeds in vegetable farms, orchards, oil palm and rubber plantations, as well as in waste land and along road sides throughout Penisular Malaysia (Holm *et.al* 1977). Goosegrass can be controlled through various management strategies, including hand weeding, cultural and mechanical methods, and chemical herbicides (DeDatta, 1981).

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Use of chemical herbicide is the major strategy for goosegrass control in most crops at present. However, each of these strategies has limitations. Intensive and repeated use of chemicals over the past several decades has led to the development of herbicide-resistant goosegrass.

The development of herbicide-resistance and increasing concerns over herbicide residues in food, soil and the environment have intensified the the search for biological control methods for goosegrass. 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, 1993).Biological control agents, especially plant pathogenic fungi, have been shown effective in control of specific problem weeds (Yunzhi and Sheng, 2004). Bioherbicides are biological herbicides in which the active ingredient is a microorganism such as fungi, bacteria or protozoan. The bioherbicide can be simply applied to a weed infected area just as a regular herbicide would be applied. Inundative control using bioherbicides research to control agricultural and environmental weeds began in the 1940s.

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1.2 Importance of study

The purpose of this study was to develop an effective and environmentally safe method for controlling grassy weeds in Malaysia. Biological control is one of the most important components of Integrated Pest Management and it is very suitable for the poor farmers who are the major producer of food in the tropics since it is a cheaper method and does not cause environmental pollution due to their short half-live in the environment.

1.3 Objectives

1. To assess the potential of *Exserohilum rostratum* as a bioherbicide agent for controlling *Eleusine indica.*
2. To assess and develop formulations to improve performance and delivery of the bioherbicide.

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CHAPTER 2

LITERATURE REVIEW

2.1 Characteristics of goosegramss

Eleusine indica (L.) Gaertn. commonly known as goosegrass, is one of the most serious weeds in vegetable farms, orchards, oil palm and rubber plantations, as well as in waste land and along road sides throughout Penisular Malaysia (Holm *et.al* 1977). It is an annual weed with a compressed, tufted stem approximately 5-20 cm in height (Figure 1 and Figure 2). It is considered as one of the most troublesome weeds, is distributed throughout tropical and temperate regions and ranked as the world's fifth worst weed species (Holm *et.al* 1977, 1979).

Native from China, it's common name is goosegrass, better known worldwide by the scientific name of *Eleusine indica* (L.) Gaertn., to avoid confusion with local names such as "pata de gallina" in Argentina and most South American countries, "negil" in Egypt, or "gbegi" in Nigeria. The truth is that it is one of the serious weedy grasses of the warm areas of the world.

Goosegrass, also called wiregrass, is an annual that grows as a compressed plant in turf. It appears as a whitish silvery mat, forming a pale green clump with flattened stems in a low rosette (Figure 2-7). Leaves have a short membranous ligule (Figure 4). Flower stalks are short, stout, and compressed. Seed heads are somewhat similar to those of dallisgrass, but short and stiff (Figure 1 and Figure 2). Goosegrass is a tough

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and clumped weed, with a silverish coloration at its center. It has spikelets in two rows with a zipperlike appearance, on 2 to 13 fingers and frequently a single one below the terminal cluster of fingers. Goosegrass reproduces by seed and one single mature plant can produce as many as 140 000 (Chin, 1979).

2.2 Management of goosegrass

Weed management is the combination of the techniques of prevention, eradication and control to manage weeds in a crop, cropping system, or environment. The choice of weed control method will depend on the area's cropping history, the grower's management objectives, the available technology and financial resources. No weed control method has ever been abandoned completely. New techniques have been added in a large-scale agriculture, but old ones are still used effectively, especially in small scale agriculture. Goosegrass can be controlled through various management strategies, including hand weeding, cultural and mechanical methods, and chemical herbicides (DeDatta, 1981).

2.2.1 Weed Prevention

A good weed management program includes watchfulness whereby a good weed manger can identify weed seeds, seedlings and mature plants. The main rule for weed prevention and main step of any good weed management program is purchase and planting of clean seed. However, this method is only workable in countries like USA

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since the law enforced by the U.S. Department of Agriculture is strictly followed by the farmers. This preventive method is only practiced in Malaysian oil palm industry since it requires huge financial resources and huge area. In Malaysia, there are still small scale farmers and they prefer mechanical weed control methods which are cheaper and easier to implement.

2.2.2 Mechanical Control

Mechanical methods have a long use history and are the primary weed control technique in many crops.

2.2.3 Hand Weeding

One of the mechanical methods of weed control is hand weeding. Hand weeding consists of hand pulling and hand hoeing is practical and efficient, but it is hard work. It is very suitable in countries like Thailand and Indonesia, where labour is still plentiful and hand weeding remains as the the most popular method supplemented with other methods. Hand pulling is very effective for annual weeds such as *Eleusine indica* but not for perennials weeds which are capable of performing vegetative reproduction. disadvantage is that hand weeding does not get the job done when it is most needed. By the time the weeds become obvious, easy to grab and pull, yield reduction due to weed competition will have occurred. Hand hoeing has been used for

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weed control for many years. It is still the method of choice for most gardens and ornamental plantings and is used regularly in many vegetable crops. Hand hoeing controls the most persistent perennials if it is done often enough. Although efficient and widely used, it takes a lot of time and human energy. If human labour is abundant, and labour cost is not high, hand pulling or hoeing is an acceptable method of weed control. If human labour is not abundant and it is expensive, hand methods are expensive and not efficient.

2.2.4 Flooding, Dredging, Dredging and Chaining

These techniques cause ecological change. If a normally dry area is flooded or a normally wet area is drained, the ecological system is changed and weed species will change. These techniques are effective only when an area is immersed or drained for 3 to 8 weeks. However, this technique is not effective on all weeds, just some of them and it creates and environment where other weeds succeed. Flooding will control established perennials, but the expense of creating dikes and obtaining water make the practice economically unfeasible (Slife, 1981).

Draining is an excellent control for weeds that grow best in wet areas such as ***Eleusine indica*** whereas chaining completely controls annuals and not the perennials that reproduce vegetatively. Chains are also used to stop passage of weeds in irrigation

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channels in many countries. Removing collected weeds from the impoundment created by the chain is a labour-intensive, unpleasant operation.

2.2.5 Chemical herbicides

Glyphosate and dinitroaniline herbicides have been widely used to control goosegrass in arable crops (Smeda and Vaughn, 1994; Vaughn, 2000). The first case of glyphosate resistant weed (*Lolium rigidum* Gaud.) was reported in Australia in 1996 (Powels *et.al.,* 1998). In Malaysia *E.indica* first evolved multiple resistance (to 2 herbicide modes of action) in 1997 and infests orchards. Recently, Teng and Teo (1999) have reported the existence of resistant species *E.indica* in vegetable areas, nurseries, orchards and oil palm plantations in several areas of Penisular Malaysia. The resistant biotype of *E.indica* was able to survive at higher glyphosate rates four times more than the commercial recommended rate of 1.08 kg ae ha^{-1} (Teng and Teo, 1999). Increasing the application rates not only increases cost but also could have harmful effect on the environment such as contamination of surface and ground water, effects on non-target organisms, human and human health concerns.

Despite the great achivements accomplished by chemical weed control in reducing weeds' effects, the approach is criticized for the appearance of herbicide resistant weeds, for many recorded water contaminations and for subjecting humans, animals, birds and insects to numerous hazards (Radosevich *et.al.,* 1997). These factors have encouraged, and even forced, weed management specialists to search for new techniques, among which biological control is a favoured alternative.

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2.2.6 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). It is also described as a selective, environment-friendly process, utilizing host-specific control agents towards targeted weeds that prevent damage to non-target crops or native plants (Rosenthal *et.al.,* 1989; Bewick, 1996; Pleban and Strobel, 1998).

Even though, the procedures for implementing biological control are relatively complex yet interest is increasing because biological control is environmentally safe and applicable to some otherwise intractable weed problems. Many people consider all insects as pests and all pathogens as diseases. Nowadays, more and more attention is being paid to the beneficial role of insects and especially pathogens, in natural and agricultural systems and their utilization in Integrated Pest Management (IPM) and biological control.

Biological control of weeds with plant pathogens has been reviewed by Charrudattan and Walker (1982), Charrudattan (1991), and Chandramohan and Charudattan (1996).

The three main techniques in biological control of weeds are including classical biological control, augmentation, and bioherbicides. Classical biological control is mainly practiced when a plant has become a weed following establishment outside its native range. This has been the most widely used and most successful method. In this

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method organisms from another country that were attacking a close relative of the target weed were imported and released (Julien, 1992).

The augmentative approach is similar to classical approach, this is used when existing natural enemies are not providing effective control. Natural enemies are mass reared and released to assist their naturally occurring population in reducing the density of the target pest. The disadvantage of of this method is that it is not self-sustaining, continued input is required, and the technique is labour intensive. It may cost effective in developing countries but has been considered prohibitively expensive in the USA (Batra, 1981).

The use of bioherbicides is an important new weed control technology. Plant pathogens, mostly fungi are isolated, tested for specificity and effectiveness in controlling the target weed. The selected pathogen is then developed as a bioherbicide with a necessary shelf-life. The product is applied to the weed area just as a herbicide would be applied. The amount of inoculum applied and dispersed is controlled, and is generally sufficient to cause 100% infection.

Fungal diseases of weeds are common; unfortunately they have not received much attention. Only recently has research focused on use of fungal pathogens as bioherbicide for weed control. A glimpse of the potential of plant pathogens in weed control can be envisioned from the empirical success of few (Templeton, 1982). There are few successful cases reported in rice fields (Julien, 1992).

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Even though, the idea of using plant pathogens to control weeds is an old approach but the seeds of the idea have laid dormant ever since (Wilson, 1969). The term biological control was first used by H. S. Smith (DeBach, 1964). Biological control of weeds in Malaysia is an active area of study with much progress being made especially since the establishment of the National Coordinating Committee on Biological Control in 1986 (Mohamed *et al.,*1992). Microbial agents, though, have been largely ignored due probably to lack of proven microbial agents and the absence of containment facilities for the testing of exotic microorganisms. Through the activities of the committee, a number of programmes on biological control have been implemented (Anwar *et al.,* 1994a; Anwar *et al.*1994b; Mislamah and Ooi, 1994; Liau *et al.,*1994).

Biological control agents, especially plant pathogenic fungi, have been shown effective in control of specific problem weeds (Zhu, Y. and Qiang, S, 2004). Bioherbicides are biological herbicides in which the active ingredient is a microorganism such as fungi, bacteria or protozoan. The bioherbicide can be simply applied to a weed infected area just as a regular herbicide would be applied.

The preferred characteristics of a potential bioherbicide pathogen is including growth and sporulation on different types of media, highly virulent, genetic stability, restricted host range, broad tolerance range, prolific prapagulate production, ability to damage its host plant and lastly innocuous in ecological effects (Templeton *et al.*,1985). Inundative control using bioherbicides research to control agricultural and environmental weeds began in the 1940s.

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2.3 *Exserohilum rostratum*

Exserohilum rostratum is a fungi which was previously placed in the genus of Helminthosporium (Alcorn, 1988), and currently forms one of three genera, namely *Drechslera, Bipolaris* and *Exserohilum.* The new classification is done according to conidial morphology, germination characteristics, and hilum morphology. The genus *Exserohilum* might be differentiated from the other two by the conidia with a exserted hilum. The hilum is defined as a scar on a conidium at the point of attachment to the conidiophore. The hilum of *Drechslera sp.* does not protrude whereas in *Bipolaris sp.*, the hilum slightly protrudes (McGinnis, *et al.*, 1986).

Exserohilum sp. identification and characterization as a fungi were limited. Depend on the conidial and hilum morphology, germination criteria and conidial dimensions reported for previously described species (Sivanesan, 1987), the fungus was identified as *Exserohilum rostratum.*

Exserohilum rostratum colonies are grey to blackish-brown, suede-like to floccose in texture, and have an olivaceous black reverse. Pigmented conidia are quite variable but approximately 200 x 8µm, straight, curved or slightly bent, rostrate shape with 6-16 septa, ellipsoidal to fusiform and are formed apically through a pore (poroconidia) on a sympodially elongating geniculate conidiophore. 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. Conidial germination is bipolar.

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Foliar lesions caused by *Exserohilum rostratum* are starw coloured with brown margins. Lesions are often dark brown at first and then become light brown. Leaf spots caused by *Exserohilum rostratum* are similar to those caused by *Bipolaris* sp. Typical spots are oval and brown with slightly yellow borders. The spots expand into larger blights that kill parts of the leaves. Under soothing environment, the disease may complete this whole cycle in a period of several days.

The severity of the disease increases with humidity and temperature. Under this high level of infection, sporulation of the fungus occurs as greyish black spore mass on the adaxial surface of necrotic. The severity level seems to be greater when grass is exposed to stress situation such as drought, herbicide injury and heavy traffic.

Mixture of three fungal pathogens *Drechslera gigantean*, *Exserohilum longirostratum* and *Exserohilum rostratum* were evaluated as potential biological control agents against goosegrass and other and other weedy grasses (Chandramohan and Charudattan, 2001). No other reports of a biocontrol agent for goosegrass have been made so far. The present study was therefore carried out to assess the potential of *Exserohilum rostratum* as a bioherbicide agent for controlling *Eleusine indica* and also to assess and develop formulations to improve performance and delivery of the bioherbicide.

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Scientific name : *Eleusine indica* (L.) Gaertn.

Common name : Goosegrass

Family : Poaceae

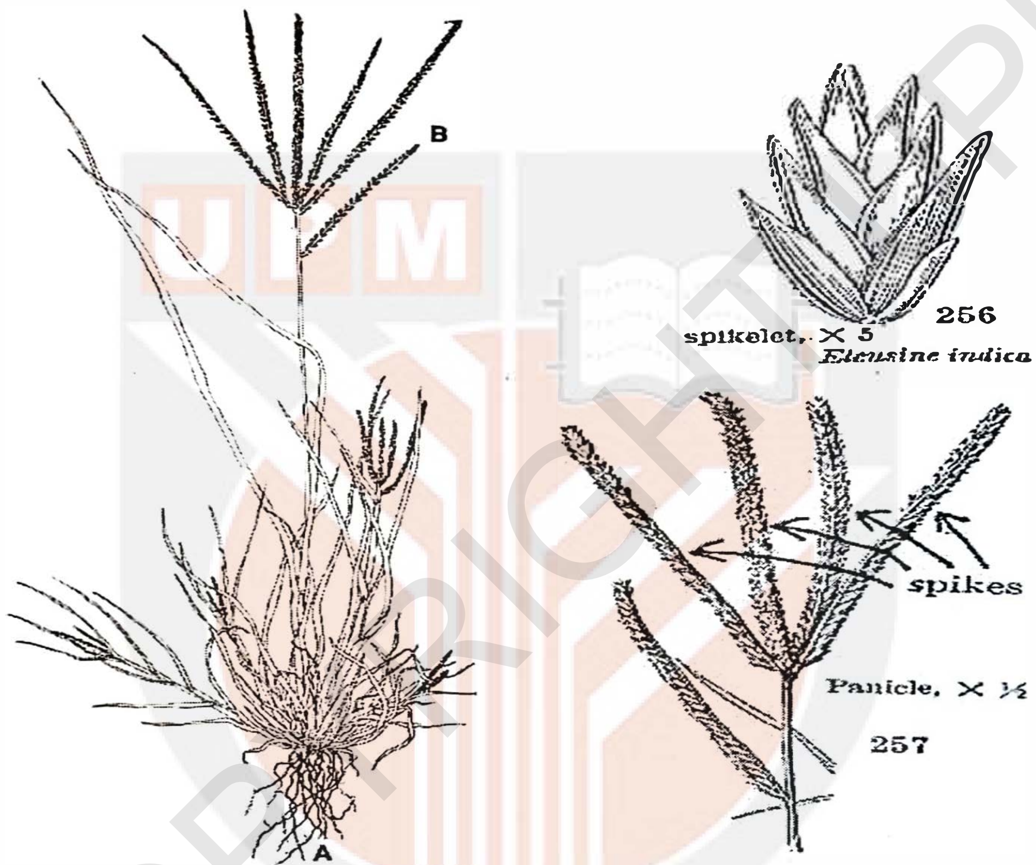

Figure 1: Morphology of *Eleusine indica* (stems branched, arise from tufts (A); flowers and seeds produced in two rows (zipper-like) along one side of the 2 to 10 finger-like branches at the top of the stem (B))

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Figure 2: *Eleusine indica* plant

Figure 3: *Eleusine indica* spikelets

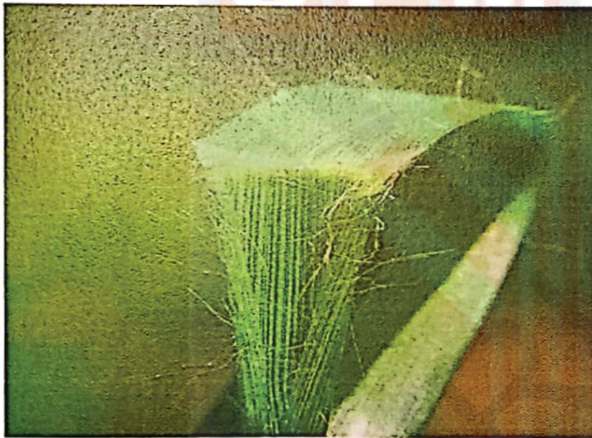
Figure 4: *Eleusine indica* ligule

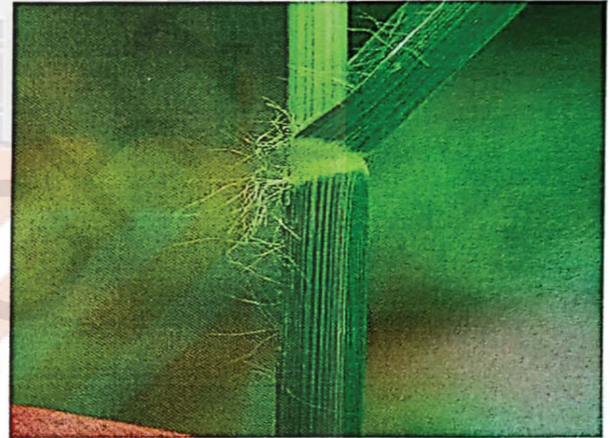
Figure5:*Eleusine indica* leaf base

Figure 6: *Eleusine indica* inflorescence

Figure 7: *Eleusine indica* base

CHAPTER 3

MATERIALS AND METHODS

3.1 Identification and characterization of fungi

Isolated fungi (*E. rostratumas*) grown in pure culture was obtained from Dr. Jugah Kadir from University Putra Malaysia Serdang campus. The pure culture was subcultured on PDA (Potato Dextrose Agar) slants and incubated at room temperature (28°C ± 2) as a stock for the study. To identify and characterize the isolate, it was grown on PDA and later used for taxonomic characterization. After 7 days incubation in a glass chamber, the conidia and conidiophores were scraped from those PDA plates using a rubber spatula. Mycelial fragments and conidia were collected and passed through a layer of cheesecloth and placed on lactophenol on a glass slide. They were identified to species level as described byAlcorn (1988).

3.2 Effect of media on fungal growth and sporulation

Four types of media were used which includes, PDA, Water Agar, V8 Agar and Graminous Based Media. A 8 mm diameter agar disc of the isolate was placed in the center of 9 cm diameter petri dishes containing for each media. The experiment was replicated four times. The plates were then arranged in a growth chamber and incubated for 7 days at room temperature (28°C ± 2). Radial mycelial growth of 24hr intervals was recorded as the mean of two perpendicular diameter minus the diameter of the inoculum plug (8 mm) for seven days. The resulting conidial suspensions were

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passed through a layer of cheesecloth and the spore concentration was determined with the aid of a haemocytometer on the seventh day.

3.3 Effect of temperature and light on fungal growth and sporulation

Light period were measured at 8, 12 and 24 hours. The temperatures were measured at 25°C, 30°C and 35°C. The plates were incubated in the growth chamber until adequate colony growth was observed for a maximum of 7days. A 8 mm diameter agar disc of the isolate was placed in the center of of 9 cm petri dish in four replicates; each containing PDA. The plates were then arranged in the growth chamber fort 7 days at room temperature (28°C ± 2). Radial mycelial growth of 24hr intervals was recorded as the mean of two perpendicular diameters minus the diameter of the inoculum plug (8 mm) for seven days. The resulting conidial suspensions were passed through a layer of cheesecloth and the spore concentration was determined by the aid of a haemocytometer on the seventh day.

3.4 Pathogenicity testing

3.4.1 Goose grass plant production

Goose grass plants used in this experiment were grown from seeds. Seeds of goose grass were directly planted into seedlings pots until they germinate. Germinated seeds were plough out from the pots leaving only two seedlings per pot. Seedlings put on test were one, two, three and four leaf stage. Medium used for planting is 2:1:1 ratio which consists of 2 parts of soil, 1 part of sand and 1 part of peat. The seedlings were

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watered regularly to soil saturation and maintained in the glasshouse at 30°C± 5°C day or night.

3.4.2 Inoculum production

For inoculum production, 8 mm diameter agar disc with mycelium is taken from the stock culture of the fungi isolation and aseptically transferred to fresh V-8 juice agar. The plates are then sealed with parafilm and incubated at room temperature (28°C ± 2). Conidia were harvested 7 days after incubation by flooding each plate with 10ml distilled water and scraping the agar surface with a rubber spatula. The resulting suspension is filtered through a layer of cheesecloth and the final conidial concentration is adjusted to 10^6 concentration by dilution with sterile distilled water.

3.3.3 Plant inoculation

Goosegrass plants are grown from one to four leaf stage in the shelter. Plants are then inoculated by spraying 10^6 conidia per/ml to runoff. The tests consisted of a single application of conidial suspension (10^6 conidia/ml) added with 5% sunflower oil and 0.05% Tween 20 as carriers; and control consisted of 5% sunflower oil and 0.05% Tween 20. The plants, in terms of every pot, were covered with plastic bag for 24 hours, to maintain 100% moisture.

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3.3.4 Disease Assessment

Susceptibility of plants was assessed daily for seven days. Disease incidence and disease severity was recorded daily. Disease severity is the proportion of plant tissue area diseased (Krantz, 1988), and disease incidence are the number of plants affected among the total plants inoculated (Horsfall and Cowling 1978). Disease progress was assessed based on the rating scale by Kadir (1997),which consist of eleven values.

Host reaction to *E. rostratum* was determined from the level of disease development on the inoculated plants. Disease was assessed as disease incidence and disease severity. Disease incidence was assessed based on the number of plants infected among the total number of plants infected among the total number of plants inoculated, expressed as the percentage of diseased plants (Horsfall and Cowling, 1978; Krantz, 1988).

Disease severity was assessed on all plants of each weed within the pots by visual estimation of the percentage of diseased (necrotic) leaves, using the disease rating scale developed by Kadir (1997). The rating scale consisted of 11 class values representing the percentage of disease severity. Disease severity was assessed daily starting one day (24 hours) after incubation.

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3.4 Host range testing

Host range determination is to ensure the safety of the biocontrol agent towards nontarget plants. The host range was determined based on the scheme developed by Wapshere (1974). Typically, plants closely related to the target weed are most rigorously tested while plant species in more distant taxonomic groups are less intensely tested (Wapshere, 1974). However, in addition two varieties of corn and paddy were also tested.

Inoculum was applied to all host plants at the same concentration as in pathogenicity testing. Inoculated plants were then covered with plastic bag for 100% moisture for 24 hours. In this experiment, plants of one, two, three and four leaf stage seedlings were used with four replications each.

The host range list include plants related to the target weed and other crop plants that were commonly associated with the target weed. Two varieties of corn which includes Ligo corn and two varieties of paddy including MR219 and MR22O were used in this experiment.

3.4.1 Crop plants production

Seedlings for crop species to be tested were grown from seeds in pots containing potting medium consisting of 2 parts soil: 1 part sand :1 part peat. The seedlings were maintained in the glasshouse and watered to soil saturation.

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3.4.2 Disease assessment for host range

Reactions of test plants were assessed on the basis of appearance of disease symptoms. Plants that developed typical disease symptoms characteristic of the pathogens being tested were considered susceptible. Those which developed symptoms such as minute flecks or nonoalescing or nonexpanding lesions were considered resistant. Nonsymptomatic plants were considered to be immune to the pathogens.

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CHAPTER 4

RESULTS

4.1 Identification and characterization of fungi

The identification of *Exserohilum rostratum* was done based on the conidia morphology, size and shape of the conidia, growth rate and the colour of the colonies. (Funder, 1968 and McGinnis *et al.*, 1986)

The chosen bioherbicide agent was identified as *Exserohilum rostratum* (Drechs.) by Leonard and Suggs. Conidia are straight, slightly bent ellipsoidal to fusiform and are formed epically through a pore on a sympodially elongating geniculate conidiophore (Figure 10). The conidia have a straight protruding truncate hilum and the septum above it is dark in colour and thickened. The interesting part of it is the end cells are paler than the other cells and the walls are finely roughened. It is a bipolar conidial germination.

Generally, colonies are grey to blackish brown in colour, suede like to floccose in texture with olivaceous reverse. The range and mean number of cells obtained per *E.rostratum* conidium in this study were shown in (Table 1; Appendix 8)

Table 1: Range and mean number of cells per conidium

	Number of cells per conidium
Range	**6-15**
Mean	**10**

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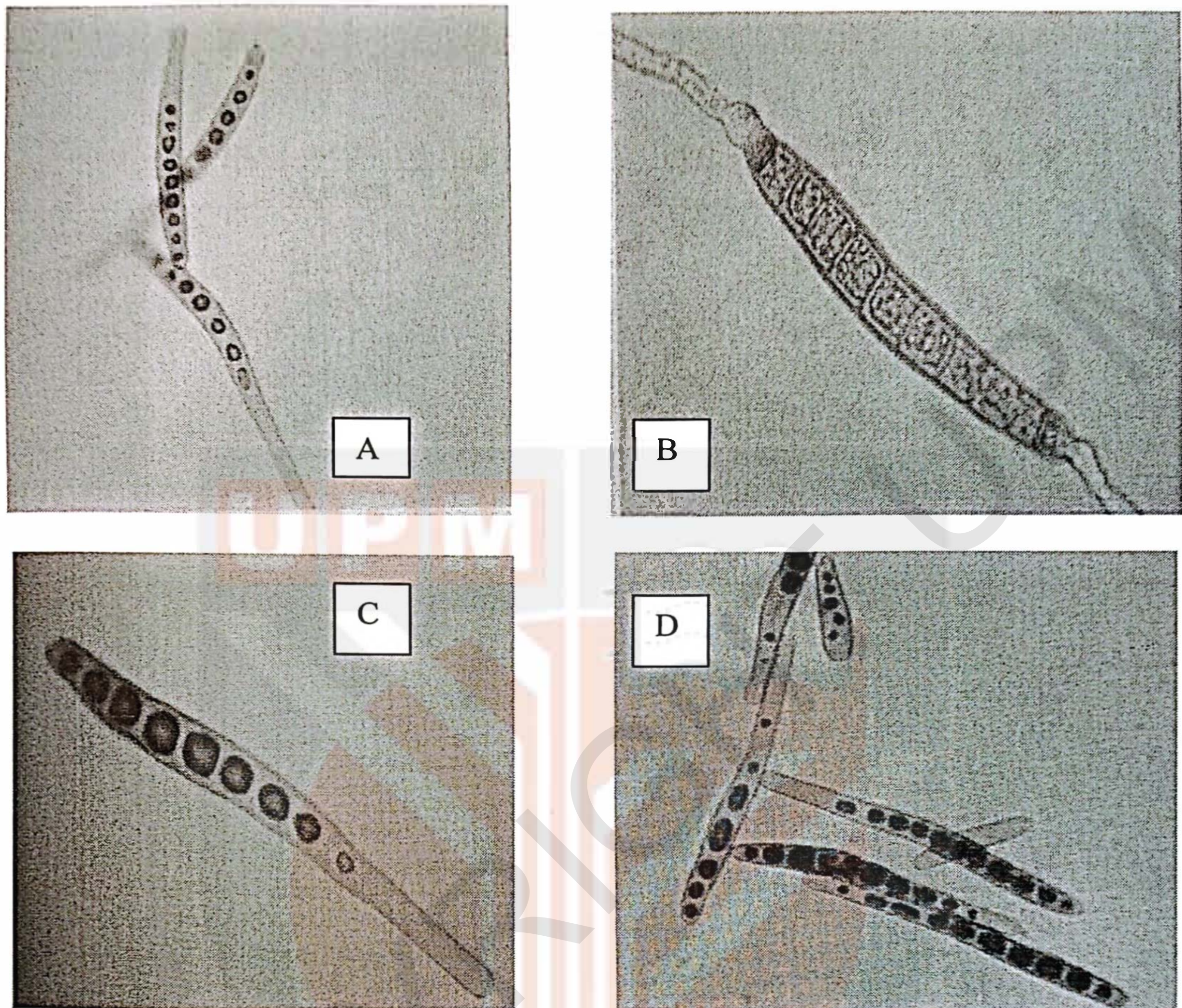

Figure 10: Morphology of *Exserohilum rostratum* (A) Conidia, (B) Bipolaris Germination, (C) Single conidium, (D) Conidia (Kundat, S. 2003)

4.2 Effect of media on fungal growth and sporulation

According to the results, all the four types of media (Figure 11) were not significantly different on the first day of incubation (Table 2; Appendix 5). This is shown by the same letters against the mean value on day 1. On day 2, except for WA all the other media showed no significant differences. On day 3, only Graminous base media and V8 agar were not significantly different whereas on day 4, Graminous based media, PDA and V8 agar were not significantly different. Finally, on day 6 Graminous based media and V8 were not significantly different, while PDA and WA were significantly

different (Figure 13). Effects of media on fungal sporulation clearly shown in the Table 3

Table 2: Effect of media on fungal growth (mm) after 6 days incubation period

	Gram	PDA	V8	WA
Day 1	7.00 a	7.00 a	5.38 a	5.63 a
Day 2	14.88 a	16.88 a	14.63 a	10.25 b
Day 3	28.00 b	31.88 a	27.13 b	17.50 c
Day 4	41.13 a	45.00 a	41.75 a	25.13 b
Day 5	54.63 b	65.63 a	55.00 b	32.50 c
Day6	66.00 b	74.25 a	67.00 b	41.25 c

Note: means within rows with the same letter are not significantly different at $p \leq 0.05$ by Tukey test

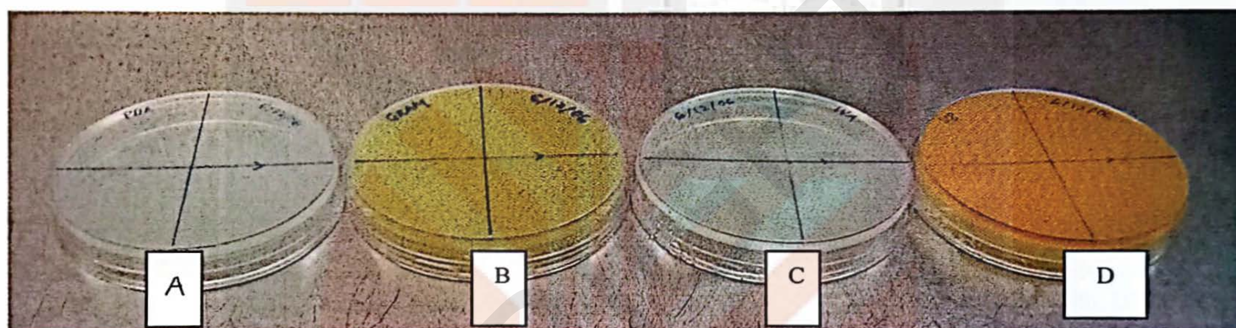

Figure 11: Four types of media being tested (A) PDA, (B)Graminous Based media (C) WA and D) V8 agar

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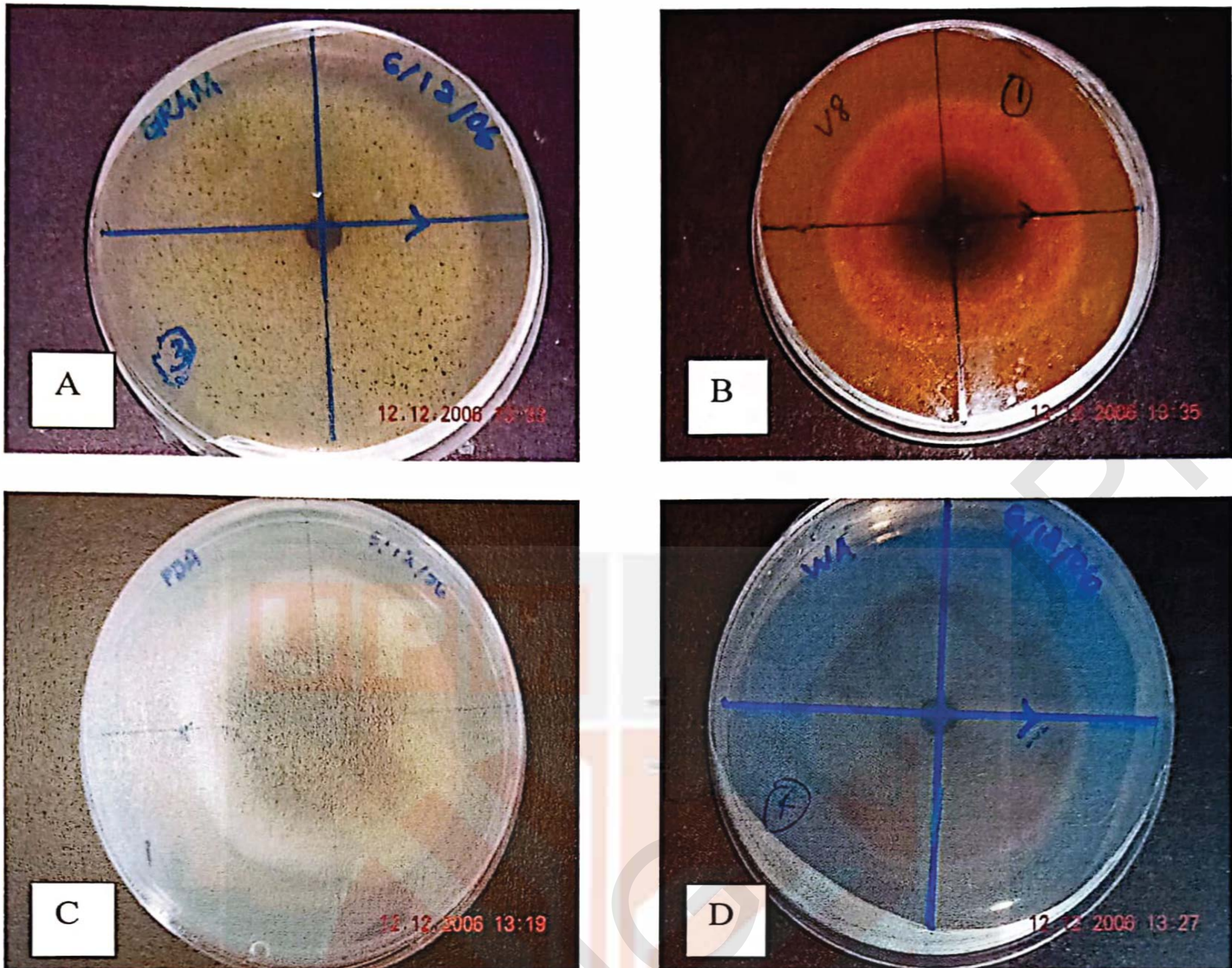

Figure12: Growth rate of *Exserohilum rostratum* after 6 days of incubation time on(A) graminous based media, (B) V8 agar, (C) PDA, (D) WA

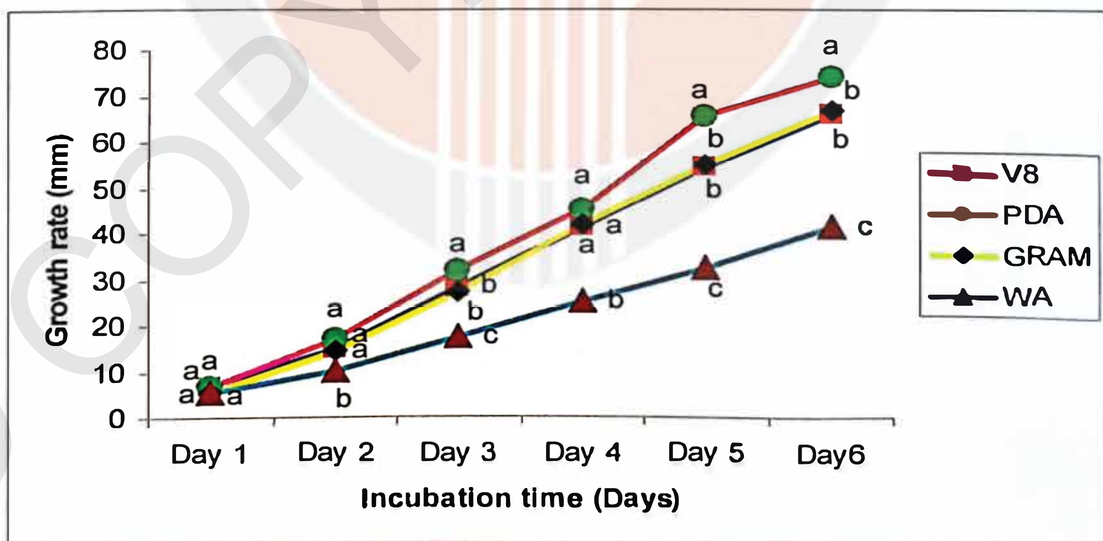

Figure 13 : Effect of media on colony growth of *Exserohilum rostratum*

Table 3: Effect of media on spore production

Number of spore produced per ml of original fungus sample	
PDA	12.0×10^6
V8	6.5×10^6
GRAM	3.5×10^6
WA	1.0×10^6

4.3 Effect of temperature and light period on colony growth and sporulation of *E.rostratum*

Temperature not only affects the growth rate (Figure 14; Appendix 3) of *Exserohilum rostratum* but also affects the spore germination (Table 4), reproduction and most of the activities of the fungus. Typical growth curve as a function of temperature are shown in Figure 14. The optimum temperature for the growth *Exserohilum rostratum* was between 30 and 35°C. At day 6, *Exserohilum rostratum* colonies incubated at 35°C reached 66.63 mm mean value, the widest value of all (Table 5). Spore production was highest at 35°C but spore production at 30°C was comparable. Incubation at 25°C resulted in lower growth rate and lower spore production.

Length of light period also influenced the growth rate (Figure 15 and 16; Table 7; Appendix 4), spore germination and reproduction of *Exserohilum rostratum*. In this study, artificial light was used. Optimum growth rate was achieved at between 12 to 24 hours light period. The highest spore production was in 12 hours light period (Table 6).

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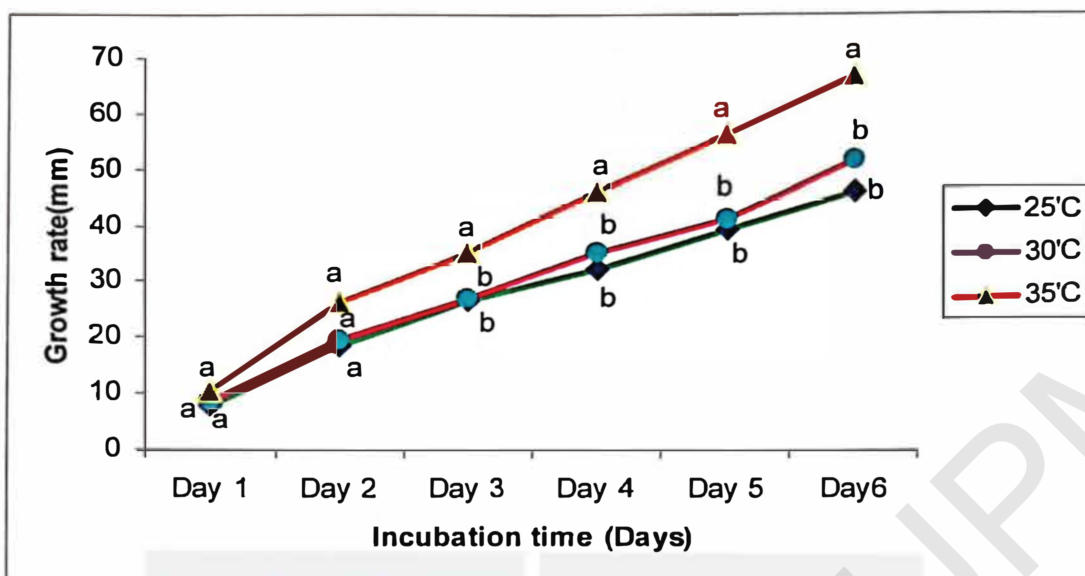

Figure 14: Effect of temperature on growth of *Exserohilum rostratum*

Table 4: Effect of temperature on spore production

Number of spore produced per ml of original fungus sample	
25°C	2.5 x 10^6
30°C	5 x 10^6
35°C	4.5 x 10^6

Table 5: Effect of temperature on fungal growth (mm) after 6 days incubation period

	25°C	**30°C**	**35°C**
Day 1	**7.85 a**	**8.63 a**	**10.25 a**
Day 2	**18.75 a**	**19.63 a**	**26.38 a**
Day 3	**26.88 b**	**27.13 b**	**35.53 a**
Day 4	**32.50 b**	**35.25 b**	**46.13 a**
Day 5	**39.63 b**	**41.50 b**	**56.13 a**
Day6	**46.00 b**	**51.62 b**	**66.63 a**

Note: means within rows with the same letter are not significantly different at $p \leq 0.05$ by Tukey test

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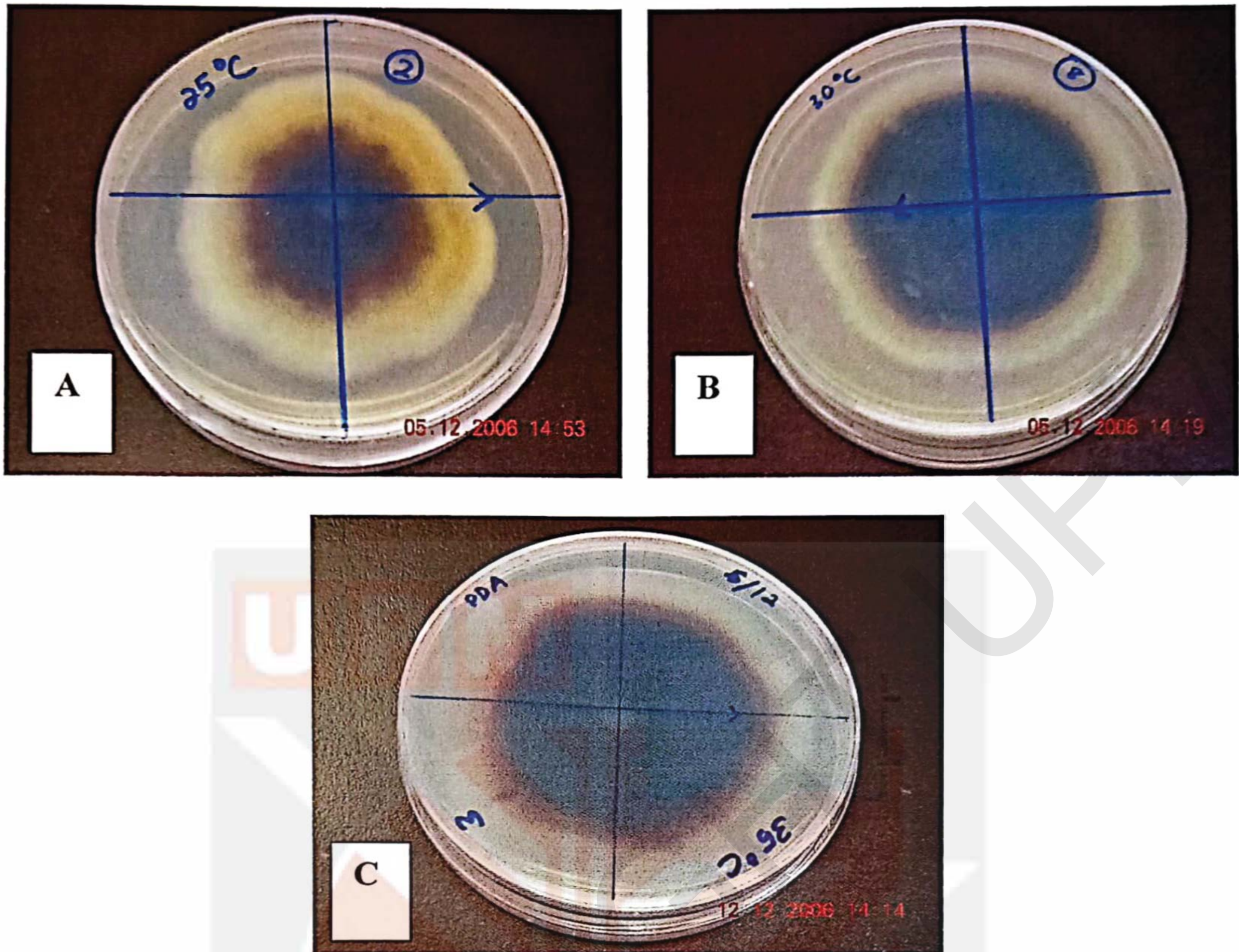

Figure 15: Growth rate of *Exserohilum rostratum* after 6 days of incubation time in 3 various temperatures (A) 25°C, (B) 30°C, (C) 35°C

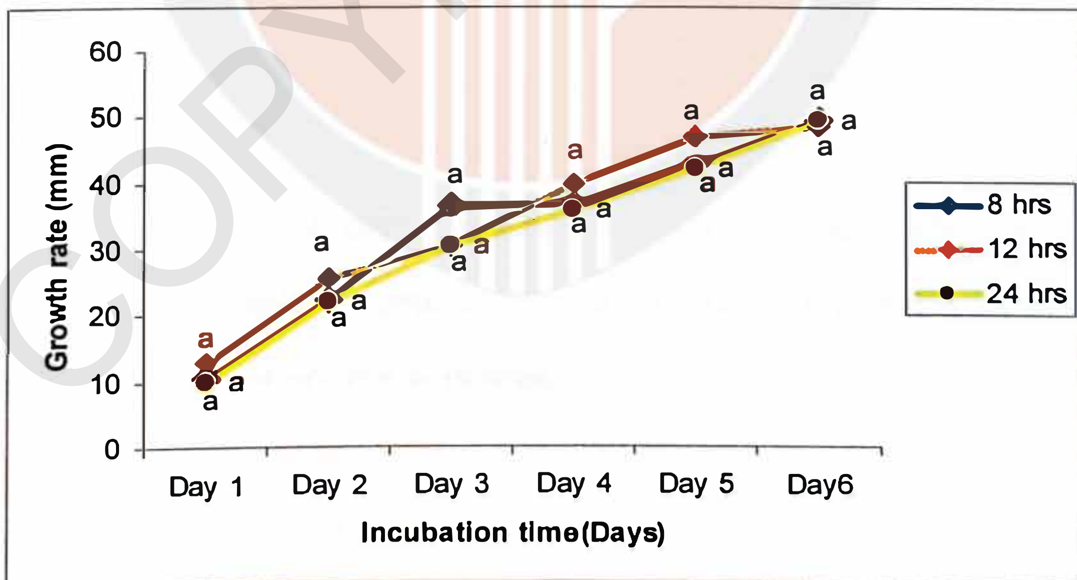

Figure 16: Effect of light period on growth of *Exserohilum rostratum*

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Table 6: Effect of light period on spore production

Number of spore produced per ml of original fungus sample	
8 hours	5.0 x 10^6
12 hours	4.5 x 10^6
24 hours	0

Table 7: Effect of light period on fungal growth after 7 days incubation period

	8hrs	**12hrs**	**24hrs**
Day 1	**10.63 a**	**12.88 a**	**9.75 a**
Day 2	**22.38 a**	**25.38 a**	**21.88 a**
Day 3	**36.25 a**	**30.38 b**	**30.25 b**
Day 4	**36.75 a**	**39.50 a**	**35.75 a**
Day 5	**42.75 a**	**46.88 a**	**42.13 a**
Day6	**49.50 a**	**48.63 a**	**49.38 a**
Day7	**51.25 a**	**53.00 a**	**54.75 a**

Note: means within rows with the same letter are not significantly different at $p \leq 0.05$ by Tukey test

4.4 Pathogenicity testing

In this study, each experiment was replicated four times with two plants per replicate (Figure 19; Appendix 7). The data were not statistically analysed since each replication only consisted of 4 inoculated pots plus one control pot. Analysis of variance (ANOVA) was not performed for this experiment since the number of control pots was not enough for comparison between inoculated seedlings and control seedlings. Thus, a simple comparison between leaf stages (Figure 17) according to disease severity and leaf severity were done.

The effect of *Exserohilum rostratum* with 10^6 conidia concentration/ml on disease development and severity was assessed as daily percentage of disease severity until it

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reached 100% disease severity. Disease did not develop on control seedlings (non inoculated plants) (Figure 17,18,20 and 21).

The disease began as small pinhead size spots; water soaked and dark brown in colour. Over day 7, the spots expanded into larger light brown blights that killed inoculated seedlings. All weedy grasses being tested were highly susceptible to *Exserohilum rostratum*. Seven days after inoculation, all the inoculated weeds developed 100% disease severity.

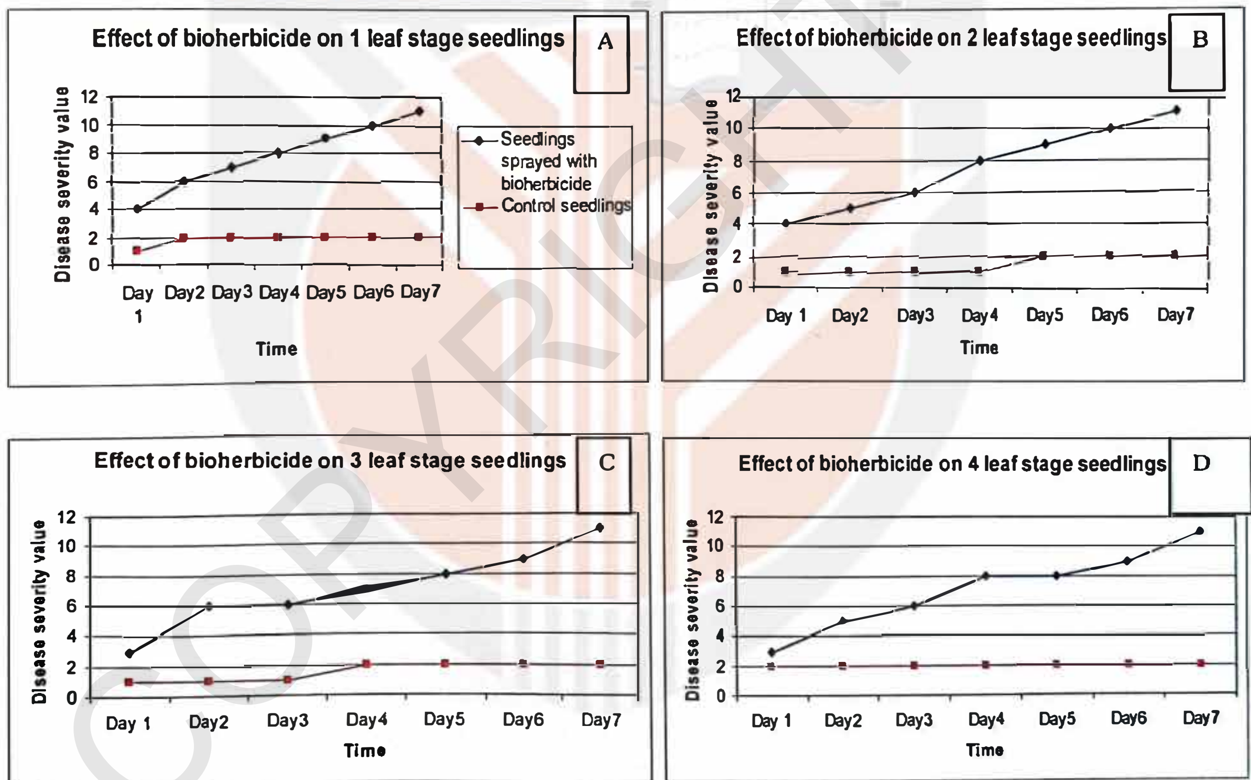

Figure 17: Effect of disease severity on inoculated and non inoculated *E. indica* at different leaf stages (A) 1 leaf stage, (B) 2 leaf stage, (C) 3 leaf stage, (D) 4 leaf stage

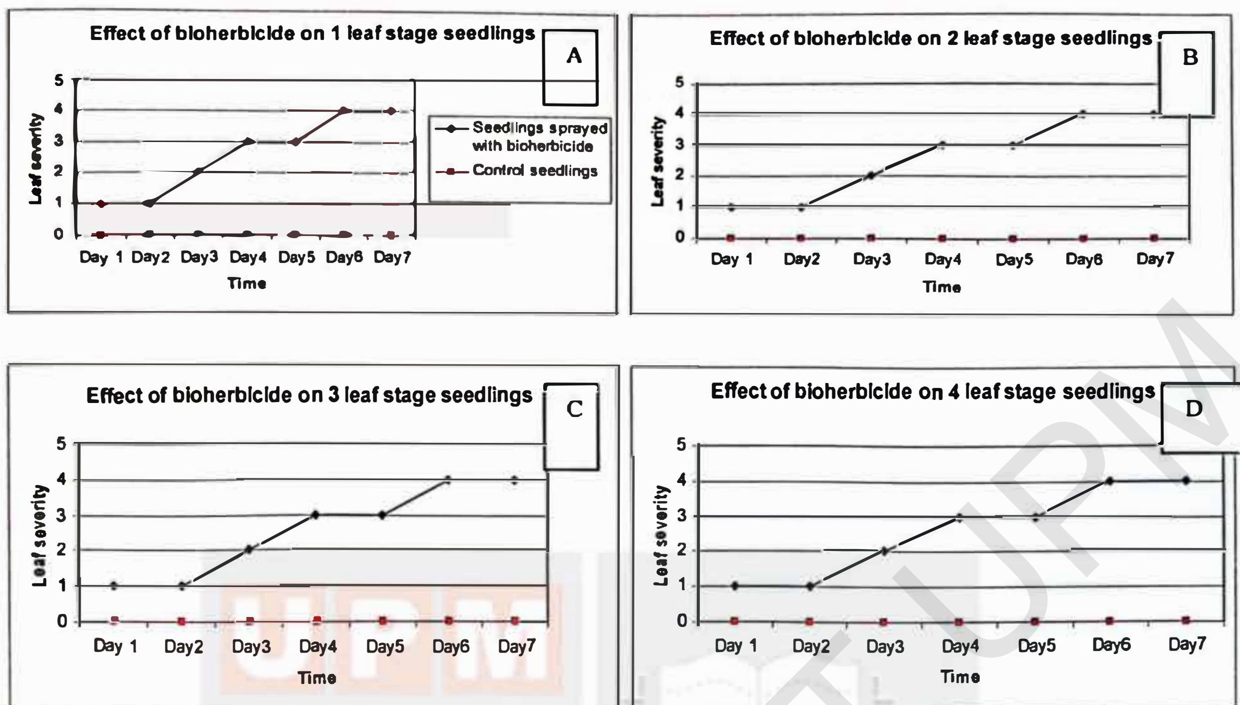

Figure 18: Effect of leaf severity on inoculated and non inoculated *E.indica* at different leaf stages (A) 1 leaf stage, (B) 2 leaf stage, (C) 3 leaf stage, (D) 4 leaf stage

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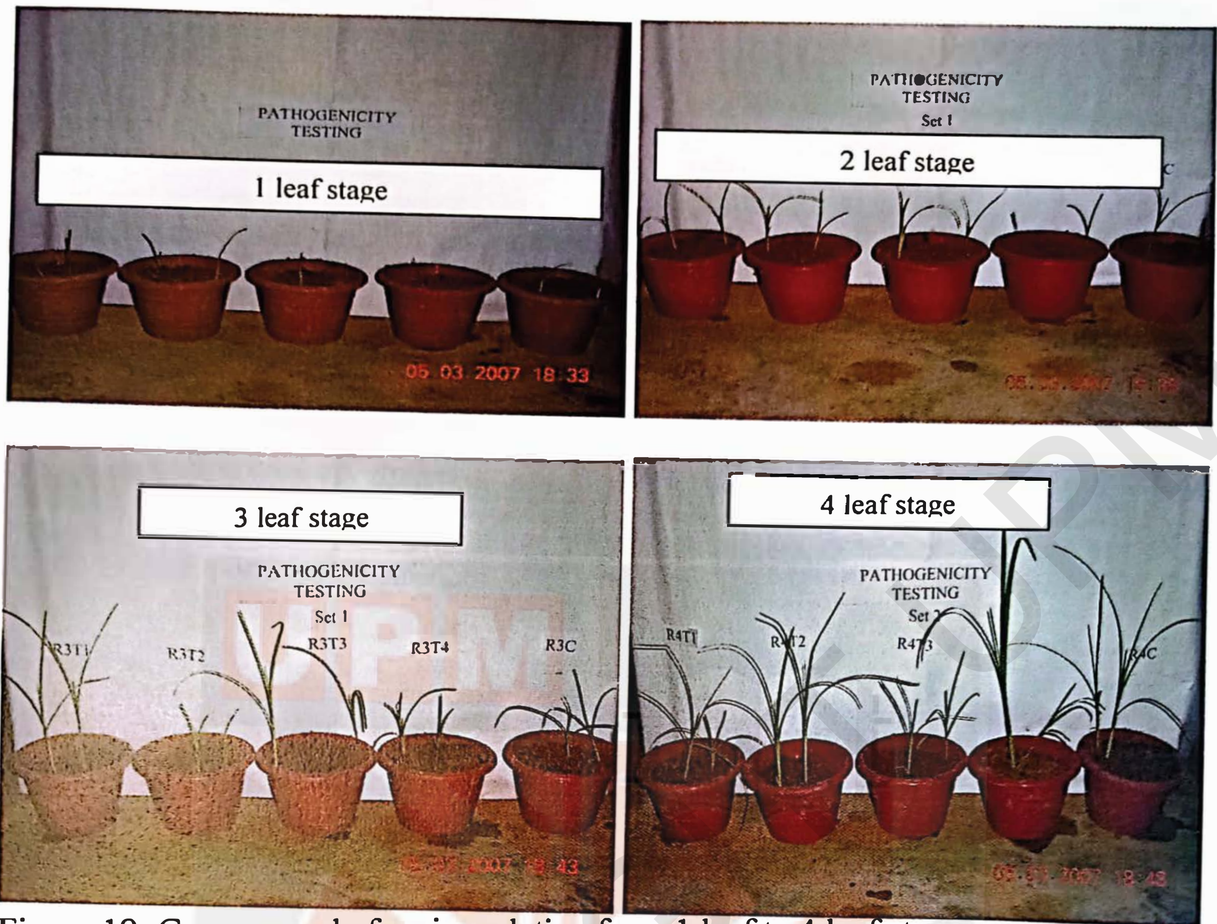

Figure 19: Goosegrass before inoculation from 1 leaf to 4 leaf stage

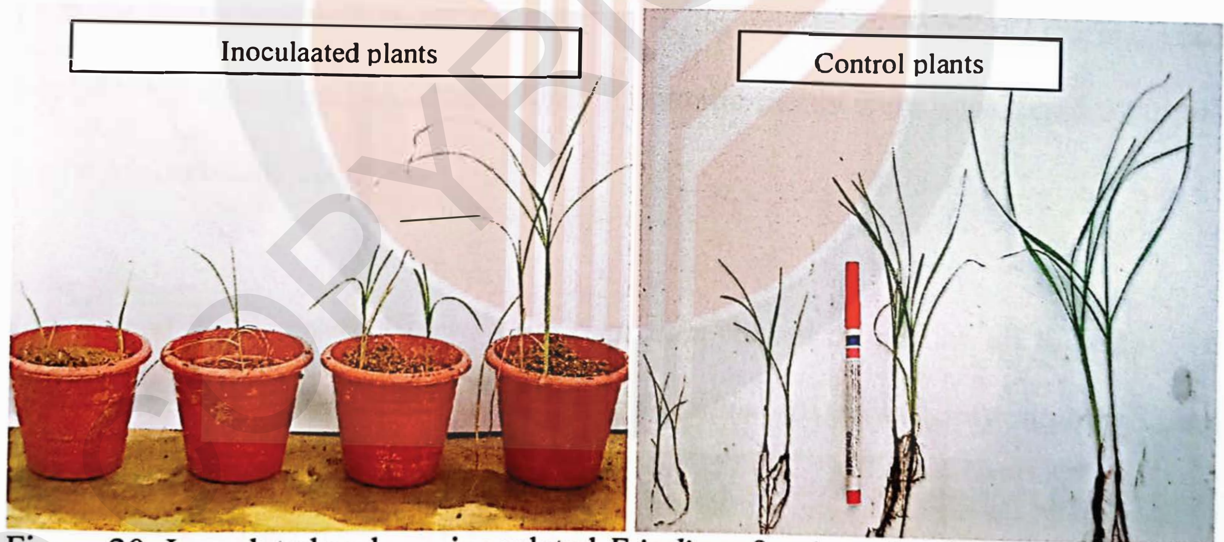

Figure 20: Inoculated and non inoculated *E.indica* after 1 week of inoculation

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Figure 21: (A) Inoculated 3 leaf stage *E.indica* and (B) Goosegrass without any fungal or surfactant treatment

4.5 Host range determination

The presence of the disease symptoms (Appendix 1 and 2) were considered to be the reactions of plants towards *Exserohilum rostratum* and it ranges from highly susceptible to immune level. Those which developed symptoms such as minute, pinhead size spots and small brown to dark brown lesions with no distinguishable center were considered resistant. Nonsymptomatic plants were considered as immune to the bioherbicide agent.

In repeated experiments, *Exserohilum rostratum* caused disease on all *Eleusine indica* from one to four leaf stages tested. *Exserohilum rostratum* only slightly infected paddy and corn (Appendix 6; Figure 22-25).

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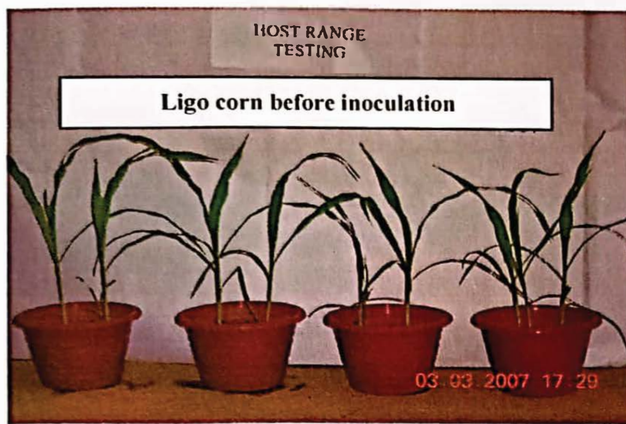


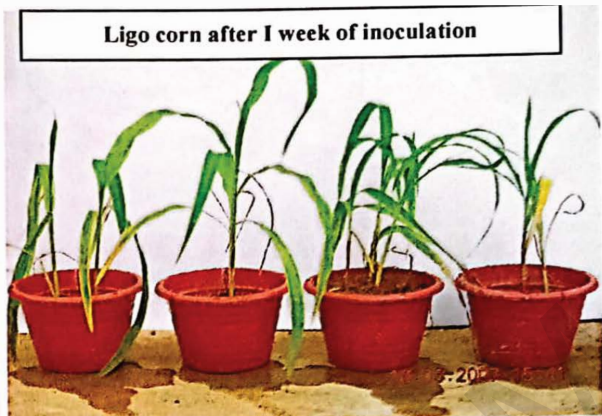
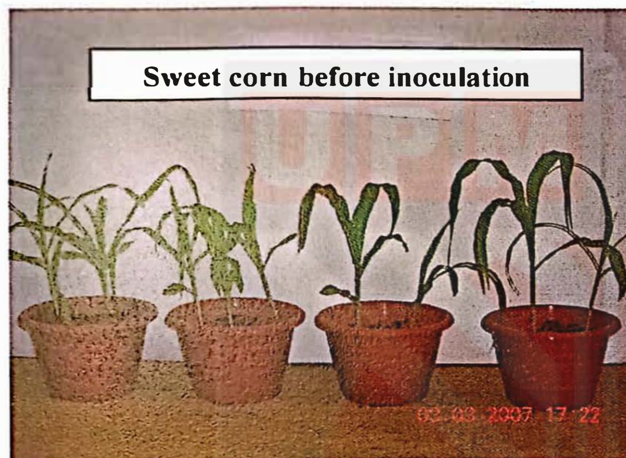


Figure 22: Symptoms of inoculation of *E. rostratum* on Ligo corn



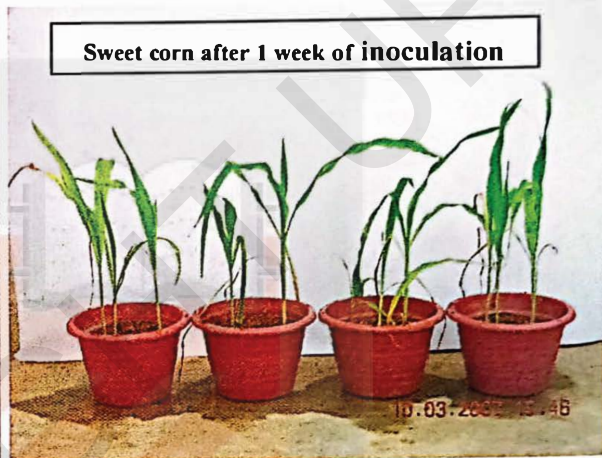
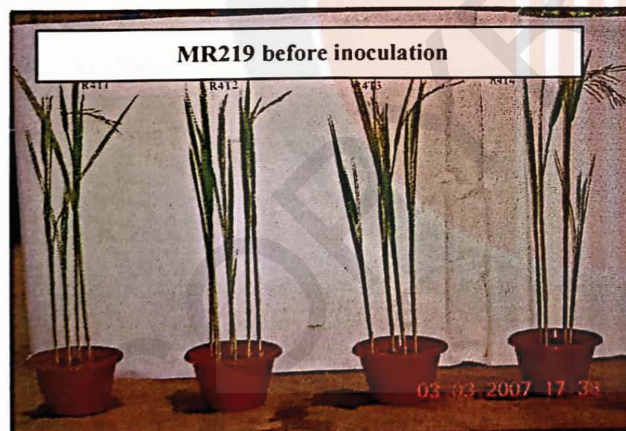


Figure 23: Symptoms of inoculation of *E. rostratum* on sweet corn



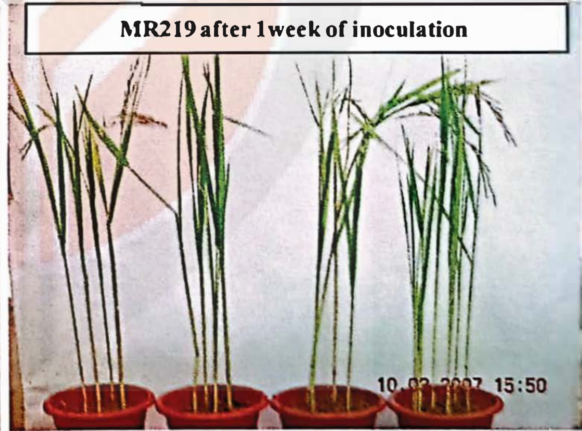


Figure 24: Symptoms of inoculation of *E. rostratum* on paddy variety MR219

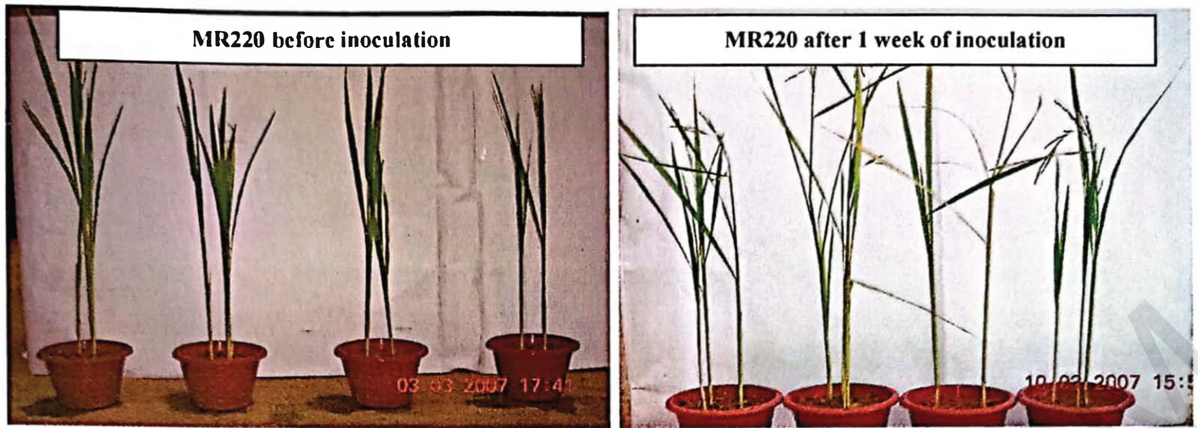


Figure 25: Symptoms of inoculation of *E. rostratum* on paddy variety MR22O

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CHAPTER 5

DISCUSSION

5.1 Identification and characterization of fungi

Since isolated *E.rostratum* obtained from Dr. Jugah Kadir from Universiti Putra Malaysia Serdang Campus, only the characterization of the fungus was determined to ensure it matches with the previous studies. From the obtained results, conidia of *E.rostratum* were straight, slightly bent ellipsoidal to fusiform and formed epically through a pore on a sympodially elongating geniculate conidiophore. This result supported by the previous studies by Sivanesan (1987) and Alcorn (1988), stated that *Exserohilum rostratum* were straight, were ellipsoidal to narrowly rostrate, with a hilum protruding from the end of the basal cell and 6-15 cells per conidium.

5.2 Effect of media on fungal growth and sporulation

Comparing the spore production of *E. rostratum* on the four types of media; PDA proven to be the best media by producing highest number of spores. This indicates that, PDA was the best media for *E.rostartum* fungal growth and spore production.

5.3 Effect of temperature and light period on colony growth and sporulation of *E. rostratum*

Togashi (1949) stated that, temperature and light period was important factors affecting growth rate of *E.rostratum.* From the results of this study, 35°C was significantly different from 30°C and 25°CT.

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From the analysis, mean value of growth rate of fungi as function of light period, did not show great differences compared to growth rate on different media. The only drastic change due to light period factor was spore production as stated by Wyss *et al.*, (1999) that the number of conidia produces was not correlated with the rate of colony growth. The theory above is supported by the results obtained from this study.

5.3 Pathogenicity testing

Disease only developed on inoculated plants and did not develop on control plants (non inoculated plants) which were only sprayed with surfactants 5 % sun flower oil and 0.01 % Tween 20. The results, strongly support previous reports which claim that *E. rostratum* contains phytotoxin which can act as a bioherbicide on certain weeds (Kundat,S. 2003).

The disease begins as small pinhead size spots; water soaked and dark brown in colour. Over 7 days, the spots expanded into larger light brown blights that killed inoculated seedlings. Seven days after inoculation, all the inoculated weeds developed 100% disease severity or mortality. These were the visible symptoms of *E. rostartum* observed on inoculated plants.

5.4 Host range determination

In repeated experiments, *Exerohilum rostratum* only slightly infected paddy varieties MR219, MR220, ligo corn and sweet corn. This results shows that, *E.rostarum* is highly target specific and does not cause any serious harm on non target

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crops. The observed symptoms on non target crops in this study were resistant reactions whereby the plants were immune or showed some pinhead size spots with no distinguishable center. These symptoms did not expand into susceptible or mortality stage as in pathogenicity testing which involved goosegrass at one to four leaf stages. This result confirms the previous study done by Chandramohan *et. al.,* (1999) that crop plants from the family Poaceae were resistant to *E. rostratum.*

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CHAPTER 6

CONCLUSION AND RECOMMENDATION

From this study, it was found that *E. rostratum* grew well in 35°C on PDA which produced the highest number of conidia compared to other ranges of temperature. There is no significant difference ($P<0.005$) on effect of light upon growth rate of *E. rostratum* but spore production differ greatly. 24 hours light period gave the best growth rate, and the 8 hours light period produced the highest value of spore production.

The selected bioherbicide agent *E. rostratum* appears to be promising in terms of pathogenicity in *E. indica*. The symptoms observed were consisted of severe foliage blighting until the stage of complete break down of the plant. Under controlled glasshouse environment and when applied at an innoculum dose of 10^6 conidium concentration, *E. rostratum* caused 100% mortality of all the four leaf stages of *E. indica*, one week after inoculation. At the same conidium concentration, corn and paddy were only slightly affected.

As a conclusion, the potential of *E.rostratum* to control *E.indica* by functioning as a bioherbicide was proven by this study.

By knowing the benefits of bioherbicide, I would like to recommend that we should consider biological control as one of the viable strategy in weed control program.

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Malaysian climate with high rainfall and humidity will make the leaf surface more suitable for microbial colonization and better control of foliar pathogens with antagonistic microorganism. Further work by plant pathologist and weed scientist is also needed to develop bioherbicide in large scale production.

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APPENDICES

Appendix 1: Disease Rating Scale (Kadir, 1997)

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 2: Scale of Leaf Severity Index (Horsfall and Cowling, 1978)

0 = Immune.

1 = Minute, pinhead sized spots.

2 = Small brown to dark brown lesions with no distinguishable center.

3 = Small eyespot shaped lesions with gray center.

4 = Typical coalescing lesions, elliptical with gray centers.

Host Reactions type 1 and 2 as resistant reactions; type 3 and 4 as susceptible reactions, the pathogen produced new conidia on the diseased leaves.

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Appendix 3: Effects of temperature on fungal growth

	Day 1		
	25°C(mm)	30°C(mm)	35°C(mm)
Rep1	7.4	9	10.5
Rep2	10	9	11
Rep3	8	9	9
Rep4	6	7.5	10.5
Average	**7.85**	**8.625**	**10.25**
	Day 2		
	25°C(mm)	30°C(mm)	35°C(mm)
Rep1	18	20	28.5
Rep2	22	20.5	27
Rep3	19	20	25.5
Rep4	16	18	24.5
Average	**18.75**	**19.625**	**26.375**
	Day 3		
	25°C(mm)	30°C(mm)	35°C(mm)
Rep1	27	27	35.5
Rep2	30	28	34.6
Rep3	27	27	36
Rep4	23.5	26.5	36
Average	**26.875**	**27.125**	**35.525**
	Day 4		
	25°C(mm)	30°C(mm)	35°C(mm)
Rep1	33.5	35	53
Rep2	33	36	43
Rep3	33.5	34.5	45
Rep4	30	35.5	43.5
Average	**32.5**	**35.25**	**46.125**
	Day 5		
	25°C(mm)	30°C(mm)	35°C(mm)
Rep1	39.5	37.5	61
Rep2	43	44.5	56.5
Rep3	38	41.5	54.5
Rep4	38	42.5	52.5
Average	**39.625**	**41.5**	**56.125**
	Day 6		
	25°C(mm)	30°C(mm)	35°C(mm)
Rep1	48.5	49.5	70.5
Rep2	50	54	65
Rep3	42	51	68
Rep4	43.5	52	63
Average	**46**	**51.625**	**66.625**

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Appendix 4: Effects of light period on fungal growth

	Day 1		
	8hrs(mm)	12hrs(mm)	24hrs(mm)
Rep 1	11	9	10.5
Rep 2	10.5	9.5	11.5
Rep 3	11	14	8
Rep 4	10	19	9
Average	**10.625**	**12.875**	**9.75**
	Day 2		
	8hrs(mm)	12hrs(mm)	24hrs(mm)
Rep 1	23	20.5	22.5
Rep 2	22	34	23
Rep 3	22.5	25	21
Rep 4	22	22	21
Average	**22.375**	**25.375**	**21.875**
	Day 3		
	8hrs(mm)	12hrs(mm)	24hrs(mm)
Rep 1	32.5	29	30
Rep 2	37.5	29.5	31.5
Rep 3	36.5	34	31.5
Rep 4	38.5	29	28
Average	**36.25**	**30.375**	**30.25**
	Day 4		
	8hrs(mm)	12hrs(mm)	24hrs(mm)
Rep 1	38.5	35	36
Rep 2	36	47.5	36.5
Rep 3	34.5	40.5	36
Rep 4	38	35	34.5
Average	**36.75**	**39.5**	**35.75**
	Day 5		
	8hrs(mm)	12hrs(mm)	24hrs(mm)
Rep 1	43	39.5	43
Rep 2	43	42	44
Rep 3	40.5	55.5	43.5
Rep 4	44.5	50.5	38
Average	**42.75**	**46.875**	**42.125**
	Day 6		
	8hrs(mm)	12hrs(mm)	24hrs(mm)
Rep 1	44.5	43.5	51.5
Rep 2	49	48	54
Rep 3	46	55	52
Rep 4	58.5	48	40
Average	**49.5**	**48.625**	**49.375**
	Day 7		
	8hrs(mm)	12hrs(mm)	24hrs(mm)
Rep 1	47.5	45	lau
Rep 2	53.5	53	60
Rep 3	49	61.5	58
Rep 4	55	52.5	43
Average	**51.25**	**53**	**54.75**

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Appendix 5: Effect of media on fungal growth

Replication	Day 1			
	PDA (mm)	WA(mm)	GRAM(mm)	V8(mm)
1	6	6	5	5.5
2	7.5	4.5	8.5	5.5
3	7	6	7	5.5
4	7.5	6	7.5	5
Average	**7**	**5.63**	**7**	**5.38**
Replication	Day 2			
	PDA (mm)	WA(mm)	GRAM(mm)	V8(mm)
1	15.5	9.5	11	14.5
2	17	9.5	15.5	14.5
3	16.5	11.5	16.5	15.5
4	18.5	10.5	16.5	14
Average	**16.88**	**10.25**	**14.88**	**14.63**
Replication	Day 3			
	PDA (mm)	WA(mm)	GRAM(mm)	V8(mm)
1	31.5	18.5	28	26.5
2	33	17.5	27.5	27.5
3	31	18	28	27
4	32	16	28.5	27.5
Average	**31.88**	**17.5**	**28**	**27.13**
Replication	Day 4			
	PDA (mm)	WA(mm)	GRAM(mm)	V8(mm)
1	44.5	24.5	40	38.5
2	46.5	25.5	41	41.5
3	43.5	24.5	41.5	40
4	45.5	26	42	47
Average	**45**	**25.13**	**41.13**	**41.75**
Replication	Day 5			
	PDA (mm)	WA(mm)	GRAM(mm)	V8(mm)
1	71	31	52	51.5
2	69.5	32	56	54
3	60.5	34	55	54
4	61.5	33	55.5	60.5
Average	**65.63**	**32.5**	**54.63**	**55**
Replication	Day 6			
	PDA (mm)	WA(mm)	GRAM(mm)	V8(mm)
1	74	41	63	59.5
2	75	43	66.5	68.5
3	73	40	67	69.5
4	75	41	67.5	70.5
Average	**74.25**	**41.25**	**66**	**67**

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Appendix 6: Host range testing

Paddy MR219	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev
Rep 1	1	2	1	2	1	2	2	3	2	3	2	4	2	4
Rep 2	1	2	1	2	1	2	2	3	2	3	2	4	2	4
Rep 3	1	2	1	2	1	2	2	3	2	3	2	4	2	4
Rep 4	1	2	1	2	1	2	2	3	2	3	2	4	2	4
Average	1	2	1	2	1	2	2	3	2	3	2	4	2	4

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Paddy MR220	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev
Rep 1	1	2	1	2	1	2	2	3	2	3	2	4	2	4
Rep 2	1	2	1	2	1	2	2	3	2	3	2	4	2	4
Rep 3	1	2	1	2	1	2	2	3	2	3	2	4	2	4
Rep 4	1	2	1	2	1	2	2	3	2	3	2	4	2	4
Average	1	2	1	2	1	2	2	3	2	3	2	4	2	4

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Ligo corn	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev
Rep 1	1	2	1	2	1	2	1	3	2	3	2	3	3	3
Rep 2	1	2	1	2	1	2	1	3	2	3	2	3	3	3
Rep 3	1	2	1	2	1	2	1	3	2	3	2	3	3	3
Rep 4	1	2	1	2	1	2	1	3	2	3	2	3	3	3
Average	1	2	1	2	1	2	1	3	2	3	2	3	3	3

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Sweet corn	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev	Leaf Sev	Dis. Sev
Rep 1	1	2	1	2	1	2	1	3	2	3	2	3	3	3
Rep 2	1	2	1	2	1	2	1	3	2	3	2	3	3	3
Rep 3	1	2	1	2	1	2	1	3	2	3	2	3	3	3
Rep 4	1	2	1	2	1	2	1	3	2	3	2	3	3	3
Average	1	2	1	2	1	2	1	3	2	3	2	3	3	3

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Appendix 7: Pathogenicity testing

1leaf stage	Disease severity		Scale of leaf severity index	
	Seedlings sprayed with fungal concentration (Average of 4 replications)	Control seedlings	Seedlings sprayed with fungal concentration (Average of 4 replications)	Control seedlings
Day 1	4	1	1	0
Day2	6	2	1	0
Day3	7	2	2	0
Day4	8	2	3	0
Day5	9	2	3	0
Day6	10	2	4	0
Day7	11	2	4	0
2 leaf stage	Disease severity		Scale of leaf severity index	
	Seedlings sprayed with fungal concentration (Average of 4 replications)	Control seedlings	Seedlings sprayed with fungal concentration (Average of 4 replications)	Control seedlings
Day 1	4	1	1	0
Day2	5	1	1	0
Day3	6	1	2	0
Day4	8	1	3	0
Day5	9	2	3	0
Day6	10	2	4	0
Day7	11	2	4	0
3 leaf stage	Disease severity		Scale of leaf severity index	
	Seedlings sprayed with fungal concentration (Average of 4 replications)	Control seedlings	Seedlings sprayed with fungal concentration (Average of 4 replications)	Control seedlings
Day 1	3	1	1	0
Day2	6	1	1	0
Day3	6	1	2	0
Day4	7	2	3	0
Day5	8	2	3	0
Day6	9	2	4	0
Day7	11	2	4	0
4 leaf stage	Disease severity		Scale of leaf severity index	
	Seedlings sprayed with fungal concentration (Average of 4 replications)	Control seedlings	Seedlings sprayed with fungal concentration (Average of 4 replications)	Control seedlings
Day 1	3	2	1	0
Day2	5	2	1	0
Day3	6	2	2	0
Day4	8	2	3	0
Day5	8	2	3	0
Day6	9	2	4	0
Day7	11	2	4	0

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Appendix 8: No of cells per conidium

No	Cells	No	Cells
1	6	31	10
2	9	32	11
3	6	33	12
4	6	34	15
5	7	35	8
6	10	36	13
7	8	37	15
8	6	38	14
9	8	39	14
10	9	40	10
11	8	41	8
12	12	42	9
13	11	43	9
14	10	44	9
15	11	45	12
16	11	46	9
17	10	47	8
18	15	48	6
19	13	49	9
20	11	50	9
21	9	51	12
22	10	52	13
23	14	53	14
24	8	54	10
25	9	55	10
26	9	56	10
27	9	57	10
28	8	58	15
29	6	59	14
30	12	60	13

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Appendix 9: ANOVA on effect of light period on fungal growth (mm) on Day 1

Source	df	SS	MS	F	Pr
Light (hours)	2	20.7917	10.3396	1.28	0.3243
Error	9	73.1250	8.1250	-	-
Total	11	93.9167			

Appendix 10: ANOVA on effect of light period on fungal growth (mm) on Day 2

Source	df	SS	MS	F	Pr
Light (hours)	2	28.6667	14.3333	1.14	0.3632
Error	9	113.5625	12.6181	-	-
Total	11	142.2292			

Appendix 11: ANOVA on effect of light period on fungal growth (mm) on Day 3

Source	df	SS	MS	F	Pr
Light (hours)	2	94.0417	47.0208	9.06	0.0070
Error	9	46.6875	5.1875	-	-
Total	11	140.7292			

Appendix 12: ANOVA on effect of light period on fungal growth (mm) on Day 4

Source	df	SS	MS	F	Pr
Light (hours)	2	30.1667	15.0833	1.15	0.3590
Error	9	118.0000	13.1111	-	-
Total	11	148.1667			

Appendix 13: ANOVA on effect of light period on fungal growth (mm) on Day 5

Source	df	SS	MS	F	Pr
Light (hours)	2	53.2917	26.6458	1.22	0.3407
Error	9	197.1250	21.9028	-	-
Total	11	250.4167			

Appendix 12: ANOVA on effect of light period on fungal growth (mm) on Day 6

Source	df	SS	MS	F	Pr
Light (hours)	2	1.7917	0.8958	0.03	0.9741
Error	9	306.8750	34.0972	-	-
Total	11	308.6667			

Appendix 12: ANOVA on effect of light period on fungal growth (mm) on Day 7

Source	df	SS	MS	F	Pr
Light (hours)	2	24.5000	12.2500	0.30	0.7445
Error	9	361.5000	40.1667	-	-
Total	11	386.0000			

Appendix 13: ANOVA on effect of media on fungal growth (mm) on Day 1

Source	df	SS	MS	F	Pr
Light (hours)	3	9.1250	3.0417	3.70	0.0430
Error	12	9.8750	0.8229	-	-
Total	15	19.0000			

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Appendix 14: ANOVA on effect of media on fungal growth (mm) on Day 2

Source	df	SS	MS	F	Pr
Light (hours)	3	93.5469	31.1823	12.77	0.0005
Error	12	29.3125	2.4427	-	-
Total	15	122.8594			

Appendix 15: ANOVA on effect of media on fungal growth (mm) on Day 3

Source	df	SS	MS	F	Pr
Light (hours)	3	447.8750	149.2917	260.58	< .0001
Error	12	6.8750	0.5729	-	-
Total	15	454.7500			

Appendix 16: ANOVA on effect of media on fungal growth (mm) on Day 4

Source	df	SS	MS	F	Pr
Light (hours)	3	953.3750	317.7917	76.08	< .0001
Error	12	50.1250	4.1771	-	-
Total	15	1003.5000			

Appendix 17: ANOVA on effect of media on fungal growth (mm) on Day 5

Source	df	SS	MS	F	Pr
Light (hours)	3	2327.0625	775.6875	63.59	< .0001
Error	12	146.3750	12.1979	-	-
Total	15	2473.4375			

Appendix 18: ANOVA on effect of media on fungal growth (mm) on Day 6

Source	df	SS	MS	F	Pr
Light (hours)	3	2486.2500	828.7500	102.53	< .0001
Error	12	97.0000	8.0833	-	-
Total	15	2583.2500			

Appendix 19: ANOVA on effect of temperature on fungal growth (mm) on Day 1

Source	df	SS	MS	F	Pr
Light (hours)	3	12.1892	4.0631	2.70	0.1159
Error	8	12.0200	1.5025	-	-
Total	11	24.2092			

Appendix 20: ANOVA on effect of temperature on fungal growth (mm) on Day 2

Source	df	SS	MS	F	Pr
Light (hours)	3	136.7443	68.3722	17.40	0.0012
Error	8	31.4375	3.9297	-	-
Total	11	168.1818			

Appendix 21: ANOVA on effect of temperature on fungal growth (mm) on Day 3

Source	df	SS	MS	F	Pr
Light (hours)	3	193.9267	96.9633	36.85	< .0001
Error	8	23.6825	2.6314	-	-
Total	11	217.6092			

Appendix 22: ANOVA on effect of temperature on fungal growth (mm) on Day 4

Source	df	SS	MS	F	Pr
Light (hours)	3	415.2917	207.6458	24.94	0.0002
Error	8	74.9375	8.3264	-	-
Total	11	490.2292			

Appendix 23: ANOVA on effect of temperature on fungal growth (mm) on Day 5

Source	df	SS	MS	F	Pr
Light (hours)	3	652.8750	326.4375	35.67	< .0001
Error	8	82.3750	9.1528	-	-
Total	11	735.2500			

Appendix 24: ANOVA on effect of temperature on fungal growth (mm) on Day 6

Source	df	SS	MS	F	Pr
Light (hours)	3	909.3750	454.6875	46.57	< .0001
Error	8	87.8750	9.7639	-	-
Total	11	997.2500			

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Potential of *Exserohilum rostratum* as a bioherbicide for controlling goosegrass (*Eleusine indica* (L.) Gaertn)" 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 the form approved by the Faculty.

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PRAMESWARI GOPAL

09 MAY 2007