Potential of *Exserohilum longirostratum* As Bioherbicide For Itchgrass

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Introduction

Rottboellia cochinchinensis or itchgrass is a major agriculture weed in many areas of the tropics and subtropics infesting both annual and perennial crops (4,5). Itchgrass was first reported in Malaysia in sugarcane plantation in the Northern States and is now reported in almost every state in west Malaysia. The presence of this weed in agro ecosystem has been reported to cause high losses in term of yield and management cost.

The method of controlling this weed is labour intensive in which the itchgrass populations are manually hand weed. Chemical herbicides cannot give satisfactory kill of the weed, as most are not selective enough for use on the graminaceous crops, which are mostly associated with this weed. Alternative control method needs to be formulated and one such alternative is the use of plant pathogen. The potential of indigenous fungal pathogens as biological agents for this weed and other weeds has not been realized in Malaysia. The present study is undertaken to investigate the potential of *E. longirostratum* as bioherbicide for *R. cochichinensis*.

Materials and Methods

Pathogenicity and host range testing were done on the 4 leaf-stage seedlings. The plants were held at about 100% relative humidity by spraying water before inoculation. Host range testing was done based on the scheme developed by Wasphere (6). The aim was to select plant species that were potential hosts of the organism in question. The plants were inoculated with 10 ml of the conidia suspension containing 0.2 % Pulse (non ionic surfactant) and *Exserohilum* conidia at the rates of 3.5×10^5 conidia/ml. A control consisting of plants sprayed with 0.2% Pulse were included. Free moisture requirement were established by inoculation 4 leaf-stage seedlings with 3.5×10^5 conidia/ml. Inoculated plants were covered with plastic bags to maintain the required leaf wetness periods. The effect of inoculum levels were studied by inoculating 4 leaf-stage seedlings with different inoculum concentration starting from 1×10^4 to 1×10^7 conidia/ml. Disease assessment was done daily based on the disease severity scale. Inoculated seedling of itchgrass, corn, and bean were examined for conidial germination, germ-tube, appressorial formation, and extend of fungal-host interaction with light and scanning electron microscopy.

Results

An indigenous isolate of *Exserohilum longirostratum* from diseased itchgrass was determined in the glass house as a potential bioherbicide for itchgrass. On susceptible itchgrass, symptoms started initially as gray lesions with watery border. The infected seedlings died 3-4 days post inoculation. *E. longirostratum* did not completely kill the older plants.

The host range of this pathogen is not specific to itchgrass but other grassy weeds are also susceptible. Plants of economic importance tested including crop plants in Poaceae family are either resistance as indicated by the presence of non-coalescing lesions with reddish border or are immune to *E. longisrostratum*.

E. longirostratum germinated on soybean but the germinating spores and germ tubes lysed before penetration. The germination process and pre-penetration structures of E. longirostratum was similar on leaf surface of itchgrass and corn except that very few appresoria were produced on corn. Conidia started to germinate 4 h after inoculation by producing a single germ tube, which always emerged from the end of the conidia. On itchgrass germ tubes differentiated at their tip into simple, globosely to spherical appresoria. The first sign of penetration on itchgrass were observed 8 h after inoculation. The level of disease severity on itchgrass was linearly related to the conidial concentration of *E. longirostratum*. The minimum conidia concentration required for 100 % control of itchgrass seedlings was 1×10^5 conidia/ml. The most susceptible growth stages of itchgrass was between 2 to 8 leaf- stage, which is within the application time of conventional post emergence chemical herbicides. E. longirostratum required a minimum of 8 h of leaf wetness period to cause serious infection of itchgrass.

Discussion

The potential of *E. longirostratum* as bioherbicide for controlling itchgrass was confirmed in the repeated greenhouse trials (1,2,3). The ability of *E. longirostratum* to induce severe infection with in 24 h after inoculation provides supporting evidence that this pathogen has the potential to be developed as a bioherbicide. The symptoms which include burnt-like appearance of the infected leaves and the speed of plant killed indicated that phytotoxins as the mode of action as most of the species in the *Helminthosporium* group were reported to produce phytotoxins which were important in plant pathogenesis(7). Host range of this pathogen was not only confined to itchgrass, but several weedy grasses. It did not cause severe disease on important Poaceae family, such as rice, sugarcane, and corn, or any other crop plants. Thus it would be safe to use this fungus as a bioherbicide in cropping situations. Histopathological studies of infection indicated parasitic relationship occurred only in the host plants and latent infection on the non host plants did not occur. This confirms the specificity and safety of this fungus. Presently, there is no bioherbicide available to control this weed. Therefore, this fungus could be commercially developed to control this weed and other grassy weeds in crop plants.

Conclusions

E. longirostratum has the potential to be developed as bioherbicide for itchgrass. The ability of this fungus to cause severe disease on susceptible host depended on the phenological growth stages. This fungus caused 100 % mortality on the younger seedlings but was only able to cause about 90 % disease severity on matured plants. In host-specificity tests, *E. longirostratum* was found to be highly pathogenic on itchgrass, other closely related grassy weeds were also susceptible. It produced small necrotic lesions on crop plants such as rice, sugar cane and corn. The timing of the germination process and morphology of pre-penetration structures were similar on itchgrass and corn, however, level of penetration was higher on itchgrass. The fungus penetrated plant cuticles directly through formation of appressoria 8 h post inoculation. Development of primary hyphae and secondary hypae was extensive on itchgrass. On corn leaves (considered as resistant), the fungus grew and penetrated the leaf surface but appresorium was not formed, and the fungal colonization was restricted to initial infection sites. The fungus grew on bean leaves but could not penetrate the cell wall on bean as indicated by lysing of the germinated conidia 8 h post inoculation. The inability of the germinating conidia to penetrate and to progress indicated that the bean is not a compatible host for this fungus. The ability to cause seedlings mortality may be associated with production of phytotoxin.

Benefits from the study

This product if developed may be able to be used as a sole weed control strategy, or it may be augmented the other existing strategies. The photytoxin that may be produced if found specific to weedy garasses, may be utilized as building block for novel biopesticide. This will cut down our dependent on chemical herbicides, and thus reduce problems associated with chemical herbicides.

Patent(s), if applicable: :

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals

Kadir, J, and Ahmad, A. **2003.** Histological study of host-pathogen interaction of *Exserohilum longirostratum* with susceptible and resistant plants. Submitted to **Biological Control.**

2. Kadir, J., Ahmad, A, Sariah, M., and Juraimi, A.S. 2003. Potential of Exserohilum longirostratum as bioherbicide for itchgrass (*Rottboellia cochinensis* (Lour) W.D. Clayton (Poaceae). Submitted to **Pertanika**

3. Kadir, J., Ahmad, A, Sariah, M., and Juraimi, A.S. 2003. Effects of growth stages, spore concentrations and leaf wetness duration on itchgrass control efficacy of *E. longirostratum*.. Submitted to **Weed Science.**

Project Publications in Conference Proceedings

1. Ahmad, A., Kadir, J., Sariah, M., and Juraimi, S. 2002. Evaluation of *Drechsclera* sp. for potential bioherbicide control of itchgrass (*Rottboellia cochinensis* (Lour) W.D. Clayton (Poaceae): Screening and hosts range test. In Biopesticides: Positioning Biopesticides in Pest Management Systems, Mulla, M.S. ed., pp 181-184.

2. Kadir, J., Ahmad, A, Sariah, M., and Juraimi, A.S. 2003. Conidial Germination and infection procsess of *Exserohilum longirostratum*: Potential bioherbicide for itchgrass. In Proceedings 9th Asian_Pacific Weed Science Society Conference, 17-21 March 2003 Manila, Philippines, pg. 433-437.

3. Kadir, J., Ahmad, A, Sariah, M., and Juraimi, A.S. 2003. Potential of *Exserohilum longirostratum* as bioherbicide for itchgrass (*Rottboellia cochinchinensis*). In Proceedings 9th Asian_Pacific Weed Science Society Conference, 17-21 March 2003 Manila, Philippines, pg. 450-455.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Azean bt. Ahmad	Potential of exserohilum longirostratum as bioherbicide for itchgrass	Biological control of weeds	M. Agric. Sc	Will be (2003)

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