UNIVERSITI PUTRA MALAYSIA

PRODUCTION AND EFFICACY OF *Exserohilum Longirostratum* AS A BIOHERBICIDE FOR THE CONTROL OF ITCH GRASS (*Rottboellia Cochinchinensis*) IN SUGARCANE FARMS

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Thesis Submitted to the School of Graduates Studies, Universiti Putra Malaysia in Fulfilment of the requirements for the Degree of Master of Agriculture Science

September 2008

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for degree of Master of Agricultural Sciences.

PRODUCTION AND EFFICACY OF *Exserohilum Longirostratum* AS A BIOHERBICIDE FOR THE CONTROL OF ITCH GRASS (*Rottboellia Cochinchinensis*) IN SUGARCANE FARMS

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CHARLES BORROMEO ALESTER ANAK ABI

September 2008

Chairperson : Associate Professor Dr. Jugah Kadir, PhD

Faculty : Agriculture

Itch grass or *Rottboellia cochinchinensis* (Lour.) W.D. Clayton is considered as on of the most important weeds in tropical countries. Integrated weed management is a tool-box of options that can be tailored to individual farm, cropping and weed situations. Biological control is one of these management options, therefore the production method and field efficacy of *Exserohilum longirostratum* as a bioherbicide to control itch grass in sugarcane was investigated. The growth and sporulation of *E. longirostratum* was excellent in media strength at recommended rate (200ml V8juice + 800ml H_2O). The optimum pH for conidia production and conidia germination was in the range of pH 6 – 7. The conidia stored in liquid suspension (water and oil) and freeze-dry failed to germinate. Air dried conidia in powder formed stored at 7^0C and 20^0C has germination rate of 80% and remained viable for 6 months in comparison to those stored at other temperature levels. The conidia

stored at 1 % Relative Humidity (RH) (silica gel) remained viable for more than 6 months with germination rate of 80%. Deterioration of conidia stored at 1% RH was slow ($^{r}{}_{L}$ = -0.41064) compared to those stored at 96.5% RH using K_2SO_4 saturated solution where the rate of deterioration was significantly fast ($^{r}{}_{L}$ = -0.73). Susceptibility of several sugarcane varieties towards *E. longirostratum* were tested using $1x10^7$/ml conidia concentration, and the results indicated that all sugarcane varieties tested were resistant to *E. longirostratum* as indicated by lower values of area under disease progress curve (AUDPC) and decreasing rates of disease progress. Sugarcane variety 95R-1004 was selected for the field trials as this variety was newly introduced to be planted at Federal Land Development Authorities (FELDA) in Chuping, Perlis.

In this study variety 95R-1004 and itch grass at 6-8 leaf stage were sprayed with 10^7,10^8 and 10^9 conidia/ml concentrations in 10% of oil emulsion. Plants were sprayed three times at interval of 1 week. The three applications treatment of *E. longirostratum* at 10^9 conidia/ml provided excellent control (100% mortality) of *R. cochinchinensis* compared to the other treatments as shown by higher AUDPC value (1168 units) and faster disease progress rate ($^{r}{}_{L}$ 0.61 unit/day). There was no significant difference in tiller numbers and the growth of sugarcane between the inoculated plots and the untreated control or plots treated with the fungus-free oil emulsion. The dry weight of itch grass was highest when treated with fungus-free oil emulsion (0.275kg) or untreated controls (0.290kg). None of the itch grass plants survived in plots treated with chemical herbicide (BASTA®), or plots with *E.*

longirostratum. The sugarcane dry weight was significantly high (1.6kg) in plots treated with *E. longirostratum* at 10^9 conidia/ml compared to the other treatments. This may be due to eradication of itch grass in this treatment earlier than in the other treatments, thus there was no competition between sugarcane and itch grass, resulting in a faster growth of the sugarcane plants. This research indicated that *E. longirostratum* has an ability to provide excellent control of *R. cochinchinensis* at 6-8 leaf stage under field conditions.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains Pertanian

PRODUKSI DAN KECEKAPAN *Exserohilum Longirostratum* SEBAGAI BIOHERBISID UNTUK RUMPUT MIANG (*Rottboellia Cochinchinensis*) DI KAWASAN PENANAMAN TEBU

Oleh

CHARLES BORROMEO ALESTER ANAK ABI

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Rottbolliea cochinchinensis atau rumput miang adalah antara rumpai yang sangat penting di Negara-Negara tropika. Pengurusan Rumpai Bersepadu ialah satu pilihan kaedah yang boleh dipadankan terhadap perladang persendirian, penanaman dan situasi rumpai. Kawalan biologi pula adalah salah satu opsyen dalam pengurusan ini, Oleh sebab itu, produksi dan kecekapan *Exserohilum longirostratum* sebagai pengawalan biologi rumpai ini di kawasan penanaman tebu telah pun diselidik. Pertumbuhan dan sporulasi *E. longirostratum* didapati sangat baik dalam konsentrasi media pada kadaran yang disyorkan (200ml V8juice + 800ml H_2O). Penghasilan dan percambahan konidia kulat ini pula didapati sesuai dalam kadar pH6 hingga pH7. Penyimpanan inokulum kulat ini dalam midium cecair (air dan minyak) didapati tidak sesuai di mana tiada percambahan konidia direkodkan. Walau

bagaimana pun, penyimpanan konidia kering udara dalam suhu 7^0C dan 20^0C mampu memberi paras percambahan yang kekal sehingga 80% selepas disimpan selama 6 bulan. Kelembapan relatif pada 1% yang dikekalkan oleh jeli silika (Silica Gel) merupakan keadaan yang terbaik untuk penyimpanan konidia kering dalam suhu bilik di mana tahap pertumbuhan mencapai 80% selepas 6 bulan. Kerosakan konidia dalam keadaan penyimpanan 1% kelembapan relative ($^r{}_L$ = -0.41064) juga sangat perlahan berbanding dengan penyimpanan dalam kelembapan tinggi (K_2SO_4 ; $^r{}_L$ = -0.73). Kepekaan dan kecederaan beberapa variati tebu terhadap kulat ini pada kosentrasi 1 x10^7/mL juga telah diperhatikan dan didapati, kesemua variati tebu resistan terhadap *E. Longirostratum* seperti yang ditunjukkan pada nilai Kawasan dibawah Keluk Perkembangan Penyakit (AUDPC) yang rendah. Walau bagaimana pun, varaiti 95R-1004 telah dipilih untuk kajian selanjutnya di lapangan kerana ia merupakan variati yang baru diperkenalkan untuk di tanam di FELDA Chuping.

Kajian keberkesanan dilapangan telah di jalankan keatas rumput miang dan tanaman tebu pada peringkat 6-8 daun dengan konsentrasi konidia *E. longirostratum* pada 10^7, 10^8, 10^9 dalam formulasi 10% minyak sawit dan 5% pelarut yang disembur pada selang seminggu setiap satu. Penyemburan tanaman dilakukan sebanyak 3 kali kekerapan dengan seminggu tempoh perantaraan memberi kesan pengawalan rumput miang yang sangat baik (100%) berbanding rawatan lain, dan ditujukan pada nilai AUDPC yang tinggi (1168^2) dan nilai perkembangan penyakit yang tinggi($^r{}_L$ = 0.619 unit/hari). Tidak terdapat perbezaan yang ketara pada pertumbuhan anak bilah dan perkembangan pertumbuhan tebu di antara plot yang dirawat dan plot yang bebas dari rawatan *E. longirostratum*. Berat kering rumput miang di dalam plot yang bebas rawatan didapati tinggi dan mempunyai perbezaan yang ketara berbanding di kawasan plot yang dirawat. Tiada berat kering rumput miang dapat direkodkan di kawasan rawatan *E. longirostratum* dan BASTA® kerana rumput miang di kawasan ini terhapus sama sekali. Walau bagaimanapun, berat kering tebu di kawasan rawatan *E. longirostratum* pada 10^9 konidia/mL didapati tinggi dan mempunyai perbezaan yang ketara berbanding kawasan rawatan lain dan kawasan bebas rawatan. Besar kemungkinan keadaan ini berlaku disebabkan rumpai miang terhapus lebih awal oleh rawatan *E. longirostratum* berbanding kawasan plot lain, maka tiada persaingan antara tebu dengan rumpai tersebut menyebabkan tebu tumbuh dengan cepat. Keputusan kajian ini menbuktikan *E. longirostratum* mempunyai keupayaan pengawalan yang sangat baik terhadap rumput miang pada tahap 6-8 daun di lapangan.

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This thesis submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Agriculture Sciences. The member the Supervisory Committee were as follows:

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DECLARATION

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

CHARLES BORROMEO ALESTER ANAK ABI

Date:

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LIST OF ABBREVIATION

m^2	Meter square
mm^2	Millimeter square
%	Percentage
PDA	Potato dextrose agar
cm	centimeter
^0C	Degree centigrade
mL	milliliter
µl	Microliter
SE	Standard error
^rL	Apparent rate values were obtain growth rate or epidemic rate by transforming percentage data using logistic model.
R2	Square of multiple correlation
Vol	Volume
DI	Disease index
∑	Sum
HR	Hypersensitive response
RH	Relative humidity
pH	Potential of Hydrogen
µ	Micro
rpm	Rotation per minute
SAS	Statistical Analysis System
w/v	Weight per volume
h	Hour
AUGC	Area Under Growth Curve
AUDPC	Area Under Disease Progress Curve
Kg	Kilogram
g	gram
P	Probability
NA	Not Applicable
a.i	Active Ingredients
ha	Hectare

CHAPTER 1

INTRODUCTION

Rottboellia cochinchinensis (Lour.) W.D. Clayton (Poaceae) or itch grass is an aggressive weed under various ecological conditions and has been labeled as a major agriculture weed in many areas of the tropics and subtropics infesting both annual and perennial crops. Its centre of origin was believed to be from Africa and Asia, but was introduced into the New World at the beginning of the century (Ellison and Evans, 1992). It is also a weed of bananas, cassava, citrus, cowpeas, papayas, groundnut, pineapple, rice, and sorghum in Cuba, Ghana, Jamaica, the Philippines, Trinidad and Venezuela but there is little reference to it in cereal crops in North American cereal. It is primarily a weed of warm-season crops in a variety of habitats around the world but can also be found growing along roadsides and in other open, well-drained sites and is an important species in old field succession (NAPPO Bulletin, 2003).

Reproduction of *R. cochinchinensis* is by seed. In the Philippines, the plant flowers all year long, and a single plant may produce 2000 -16000 seeds. Studies in Zimbabwe have shown that dense stands of the plant will produce over 600 kg of seed per season (Holm *et al.*, 1977). A single plant can produce thousand of seeds over one growing season, and densities of up to 500 plants /m^2 have been recorded (Pamplona and Mercado 1991).

Uncontrolled infestations of *R. cochinchinensis* were found to reduce yield of white food corn by about 50% (Fisher *et al.*, 1985). In addition to its effect on crop yield, *R. cochinchinensis* is a problem to laborers, as the needle-like hairs on the leaf sheaths break off in the skin and can cause painful infections. It is also an alternative host of viruses causing corn leaf gall and rice leaf gall (Agati and Calica 1949). In Malaysia *R. cochinchinensis* causes yield losses of 60% in sugarcane yield. (Tan Teck Nee, 2004; per. comm)

Land preparation and crop rotation can be the key for achieving good cultural control. Manual control also has been practiced in several countries but the cost of labour became a limitation (Chan *et. al*.,1990). Chemical herbicides can give satisfactory kill of the weed, but cost (of both product and application) and increasing incidence of herbicides resistance has become the constrains. Most are not selective enough for use on the graminaceous crops, which are mostly associated with this weed. The chemical does not persist long enough in the soil to give control of the succeeding flushes of the seedlings. Therefore, further studies on other alternate control are needed to overcome this troublesome weed. One such alternative is the use of plant pathogens which is often referred to as bioherbicides. Bioherbicides offer the possibility of an inexpensive and environmentally benign means of weed control through the utilisation of living organism to control or reduce the population of an undesirable weed. The most important characteristics of a bioherbicide are: easy to mass produced *in vitro*, high virulence, genetic stability and restricted host range. In addition, fungi are capable of active penetration of host tissue and infection is not dependent on vectors, natural openings or wounds, which are required by bacterial and viral pathogens. Thus, facultative fungal pathogens are the best candidates for spray application.

Isolates of fungi collected from *R. cochinchinensis* have been screened for host specificity and three of them were selected for further studies as potential biological control agents. An isolate of *Colletotrichum* sp. from Thailand was tested in the laboratory and in field trials as a possible candidate for development as a mycoherbicide. The results were equivocal but a synergistic response was found when a low dose of chemical herbicide was added to the fungal inoculum (Ellison and Evans, 1993). Rust, *Puccinia rottboelliae*, causes severe seedling infection in the field and preliminary host-range tests with an isolate from Kenya suggest that it is specific to *R. cochinchinensis*. Thus, this rust may have potential as a classical biological control agent in the Americas, perhaps involving a management strategy including early-season augmentation (Ellison, 1993). Investigations on *Sporisorium ophiuri*, a systemic head smut, as a possible classical biological control agent, suggested that it may have limited potential because of high inoculum requirements; however, as the sporidia are readily produced in culture, it may be possible to apply them to the soil as a form of mycoherbicide (Reeder *et al.,* 1999, Ellison and Evans, 1993). One of the problems associated with *S. ophiuri*, is that it has only one disease cycle a year and consequently, it has a slow intrinsic rate of spread within a population of *R. cochinchinensis*. The other problem is it has very narrow infection window that is it only infects *R. cochinchinensis* at flowering stage. Seeds vigor may be reduced, however, this weed is also capable of generating through rattons, and an infection of the seeds has little bearing on the dispersal and survival of this weed.

A C*urvulari*a sp. has been isolated from Trinidad and has been proven to be highly damaging to *R. cochinchinensis*, while not damaging to rice, sugarcane or pearl millet (Evans, 1999). It was able to kill *R. cochinchinensis* in a few days; however it has a wide host range including maize (Ellison, 1992). Surprisingly few insects have been recorded attacking *R. cochinchinensis* and only one unidentified gall midge was recorded in India from *R. compressa* (Barnes, 1946). In East Africa a stem borer, a lepidopteran leaf feeder and fly larva all proved to be non-specific graminaceous feeder (Evans, 1999). In Malaysia, Azean (2004) has reported the potential of *Exserohilum longirostratum* as bioherbicide for controlling this weed and this fungus can be produced on V8-juice agar with a 12h light cycle. However, the development of this pathogen as a bioherbicide requires that various limitations in biological, technological, environmental, and economic aspects to be solved. For example, effective methods to lower the cost of mass production and formulation to improve the efficacy of the pathogen are needed. Extensive studies are needed to develop methods of inoculum production and to identify additives that prolong inoculum viability. Further studies are required to confirm efficacy of this bioherbicide in commercial fields.