UNIVERSITI PUTRA MALAYSIA

POTENTIAL OF EXSEROHILUM LONGIROSTRATUM BIOHERBICIDE FOR ROTTBOELLIA COCHINCHINENSIS

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FP 2004 1

POTENTIAL OF *EXSEROHILUM LONGIROSTRATUM* AS BIOHERBICIDE FOR *ROTTBOELLIA COCHINCHINENSIS*

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 2004

BUAT.......

MAK, AYAH, ADIB, AMIN.....

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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Chairperson : Associate Professor Jugah Kadir, Ph.D.

Faculty : Agriculture

Development of *Exserohilum longirostratum* as a potential bioherbicide for controlling itchgrass (*Rottboellia cochinchinensis*) was investigated in this study. An isolate of indigenous fungus *E. longirostratum* was isolated from diseased *R. cochinchinensis* in Serdang, Selangor and was evaluated in the laboratory and greenhouse as a potential bioherbicide. This fungus was found to be highly pathogenic to *R. cochinchinensis* when the seedlings were inoculated with 3.5 X 10^5 conidia/ml. The disease symptom appeared 24 h after inoculation as discrete eyespot symptoms with watery dark border, which was eventually associated 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 Potato dextrose agar (PDA) and V8 agar with optimum temperature for growth of 28°C. Although most of *Exserohilum* spp were reported as host to member of Poaceae, but *E.longirostratum* has a narrow host range, which include several weedy grass. Corn, rice and sugarcane showed resistant reaction while dicots were immune. The pathogen penetrated plant surfaces by direct penetration through formation of appressoria on surfaces of *R. cochinchinensis* 8 h post inoculation. The appresorium 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. Extensive secondary hyhpae were produced within 32 h on *R. cochinchinensis* leaves, thus indicating that the fungus was able to establish parasitic relationship with the host. On corn leaves, the fungus grew and penetrate the leaf surface. The fungus did not produce extensive hyphae in corn tissue but were compartmentalized at the point of infection indicating resistant reaction. The fungus grew on bean leaves but could not penetrate the cell wall on bean as indicated by lysing of the conidia and germs tubes 8 h post inoculation. The inability of the germinating conidia to penetrate and to progress indicated that bean is not a compatible host for this fungus. The level of disease severity on *R. cochinchinensis* was linearly related to the conidial concentration of *E. longirostratum* with conidia concentration higher than 10^4 conidia per mililiter resulted in 100% control of the seedlings. The most susceptible age of *R. cochinchinensis* were 2- to 8- leaf stage. *E. longirostratum*, required a minimum of 8 h of dew to infect *R. cochinchinensis*. Such long dew duration could be constraint to the use of this bioherbicide in the field. However, this constraint may be circumvented by adding amendments to the formulation. Thus, the potential of *E. longirostratum* to be used as a bioherbicide to control *R. cochinchinensis* was demonstrated.

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

POTENSI *EXSEROHILUM LONGIROSTRATUM* SEBAGAI BIOHERBISID UNTUK *ROTTBOELLIA COCHINCHINENSIS*

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Kajian memajukan *Exserohilum longirostratum* sebagai bioherbisid berpotensi untuk mengawal rumpai 'itchgrass' (*Rottboellia cochinchinensis*) telah dijalankan. Pemecilan kulat dilakukan dari sampel yang diperolehi dari *R. cochinchinensis* yang mempunyai simptom penyakit di kawasan Serdang, Selangor. Tahap kepatogenan *E. Longirostratum* telah diuji di makmal dan di rumah kaca. Keputusan kajian mendapati kulat ini memberi kesan kepatogenan yang paling tinggi pada rumpai *R. cochinchinensis* apabila diinokulat dengan 3.5 X 10^5 konidia/ml. Simptom kelihatan seperti berbintik kecil berwarna hitam berair pada permukaan daun selepas 24 jam diinokulat. Lesi didapati tidak bercantum tetapi kesemua daun pokok menjadi nekrotik

dan akhirnya mati. Pertumbuhan dan perkembangan kulat ini didapati lebih sesuai di atas media Potato dextrose agar (PDA) dan V8 agar. Suhu optimum pertumbuhan kulat ini ialah 28°C. Walaupun, kebanyakan spesies *Exserohilum* dilaporkan menjadi perumah kepada keluarga 'Poaceae', tetapi *E. longirostratum* didapati mempunyai julat perumah yang agak terhad kepada beberapa spesies rumpai daun tirus. Kesannya terhadap tanaman jagung, padi dan tebu menunjukkan tindak balas resistan tetapi tumbuhan dikot tidak dijangkiti oleh kulat ini. *E. longirostratum* menembusi permukaaan daun secara terus menerusi pembentukan appresorium di atas permukaan daun *R. cochinchinensis* selepas 8 jam inokulasi. Kebiasaannya appresorium berbentuk bulat atau silinder yang menghasilkan hifa skunder dihujungnya. Kulat patogen menembusi dinding sel kutikel dan tumbuh di sebelah luar dan dalam sel tisu. Pengeluaran hifa sekunder di atas permukaan daun *R. cochinchinensis* selepas 32 jam diinokulasi menunjukkan kulat ini mempunyai hubungan parasitik dengan perumah. Di atas permukaan daun jagung pula, kulat ini tumbuh dan menembusi permukaan daun tetapi perkembangan kulat yang terhad di kawasan inokulasi menyebabkan hifa skunder tidak dihasilkan. Kulat ini tumbuh di atas permukaan daun kacang tetapi konidia dan tiub cambahnya mengecut menyebabkan kegagalan untuk menembusi dinding sel selepas 8 jam inokulasi. Ini menunjukkan kacang bukanlah perumah yang sesuai untuk kulat ini. Paras keterukan penyakit pada daun *R. cochinchinensis* adalah berkadar terus dengan konsentrasi konidia *E. longirostratum*. Konsentrasi konidia yang melebihi 10^4 konidia/ mililiter boleh menyebabkan kematian seratus peratus anak benih. Anak benih *R. cochinchinensis* yang mempunyai 2 hingga 8 helai daun sangat rentan terhadap jangkitan *E. longirostratum.* Kulat ini memerlukan sekurang-kurangnya 8 jam kelembapan untuk menghasilkan kawalan yang dikehendaki dan ini menjadi masalah jika *E. longirostratum* diguna di lapangan. Walau bagaimanapun, masalah keperluan kelembapan di lapangan boleh dielakkan dengan 'amendments' di dalam formulasi. Hasil dari kajian ini dapatlah dirumuskan *E. longirostratum* berpotensi sebagai satu bioherbisid untuk mengawal *R. cochinchinensis*.

ACKNOWLEDGMENTS

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

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

I would like to thank Ujei, Charles, Hasraena, Adam, En. Ariffin and Along for their help and moral support.

A million thanks to my family especially to my father, mother, brother and sister for their love, prayer, support and understanding. Last but not least, thanks to my beloved sweetheart, Nuhlan Ashady for his concern and love.

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LIST OF ABBREVIATIONS

m^2 =Meter square
% = Percentage
PDA = Potato dextrose agar
mm^2 = Millimeter square
cm = Centimeter
°C = Degree celcius
$\mu E/m^2$ = Micro Eustine / meter square
ml =Milliliter
CMA = Corn meal agar
µm =Micromolar
µl = Microliter
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
C = Carbon
N = Nitrogen
Vol = Volume
DI = Disease Index
∑ = Sum
HR = Hypersensitive response
M = Mortality
pH = Potential of Hidrogen
µ = Micro
rpm = Rotation per minit
SAS = Statistical Analysis System
w/v = Weight per volume
h = Hour
AUDPC= Area Under Disease Progress Curve
Kg = Kilogram
g = Gram
P = Probability
NA = Not Applicable
diam = Diameter
a.i/ha = Active ingredient / hectare
Co_2 = Carbon dioxide

CHAPTER 1

INTRODUCTION

Rottboellia cochinchinensis (Lour.) W.D. Clayton (Poaceae) or itch grass is 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, 1995). It is an extremely variable species and numerous ecotypes exist that are adapted to specific crops or locations (Pamplona and Mercado 1981a,b, 1982).

This weed is disseminated by a single plant, which can produce thousand of seeds over one growing season, and densities of up to 500 plants /m^2 have been recorded (Pamplona and Mercado 1982). In Malaysia *R. cochinchinensis* was first reported in sugarcane plantation in the Northern States and is now reported in almost every state in west Malaysia and most recently, it was reported to encroach paddy fields (Mislamah, 2000; pers. comm). The presence of this weed in agro ecosystem has been reported to cause high losses in term of yield and management cost of this weed.

The method of controlling this weed is labour intensive in which the *R. cochinchinensis* populations are manually controlled. Chemical herbicides can give satisfactory kill of the weed, but financial 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. Alternative control method needs to be formulated to control this weed. One such alternative is the use of plant pathogen which is often referred to as bioherbicide. Bioherbicide offers 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 bioherbicide are easy to mass produced *in vitro*, high virulence, genetic stability and restricted host range. In addition, fungi are capable for 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.

Fungi are the only pathogens of *R. cochinchinensis* which have been surveyed and their specificity are being examined in a joint International Institute of Biological Control and Long Ashton Research Station project covering East Africa, South America, India, Nepal, Sri Lanka and Thailand (Ellison, 1992, Ellison and Evans, 1990, 1993, Evans, 1991, Natural Resources Institute, 1992). One of the fungal pathogens that shows potential to be used as biological control agent is *Sporisorium ophiuri* (P.Henn.) Vanky (Ustilaginales). *S. ophiuri* is recorded as occurring in East Africa, Sri Lanka, Philippines and Thailand, but apparently not in the Americas and current research indicates that *S. ophiuri* is a potential agent for controlling this weed in the America. It is often locally damaging, significantly reducing vigour and virtually eliminating seeding. It host specificity is under detailed investigation (Ellison and Evans, 1993, Evans, 1991) as a potential candidate for classical biological control for areas where it does not occur. In an annual weed where seeds are the only means of propagation, a destructive seed head pathogen, such as *S. ophiuri*, is a highly promising biological control agent (Evans, 1991).

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*. Since *S. ophiuri* is soilborne, it may be potential to be utilized as a classical biological control agent. The other problem is it has very narrow infection window that is it only infects *R. cochinchinensis* at flowering stages. 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 *Curvularia* 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, 1991). 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, 1991).

Although the development of bioherbicides opens a new avenue for biological weed control but there are problems associated with bioherbicide approach. Templeton et al. (1979) list those related to the biology and ecology of pathogens such as spore formation, spore dormancy and the long incubation period of fungi, host plant tolerance and resistance and the generally narrow environmental requirements for infection. The fact that under field conditions the specific humidity and temperature requirement for spore germination and host tissue penetration often cannot be met during the period of application is a major obstacle preventing the use of bioherbicides. However, the problem of specific humidity requirement can be over come with various amendments (Kadir and Charudattan, 2000; Shabana et al., 1997).

Another problem associated with pathogen biology is that the precise conditions for optimal sporulation are still unknown for the majority of fungal pathogens. It is therefore of importance to promote investigations of the basic mechanisms is regulation of the growth and sporulation of fungal pathogens.

Although the cost for the development and registration of mycoherbicides is considerably less than that of a chemical herbicide, private industry will necessarily be preoccupied by market size, return on investments and profits. Therefore, only pathogens with the capacity to solve significant weeds problems, those effective against important herbicide resistant weeds or those for the control of which no chemical herbicide is available, are suitable candidates for bioherbicides development. Biological weed control has a future and has a contribution to make to an economic and ecologically favourable weed control.

Therefore the general objectives of this study are:

a) To determine the potential of *E. longirostratum* as bioherbicide for controlling *R. cochinchinensis*, pathogenicity, host range and spore production.

b) To determine epidemiological factors affecting disease development.

c) To study host pathogen interaction

CHAPTER II

LITERATURE REVIEW

Natural enemies invariably attack plants in their native range when physical and biotic factors are favorable, however when plants are introduced into another habitats, their natural enemies are generally left behind. Introduced plants (non-native plant species) not accompanied by their natural enemies may increase and become invasive species than they were in their native range. They may spread aggressively and become weeds on land devoted to agriculture, forestry, and grazing or recreational activities and in urban parks and garden. Human and natural disturbance that remove native vegetation also allow for the establishment of invasive species in natural communities (Harley and Forno, 1943).

Although most weeds have high population densities, some plants adversely affect mankind at quite low densities when human activities alter the environment so that the natural balance is disrupted. Some native plants may become weeds (DeLoach, 1981). Thus a weed may be either an introduced or native plant that is growing in a situation where it has detrimental effect on mankind, or on his environment. The economic impact of weeds consists of lost revenue (losses) and costs. In the agricultural sector, losses result from reduction on yield and quality caused by weeds. Inputs or costs accrue as a result of herbicide use and the employment of tillage, mowing, and cultural and biological inputs for weed management and control (Bridges, 1999). The environmental impact of non-native plant weeds results from the invasion