**UNIVERSITI PUTRA MALAYSIA**

# POTENTIAL OF EXSEROHILUM MONOCERAS AS BIOHERBICIDE FOR CONTROLING BARNYARD GRASS (ECHINOCHLOA CRUS-GALLI)

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**FP 2006 1**

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**By**

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**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Agriculture Science**

**January 2006**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agriculture Science

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**January 2006**

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Development of *Exserohilum monoceras* as a potential bioherbicide for controlling barnyard grass (*Echinochloa crus-galli)* was investigated in this study. An isolate of indigenous fungus *E. monoceras* was isolated from diseased *Echinochloa crus-galli* in Tanjong Karang, Selangor and was evaluated in the laboratory and greenhouse as a potential bioherbicide. This fungus was found to be highly pathogenic to *Echinochloa crus-galli* seedlings inoculated with 2.1 X $10^6$ conidia/ml. The disease symptom appeared 24 h after inoculation as discrete eyespot symptoms with extensive necrosis on the leaves. The lesions did not coalesce, but the leaves and entire plants turned completely necrotic and died. The fungus grew and sporulated well on V8 (half strength) agar with optimum temperature for growth of 30°C. Although most of *Exserohilum* spp were reported as pathogen to member of Poaceae, but *E. monoceras* has a narrow host range, which includes several weedy grasses.

Corn, rice and sugarcane showed resistant reaction while dicots were immune. The pathogen penetrated plant surfaces by direct penetration through formation of appressoria randomly on surfaces of *E. crus-galli* 8 h post inoculation. The appressorium being usually bulbous or cylindrical often ends with the formation of extensive secondary hyphae. The fungus penetrated the cuticle cell wall and grew intra and intercellularly within the tissues. On rice leaves, the fungus grew and penetrated the leaf surface. The fungus did not produce extensive hyphae in rice. The fungus grew on tomato and chili but could not penetrate the cell wall as indicated by lysing of the conidia and germ tubes 8 h post inoculations. The inability of the germinating conidia to penetrate and to progress indicated that tomato and chili are not compatible hosts for this fungus. The level of disease severity on *E. crus-galli* was linearly related to the conidial concentration of *E. monoceras* with conidia concentration at $10^6$ conidia per milliliter resulting in 100% control of the seedlings. Although humidity is the main concern for most mycoherbicides, *E. monoceras* provided good control of *E. crus-galli* under mini-plot trials. The fungus reduced competitive ability of *E. crus-galli.* The results demonstrate the potential of *E. monoceras* as a bioherbicide to control *Echinochloa crus-galli.* Additional further research on molecular aspects, mass conidia production, carrier formulation and amendments may further enhance the field efficacy of the pathogen.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

**POTENSI *EXSEROHILUM MONOCERAS* SEBAGAI BIOHERBISID UNTUK BARNYARD GRASS (*ECHINOCHLOA CRUS-GALLI*)**

Oleh:

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**Januari 2006**

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Kajian memajukan *Exserohilum monoceras* sebagai bioherbisid yang berpotensi untuk mengawal rumpai 'barnyard grass' (*Echinochloa crus-galli*) telah dijalankan. Pemencilan kulat dilakukan dari sampel yang diperolehi dari *Echinochloa crus-galli* yang mempunyai simptom penyakit di kawasan Tanjong Karang, Selangor. Tahap kepatogenan *Exserohilum Longirostratum* telah diuji di makmal dan di rumah kaca. Keputusan kajian mendapati kulat ini memberi kesan langsung kepatogenan yang paling tinggi pada rumpai *Echinochloa crus-galli* apabila diinokulat dengan 2.1 X $10^6$ konidia/ml. Simptom kelihatan seperti bintik kecil berwarna hitam berair pada permukaan daun selepas 24 jam inokulasi. Bintik-bintik tersebut didapati tidak bercantum tetapi kesemua daun pokok menjadi nekrotik dan akhirnya mati. Pertumbuhan dan perkembangan kulat ini didapati sangat sesuai di atas media V8 agar (separuh kepekatan).

pertumbuhan kulat ini ialah pada 30°C. Walaupun, kebanyakan spesies *Exserohilum* dilaporkan menjadi patogen kepada keluarga 'Poaceae', tetapi *Exserohilum monoceras* didapati mempunyai julat perumah yang agak terhad kepada beberapa spesies rumpai daun tirus terutamanya pada spisies *Echinochloa*. Kesannya terhadap tanaman jagung, padi dan tebu menunjukkan tindak balas resistan manakala tumbuhan dikot tidak dijangkiti oleh kulat ini. *Exserohilum monoceras* menembusi permukaan daun secara terus menerusi pembentukan appressorium di atas permukaan daun *Echinochloa crus-galli* selepas lapan jam inokulasi. Kebiasaannya appressorium berbentuk bulat atau silinder yang menghasilkan hifa skunder dihujungnya. Kulat patogen menembusi dinding sel kutikel dan tumbuh di sebelah luar dan dalam sel tisu. Di atas permukaan daun padi pula, kulat ini tumbuh dan menembusi permukaan daun tetapi perkembangan kulat yang terhad di kawasan inokulasi menyebabkan hifa skunder tidak dihasilkan. Kulat ini juga tumbuh di atas permukaan daun tomato dan cili tetapi konidia dan tiub cambahnya mengecut menyebabkan kegagalan untuk menembusi dinding sel selepas lapan jam inokulasi. Ini menunjukkan tomato dan cili bukanlah perumah yang sesuai untuk kulat ini. Paras keterukan penyakit pada daun *E. crus-galli* adalah berkadar terus dengan konsentrasi konidia *E. monoceras*. Konsentrasi konidia yang melebihi $10^6$ konidia/mililiter boleh menyebabkan kematian 100% anak benih. Masalah keperluan kelembapan di lapangan yang mempengaruhi kebolehan *E. monoceras* boleh di atasi dengan menambahkan 'amendments' di dalam formulasi. Namun kajian berkaitan molecular, penghasilan konidia secara pukal, formulasi pembawaan 'amendments' mungkin dapat mempertingkatkan keberkesanan patogen di lapangan. Hasil dari kajian ini dapatlah dirumuskan *E. monoceras* boleh mengurangkan daya saing *E. crus-galli*.

## ACKNOWLEDGMENTS

First of all, thank to God Almighty for His grace and for giving me the opportunity to undertake the Master of Agricultural Science Degree. My sincere gratitude goes to Associate Professor Dr. Jugah Kadir, as the chairman of the supervisory committee for his enormous guidance, ideas, comments, concern and understanding. I wish to extend my sincere gratitude to the other supervisory committee members, Dr. Abdul Shukor Juraimi, and Associate Professor Dr. Suhaimi bin Napis, for their valuable advice until the completion of this thesis.

I am indebted to Associate Professor Dr. Fauziah Othman, En. Rafius Zaman Haroun, Mr Ho, and others from Bioscience Institute for their assistance in SEM analysis; and to all the laboratory staff in the Department of Plant Pathology for their kind assistance.

I would like to thank Rozlianah Fitri, Azean bt Ahmad, Charles, Hasraena, Adam Supu, Ariffin Tasrif , Mohd Cholil, Zaiton bt Sapak, Chan Saw Chin, Lai Kun and all lab mates for their help and moral support.

A million thanks to my family especially to my father (Sajili Bin Jamli), mother (Lehon Bt Dilah), brother and sister for their love, prayer, support and understanding.

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## LIST OF ABBREVIATIONS

% = Percentage

PDA = Potato dextrose agar

$mm^2$ = Millimeter square

µl = Micro liter

SE =Standard Error

$r_L$ = Apparent infection rate values were obtain epidemic rate by transforming disease severity data using the logistic model

$R^2$ = Square of the multiple correlation

N = Nitrogen

Vol = Volume

DI = Disease Index

∑ = Sum

M = Mortality

pH = Potential of Hydrogen

µ = Micro

rpm = Rotation per minute

SAS = Statistical Analysis System

w/v = Weight per volume

h = Hour

AUDPC= Area Under Disease Progress Curve

P = Probability

NA = Not Applicable

diam = Diameter

a.i/ha = Active ingredient / hectare

$CO_2$ = Carbon dioxide

RY = Relative Yield

RYT = Relative Yield Total

IWMS = Integrated weed management system

WOW = water-oil-water

WA = Water Agar

LCB = Lactophenol Coton Blue

# CHAPTER I

## INTRODUCTION

*Echinochloa crus-galli* is a very serius weed in rice, which is the staple food of Malaysians and therefore a very important crop in this country. The area under (lowland) rice *cultivation* in Peninsular Malaysia is approximately 400,000 hectares (Azmi, 2002). The production of rice is concentrated in granary areas, all of which are in Peninsular Malaysia. The main growing areas are in the North-west and North-east of Peninsular Malaysia, in total accounting for 86% of domestic production (Zuki *et al.*, 1996). They are Muda in Kedah, Kemubu in Kelantan, Kerian Sungai Manik in Perak, Seberang Perai in Penang, Tanjung Karang (Barat Laut Selangor) in Selangor, Seberang Perak, Kemasin-Semerak and Besut in Terengganu with a total area of 204,927 hectares (Azmi, 2002).

Due to the labour shortage, the rice cultivation technique has changed from transplanting to direct seeding. This change has resulted in a shift in weed populations and has caused serious weed problems (Azmi, 1996). In transplanting, the half-grown plants have an advantage over their competitors (weeds), but no such advantage is accorded to the directly sown plants. Thus, grasses, such as *Echinochloa* spp., *Leptochloa chinensis*, *Ischaemum rugosum*, have become serious weeds in direct-sown rice where they are controlled mainly by herbicides (Azmi, 1996).

There are five species (*Echinochloa crus-galli Echinochloa colona, Echinochloa formosensis, Echinochloa sp. Echinochloa oryzicola*) of *Echinochloa* in Peninsular Malaysia, but only *Echinochloa crus-galli* is a major weed in direct-seeded rice (Azmi *et al*., 1991). All of them are locally known as *rumput sambau*. *Echinochloa crus-galli* has several varieties, among them var. *crus-galli* and var. *formosensis* which are look-alikes, are mainly distinguished by their awns and panicles. The var. *crus-galli* has long awns and closed or compact panicles and var. *formosensis* has short awns or sometimes awnless and has open panicles with shiny spikelets (Azmi and Itoh, 1991).

The methods for controlling this weed are difficult as manual weeding is labour intensive. Herbicides give satisfactory kill but their cost makes their application moot. Besides resistance population of *E. crus-galli* to the commonly used herbicides has already been documented. Baker and Henis (1990), has already found *E. crus-galli* var. *crus-galli* resistant to tetrazine and propanil.

There are also other problems with chemical control of *E. crus-galli* var. *crus-galli*. Most herbicides are not selective enough to control *E. crus-galli* due to the similar characteristics with the crop (rice). Continuous use of chemicals can also contaminate the environment and induce resistance in related weeds. An alternative method of control without all these problems would be highly desirable. Bioherbicide is one such alternative - using a natural enemy, like a fungus, to control the weed.

Bioherbicide not only provides pollution-free control but also has the potential for more cost effective control. However, the greatest attractions of bioherbicide are their easy production *in-vitro*, high virulence, genetic stability and restricted host range. Active penetration by the fungi into the plant tissue independent of vectors is another attraction.

Several plant pathogens have been suggested as having bioherbicide potential for *Echinochloa* spp. control. Zhang *et al.* (1996), reported *E. monoceras* which was isolated from *E. colona* has a good potential as bioherbicide. In a later study by Zhang and Watson(1997), their found that *E. monoceras* gave a 100 percent kill of *E. crus-galli*. However, more work remains to be done to formulate this fungus into potent bioherbicides.

Bioherbicide is a 'new' chapter in weed control, and according to Templeton and Heiny (1989), much remains to be learnt on the biology and ecology of the pathogens. Shabana *et al.* (1995) and Kadir and Charudattan (2000) felt that one of the major constraints to the application of bioherbicides is the humidity requirement, however this constraint can be overcome by using various amendments to stabilize the formulations.

Nevertheless, very little is known on the optimal sporulation conditions for the majority of fungal pathogens (Ahmad *et al.*, 2002). There is, therefore, the necessity to investigate the basic mechanisms regulating the growth and sporulation of the pathogens. Although the cost for development and registration of a mycoherbicide is likely to be less than that for a chemical herbicide, its (mycoherbicide) successful commercialization is still predicated on the basic business considerations of market size, return on investment and profitability (Ahmad *et al.*, 2002). Therefore, the general objective of this study are;

1. Isolate and screen the indigenous fungal pathogen of *E. crus-galli.*
2. Determine the pathogenicity and host range of the *E. monoceras.*
3. Determine plant-pathogen interaction during infection process.
4. Determine effect of *E. monoceras* on weed-rice competition.

# CHAPTER II

## LITERATURE REVIEW

### Rice (*Oryza sativa*)

Wild rice was probably used as food 10,000 to 15,000 years ago and first cultivated in South Asia or China about 9,000 years ago. Cultivated rice (*Oryza sativa* L.) probably originated from this area (Lewin and Heenan, 1984). The spread of rice cultivation to new areas gave rise to the evolution of 'new' biotypes which enabled it to grow well in a wide range of climates, environments and soil conditions (Jahromi *et al.*, 2001). It is now grown as far north as Hungary and the Czech Republic (50°N) and as far south as Uruguay and New South Wales, Australia (35°S) as a rainfed crop, in water up to 6 m deep, in flood plains and at altitudes up to 2400 m (Brennan *et al.*, 1994; McDonald, 1994).

Currently, more than one-third of the human population consumes rice for their daily diet, including Malaysians, making it one of the most important food crops in the world. Worldwide, 530 million tonnes of rice at an average yield of 3.5 tonnes per hectare are harvested from 150 million hectares annually to provide 21% of the world's calorific supply. Almost 90% of the global rice crop is produced in tropical Asia, with China and India as the major producers (Zimdahl, 1988; McDonald, 1994). The rice industry is facing a big challenge in meeting the potential demand from the increasing population of almost 100 million a year even as the cultivable area is being