UNIVERSITI PUTRA MALAYSIA

SUITABILITY OF *Exserohilum Longirostratum* (Subram.) AS A BIOHERBICIDE FOR INTEGRATED MANAGEMENT OF BARNYARD GRASS [*Echinochloa crus-galli* (L.) Beauv. spp. *crusgalli*] IN MALAYSIA

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By

NG SAW CHIN

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

August 2007

To my teacher, Assoc. Prof. Dr. Jugah Kadir who introduced me to the fungi, and to Assoc. Prof. Dr. Anuar bin Rahim, families and friends. Thank you for your supports.

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

SUITABILITY OF *Exserohilum Longirostratum* (Subram.) AS A BIOHERBICIDE FOR INTEGRATED MANAGEMENT OF BARNYARD GRASS [*Echinochloa crus-galli* (L.) Beauv. spp. *crusgalli*] IN MALAYSIA

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Chairperson: Assoc. Prof. Jugah Kadir, PhD

Faculty: Agriculture

An ideal bioherbicide should be easy and cheap to mass produce, viable and efficacious in controlling the target weed within definite time. A better understanding of the strategies of mass producing of inoculum is important to evaluate the suitability of *Exserohilum longirostratum* as bioherbicide. *Exserohilum longirostratum* has been reported as potential bioherbicide in controlling several grassy weeds; however, its control efficacy has several shortcomings. A study was conducted to determine the suitability of *E. longirostratum* as bioherbicide for controlling barnyard grass (*Echinochloa crus-galli* (L.) Beauv. spp. c*rusgalli*) both in the glasshouse, and in the field. In the Carbon:Nitrogen (C:N) ratio test, 10:1 (C:N) produced the highest biomass (0.75g/100mL) and, 7.5:1 and 10:1 (C:N) resulted in highest production of healthy spores (6.4 x 10^5 spore per mL and 6.6 x 10^5 spore per mL respectively) compared with control treatment without external carbon and nitrogen source (1.4 x 10^5 spores per mL). The later produced about 4 times less than in the 7.5:1 and 10:1 C:N ratio test. An

early infection of target plant by the pathogen may assure a successful disease development. A 10^5 spores/mL with 30% oil and a 5 day-old (DO) mycelium culture in the ratio of 1:5 (wt:vol) with 30% oil infected high disease severity on barnyard grass (*Echinochloa crus-galli* (L.) Beauv. spp. *crusgalli*). Both inoculum forms are effective in causing severe disease on the weed. The overall disease progress rate for the conidia on barnyard grass (r_{Lc}= 2.50 unit/day) was not significantly different with disease progress rate for mycelium (r_{Lm}= 2.27 logit/day**).** With the presence of herbicide resistant biotype of barnyard grass, pre-disposing the barnyard grass with sublethal dose of chemical herbicides will enhance better control of the weed and are crucial in integrated weed control. Base on this study, *E. longirostratum* was able to grow in some of the herbicides tested at the sublethal dose. Pretilachlor and cyhalofop/buthyl supported high germination of *E. longirostratum* compared with other chemical herbicides at all doses and to non-chemical amended control. These chemicals also supported > 60% appressorium formation at 0.25x of the recommended rate compared with the control. Compatibility of *E. longirostratum* with chemical herbicide may increase fungus efficacy on weed control. Sublethal rate of herbicide combined with pathogen may lead to synergistic effect, potentially increasing weed control and reducing producing costs. Understanding the course of the infection and development of *E. longirostratum* aids in elucidating the mechanism of host death and in determining the suitability of *E. longirostratum* as the biocontrol agent on barnyard grass. Germination of *E. longirostratum* took place about 4 hours after inoculation on both barnyard grass (67%) compared with rice (51.25%) 24 hours after inoculation. Many of the germ tubes extended along the surface of the junction of the epidermal cells.

Appressoria formation were significantly higher on barnyard grass (92.4%) compared with only 10.9% on rice. Most infection process ceased at the stage of germ tubes elongation in rice. The fungus fails to cause infection. This observation indicated that rice is not a compatible host for this fungus. This conclusion was further supported by the result of the mini plot trial. Rice plants produced more tillers and high biomass compared with the untreated counterpart. However, barnyard grass was effectively controlled by *E. longistratum* with or without pretilachlor added as auxiliary. High mortality of barnyard grass caused its numbers of tillers and dry weight dramatically low compared to rice. Barnyard grass reduced >90% of dry weight from the control. The result of this research indicated *E. longirostratum* has the potential to be used as bioherbicide for integrated management of barnyard grass in rice field.

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

KESESUAIAN *Exserohilum longirostratum* (Subram.) SEBAGAI BIOHERBISID UNTUK PENGAWALAN INTEGRASI TERHADAP RUMPUT SAMBAU (*Echinochloa crus-galli* (L.) Beauv. spp. *crusgalli*) DI MALAYSIA

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Bioherbisid yang ideal sepatutnya mudah dan murah untuk dihasilkan dalam kuatiti yang banyak dan berkesan untuk mengawal sasaran rumpai dalam masa yang tertentu. Pemahaman yang dalam terhadap strategik untuk menghasilkan inokula dalam kuantiti yang banyak adalah penting dalam penganggaran kesesuaian *Exserohilum longirostratum* sebagai bioherbisid. *Exserohilum longirostratum* telah dilaporkan sebagai bioherbisid yang berpotensi untuk mengawal beberapa jenis rumpai berdaun; akan tetapi, masih mempunyai beberapa kelemahan dari segi keberkesanannya. Satu kajian telah dijalankan dalam rumah kaca dan di ladang untuk menentukan kesesuaian *E. longirostratum* sebagai bioherbisid untuk mengawal rumput sambau (*Echinochloa crus-galli* (L.) Beauv. spp. *crusgalli*). Dalam kajian nisbah Carbon:Nitrogen (C:N) rasio, 10:1 (C:N) menghasilkan biomas yang paling tinggi (0.75g/100mL) dan, 7.5:1 dan 10:1 (C:N) menghasilkan paling banyak spora yang sihat (6.4×10^5 spora per mL and 6.6×10^5 spora per mL) berbanding dengan kawalan tanpa sebarang sumber karbon

dan nitrogen (1.4 x 10^5 spora per mL). Yang kemudian menghasilkan 4 kali kurang daripada kajian C:N rasio 7.5:1 dan 10:1. Serangan awal sasaran rumpai oleh patogen akan menentukan keberkesanan perkembangannya sesuatu penyakit. 10^5 spora/mL dengan 30% minyak dan 5 hari-matang (DO) kultur mesilium dalam nisbah 1:5 (wt:vol) dengan 30% minyak menjangkit serius penyakit pada rumput sambau (*Echinochloa crus-galli* (L.) Beauv. spp. *crusgalli*). Kedua-dua bentuk inokula berkesan untuk menghasilkan penyakit yang serius pada sasaran rumpai. Perkembangan keseluruhan spora pada rumput sambau (r_{Lc}= 2.50 unit/day) adalah tiada perbezaan ketara berbanding dengan mesilium (r_{Lm}= 2.27 logit/day). Kewujudan rumput sambau yang rintang terhadap herbisid, pengurusan awal rumput sambau dengan dos rendah herbisid akan menambah keberkesanan pengawalan rumpai dengan bioherbisid dan adalah penting dalam pengawal rumpai secara berintegrasi. Merujuk kepada kajian ini, *E. longirostratum* mampu hidup dengan sesetengah herbisid yang dikaji pada dos sampingan. Pretilachlor dan cyhalofop/buthyl menyokong kadar pertumbuhan *E. longirostratum* yang tinggi berbanding dengan herbisid yang lain pada semua dos yang dikaji dan kawalan tanpa herbisid. Herbisid kimia tersebut juga menyokong pertumbuhan >60% pembentukan apresoria pada 0.25x daripada dos cadangan berbanding kawalan. Kesepadanan *E. longirostratum* dan herbicid kimia akan meningkat keberkesanan dalam pengawalan rumpai. Dos sampingan herbisid kimia dengan patogen akan membangkit kesan tambahan, berpotensi meningkat pretasi pengawalan rumpai dan mengurangkan kos penghasilan. Pemahaman proses menjangkit dan perkembangan *E. longirostratum* membantu dalam menerangkan mekanisme kematian hos dan penentuan kesesuaian *E. longirostratum* sebagai agen biokawalan

rumput sambau. Pertumbuhan *E. longirostratum* mangambil masa 4 jam selepas diinokulasi pada rumput sambau (67%) berbanding dengan padi (51.25%) pada 24 jam selepas inokulasi. Banyak germ tube berkembang sepanjang permukaan simpang tisu epiderma. Pembentukan apresoria adalah ketara banyak pada rumput sambau (92.4%) berbanding dengan 10.9% pada padi. Kebanyakan proses jangkitan berhenti setakat pemanjangan germ tube pada padi. Kulat tersebut gagal menyebabkan jangkitan. Pemerhatian tersebut menerangkan bahawa padi bukan hos sepadan kepada kulat tersebut. Kesimpulan ini dapat dikukuhkan lagi dengan keputusan daripada kajian mini plot. Padi meningkat dalam pengeluaran anak padi dan biomas kering berbanding dengan pokok yang tidak disembur. Bagaimanapun, rumput sambau dikawal dengan berkesan oleh *E. longirostratum* dengan atau tanpa pretilachlor sebagai tambahan. Kematian yang tinggi pada rumput sambau menyebabkan pengeluaran anak rumput dan biomas kering jelas lebih rendah berbanding dengan padi. Biomas kering sumput sambau berkurangan >90% daripada kawalan. Keputusan daripada kajian ini menunjukkan *E. longirostratum* berpotensi digunakan sebagai bioherbisid dalam pengawalan rumput sambau secara berintegrasi di sawah padi.

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I certify that an Examination Committee has met on 19th December 2007 to conduct the final examination of Ng Saw Chin on her Master of Agriculture Science thesis entitled "Suitability of *Exserohilum longirostratum* (Subram.) as bioherbicide for Integrated Management of Barnyard grass (*Echinochloa crus-galli* (L.) Beauv. spp. crusgalli) in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (High Degree) Regulations 1981. The Committee recommends that the candidate be awarded the degree of Master of Agriculture Science.

Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Agriculture Science. The members of the Supervisory Committee were as follows:

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Date: 8 May 2008

DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NG SAW CHIN

Date:

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LIST OF ABBREVIATIONS

%	Percentage
>	More than
^{0}C	Degree Celsius
±	Plus minus
r_L	Disease progress rate / epidemic rate
wt / w	Weight
vol / v	Volume
g	Gram
ha	Hectare
r^2	coefficient
m^2	Meter square
L	Liter
h / hr	Hour
mL	Milliliter
LC	Lethal concentration
sec	Second
LS	Leaf-stage
t/ha	Metric tonne /hectare
rpm	Rotation per minute
C:N	Carbon-to-Nitrogen ratio
AUDPC	Area Under Disease Progress Curve
LSD	Least significant different

PDA	Potato Dextrose Agar
DO	Day-old
SEM	Scanning Electron Microscope
ppm	Part per million

CHAPTER I

GENERAL INTRODUCTION

In Malaysia, wetland rice is facing increasing weed problems of the change in its planting method from transplanting to direct-seeding. Among the grassy weeds, barnyard grass (*Echinochloa crus-galli* complex) has become one of the most troublesome. Barnyard grass is not only a weed of rice, but also to many other crops, especially those grown on moist, fertile soils. Its genetic similarity and similar growth requirements to rice makes it a formidable competitor to the crop causing untold losses in yield by its competition. It has been reported that barnyard grass reduced rice yield by 21-40% (Azmi, 1988, Tjitrosemito, 1994, Tasrif *et al.*, 2004). To compound the problem, the weed is also host to many insect pests and pathogens, indirectly causing yet more losses in yield and produce quality of rice.

The present method of weed control is largely by herbicides, but the method, once economic and effective, has now loaded with problems. The continued use of herbicides induced resistance in the weeds and the chemical residues wrought environmental havoc. Nevertheless, herbicides continue to be used for the less of other choices. It is, therefore, imperative that safer and more environmentally-friendly alternatives be developed for greater effectiveness and to minimize the wanton destruction of the environment.

Biological control of weeds using plant pathogens has gained acceptance as a practical, safe and environmentally-friendly management practice to the agro-ecosystem. Bioherbicides are commonly formulated using microorganisms as the active ingredient, and applied just as a chemical herbicide. Therefore, the suitability and stability of the pathogen is of paramount importance to ensure efficacy of the product. The plant pathogen used must be host-specific so that no harm is caused to the crop and non-target plants. It should also have a long storage life and be easy to apply.

For bioherbicides to be practical, it must first be possible to mass produce the pathogen which, *inter alia*, means that it must be able to remain genetically stable through the production process. An inoculum easy to produce is therefore desired as the active ingredient to lower the production cost and increase the productivity. In the culture, the carbon-to-nitrogen (C:N) ratio is very important for the growth and sporulation of the inoculum, and it can also affect the viability and pathogenicity of the bioherbicide propagules.

Bioherbicides require moisture to keep the pathogen alive for maximum infection. For example, Ahmad *et al.* (2004) reported that *E. longirostratum* needs a minimum of 8 hours' dew for severe infection. This has always posed a problem in formulating bioherbicides, and a wetting agent and oil emulsion may have to be incorporated to retain the moisture. Ng *et al.* (2004) applied *E. longirostratum* in vegetable oil to control barnyard grass seedlings, and the treatment managed a control up to 97%. In addition, the dew requirement was reduced from 24h to 8h.