

**EVALUATION OF *Exserohilum prolatum* AS A POTENTIAL  
MYCOHERBICIDE FOR DIFFERENT BIOTYPES OF ITCHGRASS  
(*Rottboellia cochinchinensis* (Lour) Clayton)**

**By**

**HALA ELTAHIR MAHMOUD ALLOUB**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of Requirements for the Degree of Doctor of Philosophy**

**April 2006**

## **DEDICATION**

With love to my mother, father, sisters and brothers for their love, moral support and encouragement and in memory of my aunts Fatima and Najat who did not live to see this work.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

**EVALUATION OF *Exserohilum prolatum* AS A POTENTIAL MYCOHERBICIDE FOR DIFFERENT BIOTYPES OF ITCHGRASS (*Rottboellia cochinchinensis* (Lour) Clayton)**

By

**HALA ELTAHIR MAHMOUD ALLOUB**

**April 2006**

**Chairman : Abdul Shukor Juraimi, PhD**

**Faculty : Agriculture**

Itchgrass or *Rottboellia cochinchinensis* is a noxious and widely spread annual grass weed in the tropics and subtropics. In Malaysia itchgrass is a major problem in the sugarcane plantations in the states of Perlis and Kedah. Preliminary and extended glasshouse experiments were conducted to classify itchgrass present in Peninsular Malaysia based on morphological characteristics to aid in its biological control. The results categorized 13 populations, collected from six infested states, into three biotypes. Biotype 1 found in association with banana, cocoa and oil palm or along the roadsides in Johor, Pahang and Perak. Biotype 2 found in association with maize, upland rice, rubber, sugarcane and tobacco in Kedah, Perlis and Selangor. Biotype 3 was found only along roadsides of Padang Kartong.

Fungi associated with itchgrass were isolated from diseased plants collected from 14 different infested locations. Ten fungi were pathogenic to the three itchgrass biotypes. Primary screening of the fungi showed that isolates of *Botryodiplodia*

*theobrome*, *Curvularia eragrostidis*, *C. fallax*, *C. lunata*, *C. pallescens* and *Fusarium oxysporum* were highly pathogenic to most of the plants tested. Isolates of *Exserohilum longirostratum*, *E. prolatum*, *Fusarium moniliforme* and a *Phoma* sp. showed some degree of specificity to itchgrass. Further screening of *E. prolatum* against 32 plants belonging to 12 families showed complete kill of itchgrass, while all other plants tested were immune, resistant or tolerant to the isolate.

Factors affecting the efficacy of *E. prolatum* were studied. Fungal growth and sporulation were evaluated in five culture media under three light durations. Highest colony diameters were obtained with CMA, PDA and CMA under the 12 hr light, however highest spore concentration was obtained with V8A under 24 hr light. Different additives were also evaluated for *E. prolatum* spore viability. The additives include suspensions of conidia in distilled water, conidia in three different concentrations of Tween 20 surfactant, conidia in three different palm oil concentrations, three combinations of Tween 20 concentrations and palm oil and conidia in five different concentrations of two herbicides glyphosate (Round Up<sup>®</sup>) and ammonium glufosinate (Basta 15<sup>®</sup>). The results showed high spore viability with Tween 20 and palm oil suspensions. However, both the herbicides greatly reduced fungal viability compared to the control.

Factors affecting *E. prolatum* disease development were also studied. The results showed that increasing spore concentration increased disease severity, optimum wetness duration was 24 hr, combination of surfactant and palm oil increased disease

severity and reduced dry weight under 8 and 12 hr wetness duration and disease severity decreased with increasing plant age.

The potential of *E. prolatum* as a mycoherbicide was evaluated under natural conditions in two field experiments in a non crop situation and in association with a maize crop. Application frequency and inoculum concentration greatly influence itchgrass control. Three application frequencies had significantly higher percentage of control compared to single and double applications. Within the three frequencies of application, increasing inoculum concentration increased itchgrass control compared to untreated check. The results indicate that *E. prolatum* has a good potential as a biocontrol agent for itchgrass.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENILAIAN KE ATAS *Exserohilum prolatum* MIKOHERBISID  
BERPOTENSI TERHADAP BIOTIP RUMPUT MIANG (*Rottboellia  
cochinchinensis* (Lour) Clayton) BERBEZA**

Oleh

**HALA ELTAHIR MAHMOUD ALLOUB**

**April 2006**

**Pengerusi : Abdul Shukor Juraimi, PhD**

**Fakulti : Pertanian**

Rumput miang atau *Rottboellia cochinchinensis* ialah rumpai tahunan noksius yang tersebar secara meluas di kawasan tropikal dan sub tropikal. Di Malaysia rumput miang merupakan masalah utama di kawasan ladang tebu di Perlis dan Kedah. Pra-kajian dan seterusnya kajian rumah kaca telah dijalankan untuk mengelaskan rumput miang yang terdapat di Semenanjung Malaysia berdasarkan ciri-ciri morfologi untuk membantu dalam pengawalannya secara biologi. Keputusan kajian ini telah mengkategorikan 13 populasi yang dikumpul dari enam negeri kepada tiga biotip. Biotip 1 yang dijumpai bersama dengan tanaman pisang, koko dan kelapa sawit atau di sepanjang jalan di Johor, Pahang dan Perak. Biotip 2 merangkumi populasi yang dijumpai bersama tanaman jagung, padi huma, getah, tebu dan tembakau di Kedah, Perlis dan Selangor. Biotip 3 ialah populasi yang berasingan dan hanya dijumpai di sepanjang jalan Padang Kartong, Perlis.

Kulat yang dijumpai pada rumput miang diasingkan dari tumbuhan berpenyakit yang dikumpul dari 14 kawasan yang berbeza. Sepuluh kulat didapati patogenik kepada tiga kumpulan rumput miang. Penilaian awal keberkesanan kulat patogenik *Botryodiplodia theobromae*, *Curvularia eragrostidis*, *C. fallax*, *C. lunata*, *C. pallescens* dan *Fusarium oxysporum* didapati sangat patogenik terhadap kebanyakan tumbuhan yang diuji. Isolat *Exserohilum longirostratum*, *E. prolatum*, *Fusarium moniliforme* dan *Phoma herbarum* pula menunjukkan aktiviti yang spesifik terhadap rumput miang. Kajian lanjut keberkesanan *E. prolatum* terhadap 32 tumbuhan dari 2 famili menunjukkan kematian sepenuhnya rumput miang, sementara tanaman lain yang diuji mengalami sama ada lali, resistan atau toleran terhadap isolat.

Faktor yang mempengaruhi keberkesanan *E. prolatum* dikaji. Pertumbuhan kulat dan penghasilan spora dinilai dalam lima media kultur dibawah tiga tempoh pencahayaan. Diameter koloni tertinggi didapati dari media CMA, PDA dan CMA dibawah 12 pencahayaan, tetapi kepekatan spora tertinggi didapati dari media V8A dibawah 24 jam pencahayaan. Kesan beberapa bahan aditif turut dinilai ke atas viability spora *E. prolatum*. Bahan aditif tersebut termasuk ampaian konidia dalam air suling, konidia dalam tiga kepekatan surfaktan tween 20 yang berbeza, konidia dalam tiga kepekatan minyak kelapa sawit yang berbeza, tiga kombinasi kepekatan tween 20 dan minyak kelapa sawit, dan konidia dalam lima kepekatan herbisid glifosat (Round Up<sup>®</sup>) dan glufosinat ammonium (Basta 15<sup>®</sup>). Hasil kajian menunjukkan viabiliti spora yang tinggi pada ampaian tween 20 dan minyak kelapa sawit. Walau bagaimanapun kedua-dua herbisid telah menurunkan viabiliti spora dengan teruk berbanding kawalan.

Kajian kesan faktor-faktor yang mempengaruhi perkembangan penyakit *E. prolatum*, keputusan kajian menunjukkan meningkatnya kepekatan spora akan meningkatkan keterukan penyakit, jangkamasa kelembapan optimum ialah 24 jam, kombinasi surfaktan dan minyak kelapa sawit mencatatkan keterukan penyakit dan merendahkan berat kering pada jangka masa kelembapan 8 dan 12 jam tetapi keterukan penyakit berkurangan dengan meningkatnya umur tumbuhan.

Potensi *E. prolatum* sebagai mikroherbisid telah dinilai di bawah keadaan semulajadi di dua kajian ladang, iaitu yang tiada tanaman dan bersama tanaman jagung. Keputusan kajian menunjukkan kekerapan rawatan dan kepekatan inokulum didapati sangat mempengaruhi pengawalan rumput miang. Kekerapan rawatan sebanyak tiga kali menghasilkan peratus kawalan lebih tinggi dan signifikan berbanding sekali dan dua kali rawatan. Pada kekerapan rawatan tiga kali peningkatan kepekatan inokulum lebih meningkatkan peratus kawalan rumput miang berbanding kawalan. Keputusan kajian menunjukan *E. prolatum* mempunyai potensi sebagai agen kawalan biologi rumput miang.



## ACKNOWLEDGEMENTS

First my praise to almighty Allah for giving me the will and power to complete this study and peace be upon his final Prophet and Messenger Mohamed.

I would like to express thanks to my Supervisory Committee for their input and helping me to have such a great opportunity in such a great place. My sincere appreciation to Dr. Abdul Shukor Juraimi, head of the supervisory committee for his guidance, patience encouragement, and continuous support during the study. Special thanks are due to Assoc. Prof. Dr. Jugah Kadir, Assoc. Prof. Dr. Rajan Amartalingam and Dr Soetikno Sastroutomo for their constructive discussion, advice, comments and suggestions.

I wish to thank Prof. Dr Mohd Said Saad, and Assoc. Prof. Dr Anuar Abdul Rahim for their help with the statistical analysis. I am also indebted to Dr Zakaria Wahab, Head of the Department of Crop Science for his help and for providing banana seedlings, Prof. Dr. Ghizan Salleh for supplying maize seeds and to all other staff of the Faculty of Agriculture, UPM for their generous cooperation.

I am grateful to the University of Gezira (Sudan) for kindly giving me study leave to complete this study. My sincere gratitude is also extended to partial financial support provided under the Felda Foundation grant.

I wish to express my great appreciation to staff members of Crop Protection and Quarantine, Department of Agriculture (DOA), Malaysia especially to Head of Weed

Science Section, Mrs Mislamah Abu Bakar and to Mr Francis Nitto for their invaluable assistance.

Further, I would like to thank Rubber Plantation Bhd, Golden Hope Bhd, Perlis Sugarcane Plantation Bhd for supplying rubber, oil palm seedlings and sugarcane cultivars stems, CAB International Southeast Asian Regional Office for allowing me to use the facilities of their laboratories and CABI Bioscience-UK centre for identification of the isolates.

This work would not have been possible without the help of En Haji Yasir, En Yakob, En Ibrahim, En Haji Ismail, Pn Zawiah, En Mohammed Mat Daud, Pn Siti Ramlah Jaffar, staff of Plant Pathology Laboratory, Soil Science Laboratory, field 10, the director of the University Meteorology station and my friends Mahasin, Abd Elrahman, and Adroub.

I am grateful to my fellow graduate students for helping throughout my study and to all Malaysian friends for the excellent hospitality.

Lastly, I would like to thank my mother, father, sister and brothers in Sudan for their love, inspiration, encouragement and moral support.

I certify that an Examination Committee has met on 12 April 2006 to conduct the final examination of Hala Eltahir Mahmoud Alloub on her Doctor of Philosophy thesis entitled “Evaluation of *Exserohilum prolatum* as a Potential Mycoherbicide for Different Biotypes of Itchgrass (*Rottboellia cochinchinensis* (Lour) Clayton)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommended that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Rosli Mohamad, PhD**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Sariah Meon, PhD**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Zainal Abidin Mior Ahmed, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Baharuddin Salleh, PhD**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(External Examiner)

---

**HASANAH MOHD. GHAZALI, PhD**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

**Abdul Shukor Juraimi, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Jugah Kadir, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Rajan Amartalingam, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Soetikno Sastroutomo, PhD**

Assistant Professor  
CAB International Southeast Asian Regional Office  
MARDI  
(Member)

---

**AINI IDERIS, PhD**

Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

**DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

---

**HALA ELTAHIR MAHMOUD ALLOUB**

Date:

## TABLE OF CONTENTS

|   | <b>Page</b> |
|---|-------------|
| <b>DEDICATION</b>                                   | 2           |
| <b>ABSTRACT</b>                                     | 3           |
| <b>ABSTRAK</b>                                      | 6           |
| <b>ACKNOWLEDGEMENTS</b>                             | 9           |
| <b>APPROVAL</b>                                     | 11          |
| <b>DECLARATION</b>                                  | 13          |
| <b>LIST OF TABLES</b>                               | 18          |
| <b>LIST OF FIGURES</b>                              | 20          |
| <b>LIST OF PLATES</b>                               | 22          |
| <b>LIST OF ABBREVIATIONS</b>                        | 23          |
| <b>CHAPTER</b>                                      |             |
| <b>I INTRODUCTION</b>                               | 25          |
| <b>II LITERATURE REVIEW</b>                         | 31          |
| Impact of Weeds on Agriculture                      | 31          |
| Impact of Herbicide                                 | 32          |
| Biological Control of Weeds using Fungal Pathogens  | 34          |
| Classical Approach                                  | 35          |
| Augmentation Approach                               | 37          |
| Inundative Approach                                 | 37          |
| Integrated Weed Management                          | 40          |
| The Target Weed: <i>Rottboellia cochinchinensis</i> | 42          |
| Taxonomy  | 42          |
| Synonyms  | 42          |
| Common Names  | 43          |
| Characteristics                                     | 43          |
| Distribution and Habitat                            | 45          |
| Biology and Ecology                                 | 47          |
| Itchgrass Biotypes                                  | 49          |
| Economic Importance of Itchgrass                    | 51          |
| Management of Itchgrass                             | 53          |
| The Pathogen: <i>Exserohilum prolatum</i>           | 60          |
| Taxonomy  | 60          |
| General Characteristics                             | 61          |
| Host Range of <i>Setosphaeria prolata</i>           | 63          |
| <i>Exserohilum spp</i> as Biocontrol Agents         | 63          |

|            |   |     |
|------------|---|-----|
| <b>III</b> | <b>INVESTIGATIONS ON THE SEED MORPHOLOGY, GERMINATION AND SEEDLING GROWTH OF ITCHGRASS POPULATIONS IN PENINSULAR MALAYSIA</b> | 65  |
|            | Introduction  | 65  |
|            | Materials and Methods   | 65  |
|            | Observations on Seed Morphology   | 66  |
|            | Seed Germination  | 66  |
|            | Seedling Growth   | 66  |
|            | Statistical Analysis  | 67  |
|            | Results   | 67  |
|            | Observations on Seed Morphology   | 67  |
|            | Seed Germination  | 67  |
|            | Seedling Growth   | 70  |
|            | <br>  |     |
|            | <b>MORPHOLOGICAL CLASIFICATION OF ITCHGRASS POPULATIONS IN PENINSULAR MALAYSIA</b>  | 72  |
|            | Introduction  | 72  |
|            | Materials and Methods   | 72  |
|            | Statistical Analysis  | 74  |
|            | Results   | 75  |
|            | Discussion  | 86  |
| <br>       |   |     |
| <b>IV</b>  | <b>POTENTIAL INDIGENOUS FUNGI FOR BIOLOGICAL CONTROL OF ITCHGRASS: PATHOGENICITY AND HOST RANGE SCREENING</b>                 | 88  |
|            | Introduction  | 88  |
|            | Materials and Methods   | 90  |
|            | Collection, Isolation and Identification of Fungi   | 90  |
|            | Inoculum Production   | 91  |
|            | Pathogenicity of Isolated Fungi   | 92  |
|            | Primary Host Range  | 95  |
|            | Extended Host Range   | 96  |
|            | Statistical Analysis  | 97  |
|            | Results   | 97  |
|            | Collection, Isolation and Identification of Fungi   | 97  |
|            | Pathogenicity of Isolated Fungi   | 101 |
|            | Primary Host Range  | 104 |
|            | Extended Host Range   | 112 |
|            | Discussion  | 114 |
| <br>       |   |     |
| <b>V</b>   | <b>FACTORS AFFECTING EFFICACY OF <i>Exserohilum prolatum</i> FOR CONTROL OF ITCHGRASS</b>                                     | 117 |
|            | Introduction  | 117 |
|            | Materials and Methods   | 118 |
|            | A) Factors Affecting Conidial growth and Viability  | 118 |
|            | Effect of Light and Media on Colony Growth  |     |

|             |  |     |
|-------------|--|-----|
|             | and Sporulation  | 118 |
|             | Effect of Additives on Spore Viability   | 119 |
| B)          | Factors Affecting Disease Development  | 119 |
|             | Effect of Conidial Concentration   | 119 |
|             | Effect of Wetness Duration   | 120 |
|             | Effect of Plant Growth Stage   | 121 |
|             | Statistical Analysis   | 121 |
|             | Results  | 122 |
| A)          | Factors Affecting Conidial growth and Viability  | 122 |
|             | Effect of Light and Media on Colony Growth and Sporulation   | 122 |
|             | Effect of Additives on Spore Viability   | 125 |
| B)          | Factors Affecting Disease Development  | 128 |
|             | Effect of Conidial Concentration   | 128 |
|             | Effect of Wetness Duration   | 128 |
|             | Effect of Plant Growth Stage   | 136 |
|             | Discussion   | 141 |
| <b>VI</b>   | <b>FIELD EFFICACY OF <i>Exserohilum prolatum</i> FOR BIOLOGICAL CONTROL OF ITCHGRASS</b>               | 146 |
|             | Introduction   | 146 |
|             | Materials and Methods  | 149 |
|             | Inoculum Production  | 149 |
|             | Field Experiments  | 151 |
|             | Evaluation   | 154 |
|             | Statistical Analysis   | 155 |
|             | Results  | 155 |
|             | Experiment 1   | 155 |
|             | Experiment 2   | 161 |
|             | Discussion   | 173 |
| <b>VII</b>  | <b>GENERAL DISCUSSION</b>  | 176 |
|             | Introduction   | 176 |
|             | Classification of Itchgrass in Peninsular Malaysia   | 176 |
|             | Evaluation of Indigenous Fungal Plant Pathogens for Potential Control of Itchgrass                     | 177 |
|             | Factors Affecting Efficacy of <i>Exserohilum prolatum</i> for Control of Itchgrass                     | 179 |
|             | Field Efficacy of <i>Exserohilum prolatum</i> for Biological Control of Itchgrass                      | 184 |
| <b>VIII</b> | <b>CONCLUSIONS</b>   | 187 |
|             | Classification of Itchgrass in Peninsular Malaysia   | 187 |
|             | Potential Indigenous Fungal Plant Pathogens for Biological Control of Itchgrass in Peninsular Malaysia | 187 |
|             | Factors Affecting Efficacy of <i>Exserohilum prolatum</i> for Control of Itchgrass                     | 188 |



|  |     |
|--|-----|
| Field Efficacy of <i>Exserohilum prolatum</i> for Biological<br>Control of Itchgrass | 189 |
| <b>BIBLIOGRAPHY</b>  | 191 |
| <b>APPENDICES</b>  | 211 |
| <b>BIODATA OF THE AUTHOR</b>   | 231 |