Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 6 (1): 21-28, 2007
ISSN 1682-3974

Fungal Pathogen of *Rottboellia cochinchinensis* and its Potential as Bioherbicide

[1]Jugah B. Kadir, [2]A. Ahmad, [1]M. Sariah and [3]A.S. Juraimi
[1]Department of Plant Protection, Faculty of Agriculture,
Universiti Putra Malaysia, 43400 Serdang, Selangor,
[2]Department of Agriculture,
[3]Department of Crop Science, Faculty of Agriculture,
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Abstract: The possibility of using an indigenous plant pathogen to control itchgrass (*Rottboellia cochichinensis*) was investigated in this study. A fungal pathogen of itchgrass, *Exserohilum longirostratum*, was determined in the laboratory and greenhouse as a potential bioherbicide. The disease symptoms on inoculated plants appeared 24 h post inoculation as discrete spot with watery dark border, eventually expand and cause extensive necrosis on the leaves, resulted in burnt-like symptom. When applied as a post emergence foliar spray, the fungus inflicted high percentage of mortality to young itch grass seedlings It did not killed older plants but was capable of reducing biomass by about 56% when the plants were inoculated with 3.5×10^5 conidia mL^{-1}. Media containing carbon: nitrogen ratio of 10:1 as in V8 juice agar and PDA produced more conidia compared to medium (CMA) containing carbon:nitrogen ratio of 40:1. Light and temperature had major influence on fungal sporulation, exposing the fungus to longer duration (12 h) of light significantly increases conidia production. The optimal temperature for growth and sporulation of this fungus is in the range of 25 to 30°C.

Key words: *Exserohilum longirostratum*, *Rottboellia cochinchinensis*, bioherbicide, itchgrass

INTRODUCTION

Itchgrass (*Rottboellia cochinchinensis* (Lour.) W.D. Clayton) is an important weed in most major crops of the tropic and subtropics (Holm *et al*., 1977). Its ability to grow fast coupled with its spreading habits makes it a vigorous competitor with crops. Besides becoming weeds in crops, this weed becomes problematic due to the presence of irritating hairs on its leaf sheath and blades, which are easily detached on contact making it unpalatable for grazing animals and causing painful infection to workers. It was first reported in a sugar plantation in Malaysia in 1980. Detection of this weed at the very young stage was not possible, as it resembles sugar cane. By 1985, about 80 ha of the sugarcane planting had this weed problem (Chan *et al*., 1990). Presently, this weed has been reported infesting sugarcane and rice fields and is continuing to pose threat to other crops. Base on the recent observation, this weed has spread to almost all the states in Peninsular Malaysia.

Manual removal, mechanical cultivation, cultural practices, or chemical herbicides can control this weed; however, use of physical and/or chemical methods of weed control alone is not feasible, or sufficient in every situation. This creates the opportunity for the use of biological control agent as an alternative for weed control. The use of an inundative method, also called bioherbicide strategy, where an organism is applied to achieve rapid reduction in weed populations, has proven successful in some instances. The discovery and development of host-specific fungal pathogens as bioherbicides has been proposed to provide a nonchemical option to manage this weed. Currently, *Sporisorium ophiuri* (P. Henn). Vanky (Ustilaginales), was reported as a potential biological control agent (Ellison and Evan, 1990) for this weed. This pathogen is an obligate parasite and has only one disease cycle per year. Since this pathogen is not reported in Malaysia, its potential as biological control agent is still doubtful. Although a number of pathogens have been found to occur *on R. cohinchinensis*, there have been no reports on bioherbicides that are registered for the control of *R. cochinchinensis* at present. The objectives of this study were to determine the bioherbicide potential of the *Exserohilum longirostratum* for controlling *R. cohinchinensis* and to determine the optimum condition for growth and sporulation of this pathogen.

Corresponding Author: Jugah Kadir, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

MATERIALS AND METHODS

Isolation and identification: Diseased leaves of *R. cochichinensis* were collected from various locations in Malaysia and were placed in paper bags inside plastic bags and kept in the refrigerator. The leaf pieces were cut into 4 mm^2; surface sterilized with 0.5% sodium hypochlorite solution, rinsed twice with sterile water and plated either on 1% water agar or incubated on wet filter paper (Kadir and Charudattan, 2000). After 3 days of incubation, fungi cultures displaying characteristics of *Exserohilum* sp. were transferred to fresh potato dextrose agar (PDA; Difco, Detroit, MI). Pure cultures of the recovered fungi were prepared from single conidium and maintained on half strength PDA slants as stock cultures. The isolated fungi were identified to the genus based on their conidial morphology and growth characteristics on various growing media. Further identification and characterization were done on isolate that was confirmed to be highly pathogenic to *R. cochichinensis* by applying Koch's postulates. Cultures grown on PDA as well as sporulating on infected plants in the greenhouse were used for taxonomic characterization of the conidia.

Leaves from plants naturally infected by *R. cochichinensis* in the field were also included to determine the variability in conidia shapes and sizes. Infected leaves were sterilized in 5% sodium hypochlorite and incubated on moist filter paper in petri plates for two days. The conidia and conidiophores were scraped from these leaves with a scalpel and transferred onto glass slides to be viewed under the microscope. The measurements were taken by using a calibrated ocular micrometer by counting 100 conidia and conidiophores from each plate or infected leaf.

Pathogenicity testing

Plant production: The itchgrass plants used in this experiment were grown from seeds collected from a farm in University Putra Malaysia. The plants were grown to uniform growth stage by planting the seeds in 10% Vitagrow® solution {Vitagrow (Fertilisers) Limited, PO Box161, Southport Lancashire, PR9 8GH, UK}. When the seedlings emerged, they were transplanted to 10 cm-diam ×10.6 cm-tall plastic pots containing mixture of commercial soil conditioner (KOSAS peat) and top soil (3:1; vol:vol). Five plants were grown in each pot. The pots were kept on a greenhouse bench at a light intensity of 400 $\mu E\ s^{-1}\ m^{-2}$ at midday under nonlimiting conditions of water and nutrients.

Inoculum production: A small mycelial plug from a stock culture was aseptically transferred to fresh modified V8 agar (200 mL V8 juice, 800 mL water, 14 g agar; Dinghra and Sinclair, 1995). The plates were incubated for 1-2 days (25°C, 12 h/12 h light/dark, 35±5 $\mu E\ m^{-2}$) until adequate colony growth was observed. Mycelial plugs from the margins of a young growing colony were transferred to fresh plates of V8 agar (6 plugs per plate). The plates were incubated again for 6 days. To harvest the conidia, the agar plates were flooded with 10 mL distilled water and the spores were scraped off from the plates with a rubber spatula. The resulting conidial suspensions were passed through a single layer of cheesecloth. The conidia were counted using Brite-Line phase contrast hemacytometer (Reichert Scientific Instruments, Buffalo, N.Y., USA) and the spore suspension was adjusted to the desired concentration by dilution in water.

Plant inoculation: The 4 leaf stage seedlings were held at about 100% relative humidity by spraying with water. The seedlings were subsequently inoculated with a conidial suspension (3.5×10^5 spores mL^{-1}) containing 5.0% Maxigreen® (nonionic surfactant plus sticker agent; Saleswide Sdn. Bhd., Puching, Kuala Lumpur). Each pot received about 10 mL of the conidia suspension, which is translated to about 5.2 million spores per pot. Maxigreen® at concentration of 5.0% was use as additive to maintain uniform conidia distribution on the leaves and as surfactant to break the protective layers found on the leaves. Inoculated plants were then covered with polyethylene bags for 24 h to maintain humidity. The bags were then removed. The pots were arranged randomly in the greenhouse with temperature of 32±2°C. A control consisting of plants sprayed with 5.0% Maxigreen® was included. Disease incidence and disease severity were recorded every day for 7 days after inoculation using methods of Kadir and Charudattan (2000). Diseased leaves were collected and the fungus was re-isolated from symptomatic lesions to confirm Koch's postulate.

Disease assessment: Disease assessment was base on the number of plants affected among the total plants inoculated (disease incidence), expressed as the percentage of disease plants (Horsefall and Cowling, 1978; James, 1974; Kranz, 1988) and plant reaction to disease based on disease severity (area of plant tissue that is diseased (Kranz, 1988)).

Disease progress was assessed on the inoculated plants in each pot by estimating the disease development. The latter was expressed as disease severity using disease severity numerical rating where :0 = healthy; 1 = 10% disease severity; 2 = 20% disease severity; 3 = 30% disease severity; 4 = 40% disease severity; 5 = 50% disease severity; 6 = 60% disease severity; 7 = 70% disease severity; 8 = 80% disease severity; 9 = 90% disease severity and 10 = plant death.

Effect of light and culture media on growth and sporulation of the isolate: A 5 mm agar plug of the isolate obtained from a 5 day old culture was placed in the center of each PDA, V8 juice agar and Corn Meal Agar (CMA;

Oxoid, Unipath Ltd., Basingstoke, Hampshire, England). The plates were sealed with parafilm and were incubated in the incubator at 30°C. The plates were exposed to 4, 8, 12 and 24 h of light, which was supplied by the florescence lamp (35±5 μE m^{-2}). The light duration was controlled by a 24 h regiment time controller (Theben-Werk Zeitantomatik Gmoh, Hohenbergstrabe 32, D-72401 Heigerloch, Germany). A control consisting of plates exposed to 24 h of darkness were included. Radial growth of the colony was measured using a caliper every day for 7 days. Conidia production was assessed 14 days after media inoculation. The conidia were harvested by flooding the surface of the media with 10 mL sterile water and another 5 mL sterile water to wash the remaining conidia from the surface of the media. The conidia were counted using Brite-Line phase contrast hemacytometer (Reichert Scientific Instruments, Buffalo, N.Y., USA).

Effect of temperature on fungal growth sporulation: A 5 mm plug was taken from the margin of an actively growing isolate and was inoculated on PDA plates, V8 juice agar and CMA agar. The plugs were placed in the centre of each petri plate and the plates were sealed with parafilm. After inoculation, the plates were incubated in the incubators at various temperatures: 15, 20, 25, 30 and 35°C. The radial growth of the isolate was measured using caliper every day for 7 days. Conidia production was assessed as mentioned earlier and the conidia were counted using hemacytometer.

Data analysis: All experiments were performed twice using a completely randomized design with four replications. All percentage data were transformed by arcsine transformation before analysis (Gomez and Gomez, 1984). Data from both trials were pooled if a test of homogeneity of variance by Bartlett test (Gomez and Gomez, 1984) justified such pooling. Data were subjected the standard SAS procedure (SAS Institute, Cary, NC). Means separation were done if treatments showed significant differences.

RESULTS

Identification and characterization: Few genera of fungi were consistently isolated from the diseased *R. cochinchinensis*, however, only one fungus fulfilled the Koch's postulate. The fungus was highly pathogenic on diseased *R. cochinchinensis*. The symptoms produced on *R. cochinchinensis* when inoculated with this fungus under green house conditions were similar to those seen in the filed. The conidiophore emerged through the epidermal cells, singly, 0-1 septate and was olivaceous brown. The conidia were borne terminally and intercalary. The conidia were olivaceous brown, broadest around the basal area, narrowing towards the apex into a long beak, ends rounded, end cells often cut-off by dark, thick, septum often referred to as hilum (Fig. 1). The conidia consisted of 10-26 disto-septate and measured 97.8-340.6 X 11.2-20 μm (Table 1).

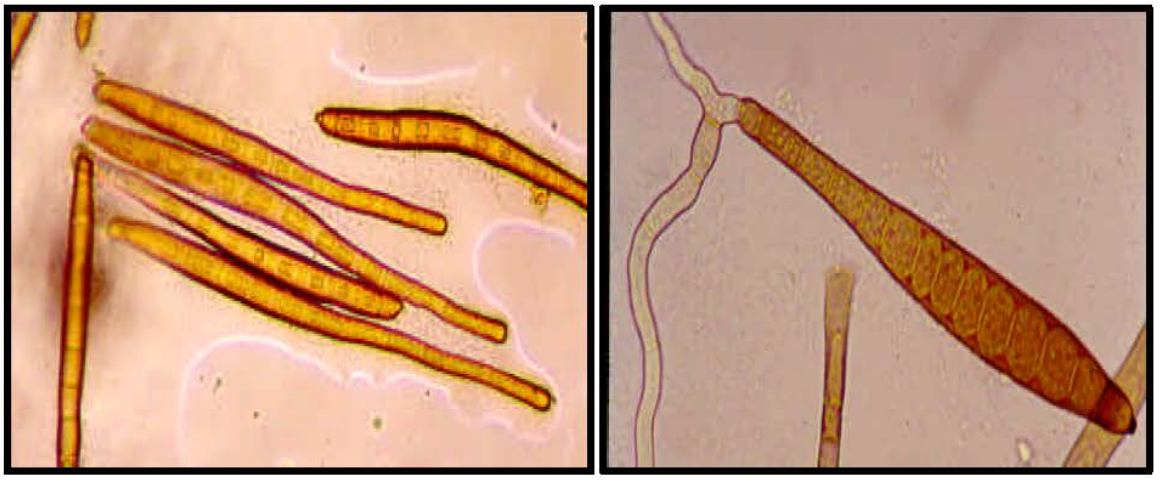

Fig. 1: Morphology of the conidia (A) and germination pattern of conidia (B) of *E. longirostratum*

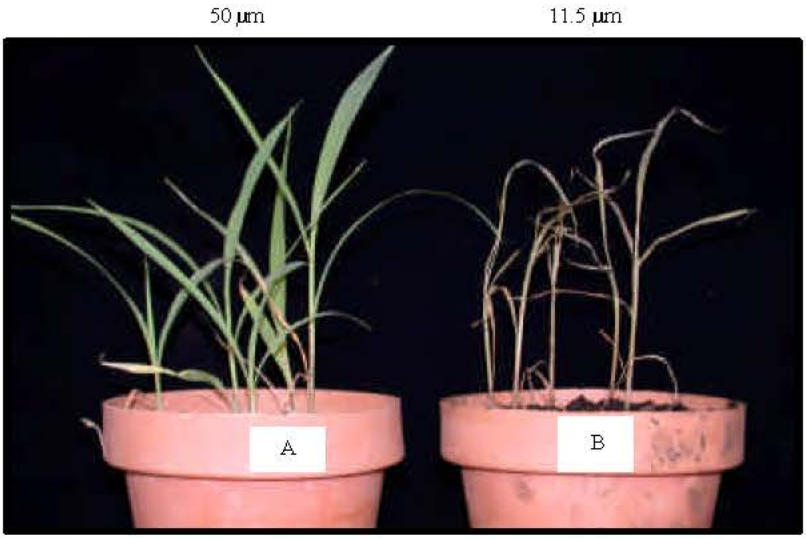

Fig. 2: Effect of *E. longirostratum* on seedling of *R. cochinchinensis*; healthy noninoculated control (A) and diseased seedlings 4 days after inoculation with 3.5×10^5 spores mL^{-1} (B)

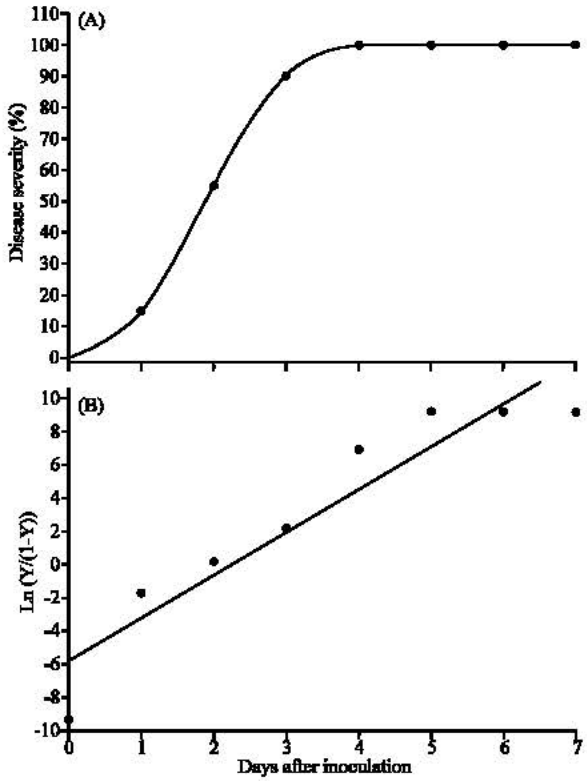

Fig. 3: Disease progress curve of seedling blight caused by *E. longirostratum* on *R. cochinchinensis* seedlings; untransformed disease severity value (A). Regression of transformed disease severity using Logistic model Ln (Y/(1-Y)) (Berger, 1981) (B). The equation for the line is Y = -5.77 +2.58X, (R^2 = 0.89)

Table 1: A comparison of conidial dimensions of isolated fungus with those described in the literature

Fungi	Length (µm)	Width (µm)	No. of cells/ conidium
Drechslera longirostard[a]	63-462	12-26	6-26
Exserohilum rostratum[b]	15-190	7-29	18
Exserohilum monoceras[b]	60-150	15-25	4-10
Exserohilum longirostrartum[b]	100-434	12-20.5	6-26
Exserohilum longirostratum[c]	97.8-340.6	11.2-20	10-26

[a]Chidambaram *et al.* (1973), [b]Sivanesan (1987), [c]Isolated from diseased leaves (fungus in the present study)

The germination was usually unipolar with the based hyphae-growing semi axially to the orientation of the conidia (Fig. 1). There was a slight difference in the dimension of the conidia as reported in the literature (Sivanesan, 1987); however, it fit well within the described range for *Exserohilum longirostratum* (Subram) Sivan (Alcorn, 1988; Sivanesan, 1987) [=*Drechslera longirostratum* (Subram) Subram].

Pathogenicity testing: *E. longirostratum* was very pathogenic on *R. cochinchinensis* and the pathogenicity of this fungus was confirmed by fulfilling Koch's postulates. Disease symptom started with minute specks, which later formed a discrete lesion. As the disease progressed, the area around the discrete lesions turned yellow. Tips and edges of infected leaves turned dark green to brown and eventually dried up, giving the leaf a folded appearance (Fig. 2). Older leaves exhibited more severe disease symptoms as large necrotic areas with yellow borders and subsequent blighting of most of the leaf blade, beginning at the tips and edges of leaves, were

observed within 72 h after inoculation. After this date 100% of the inoculated seedlings were heavily diseased with 100% disease severity and the seedlings were dead (Fig. 2). No secondary spread was observed in the green house. Control plants that were sprayed with 5.0% Maxigreen® did not exhibit any damage on the leaves and they remain healthy through out the experiment.

The disease progress of *E. longirostratum* on *R. cochinchinensis*, as measured by the disease severity, is as shown in (Fig. 3). The disease has a short incubation period (few hours). During the initial stage of disease development, disease progress was slow, but the progress was rapid a day after inoculation and reached the maximum 100% disease severity 4 days after inoculation. After this date 100% of the inoculated seedlings were dead. The disease progress was best described by the logistic growth model (Fig. 3) and the overall apparent infection rate in the two trials average was r_L= 2.58 unit day^{-1} (SE = 0.001, R^2 = 0.89; $p<0.005$). Under greenhouse conditions this fungus is not capable of causing secondary infection. No disease developed on the noninoculated control.

Effect of light and culture media on fungal growth and sporulation: Light has no effect on the radial growth of *E. longirostratum* (Table 2); however, it has significant effect on conidia production (Table 3). Conidia production was induced by exposing the fungus to 24 h light in all media tested. Exposing the fungus to alternate short dark and light cycle (4 h dark and 4 h light) has significantly

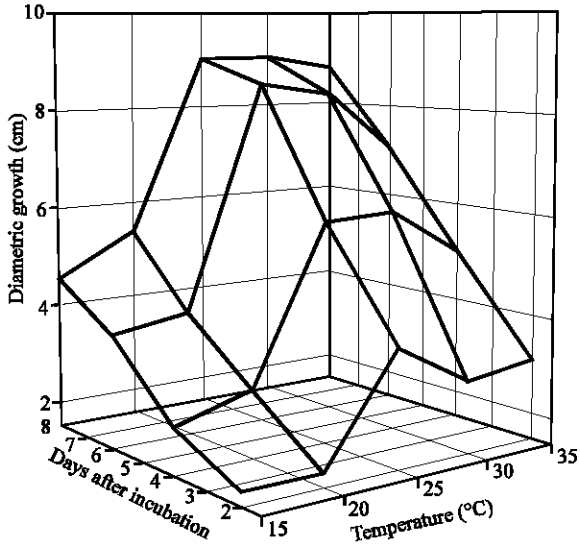

Fig. 4: Effect of incubation temperatures on radial growth of *E. longirostratum* when cultured on PDA. Each point was represented by the average of ten replicates

Table 2: Effect of light on mean radial growth of *E. longirostratum* when cultured on various media. Mean radial growth was expressed as the total area under the growth curve for analysis purposes

Exposure time to light (h)	Total area under the growth curve[x] — Media: PDA	V8 agar	CMA
0	9.72^{b}	8.211^{b}	7.834^{c}
4	7.54^{b}	8.721^{b}	8.807^{bc}
8	10.33^{b}	10.931^{ab}	10.846^{b}
12	11.6^{b}	12.330^{a}	12.471^{a}
24	73.39^{a}	13.352^{a}	13.559^{a}
Mean	22.516^{A}	10.709^{A}	10.704^{A}

[x]Cultures were grown from standard-sized inoculum disc for 7 days. Total area under the growth curve were obtained by the following formula: Area under the growth curve = $\Sigma(Y_{i+1} + Y_i)/2 \times t_{i+1}-t_i$ and were the average from two trials, each with ten replicates per light period. Means within column denoted by different small letter(s) and means within rows denoted by different capital letter(s) are significantly different at $p<0.05$ according to Fisher's protected LSD

Table 3: Sporulation of *E. longirostratum* on various media when exposed under various light regimes

Exposure time (h)	Mean number of conidia (conidia mL^{-1})[x] — Media: PDA	V8	CMA
0	385750^{b}	546000^{b}	60000^{b}
4	16500^{e}	216750^{d}	43000^{c}
8	20800^{d}	243000^{d}	52000^{c}
12	243750^{c}	292250^{c}	55750^{b}
24	514000^{a}	695000^{a}	80250^{a}
Mean	303300^{B}	398600^{A}	58200^{C}

[x]Cultures were grown from standard-sized inoculum disc for 7 days. Mean number of conidia were average from two trials, each with ten replicates per light period. Means within column denoted by different small letters and means within rows denoted by different capital letter(s) are significantly different at $p<0.05$ according to Fisher's protected LSD

Table 4: Effect of incubation temperatures on mean radial growth of *E. longirostratum* when cultured on various media. Mean radial growth was expressed as the total area under the growth curve for analysis purposes

Temperature (°C)	Total area under the growth progress curve[x] — Media: PDA	V8	CMA
15	4.925^{b}	5.328^{b}	3.769^{b}
20	5.644^{b}	5.513^{b}	5.728^{b}
25	11.344^{a}	9.856^{a}	8.775^{a}
30	10.709^{a}	9.806^{a}	11.056^{a}
35	10.438^{a}	8.934^{a}	10.359^{a}
Mean	8.612^{A}	7.888^{B}	7.938^{B}

[x]Cultures were grown from standard-sized inoculum disc for 7 days. Total area under the growth curve were obtained by the following formula: Area under the growth curve = $\Sigma(Y_{i+1} + Y_i)/2 \times t_{i+1}-t_i$ and were the average from two trials, each with ten replicates per temperature. Means within column denoted by different small letter(s) and means within rows denoted by different capital letter(s) are significantly different at $p<0.05$ according to Fisher's protected LSD

($p<0.05$) lower conidia production as compared to exposing the fungus to longer light period or longer dark period (12 h dark and 12 h light) (Table 3). The highest

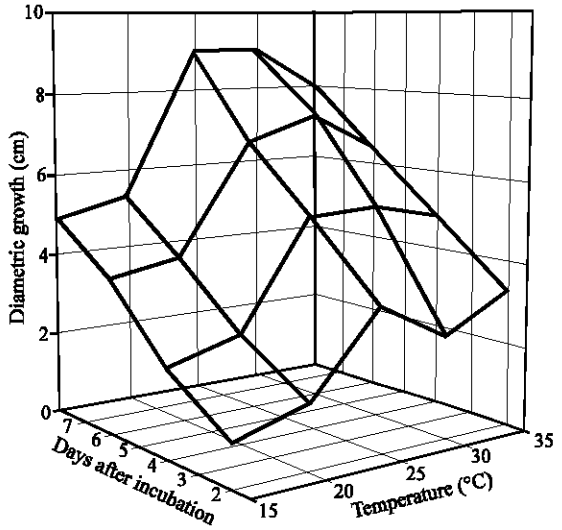

Fig. 5: Effect of incubation temperatures on radial growth of *E. longirostratum* when cultured on V8 juice agar. Each point was represented by the average of ten replicates

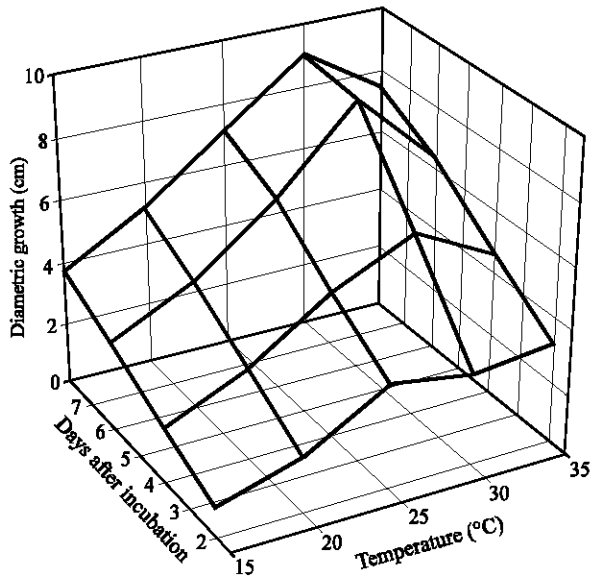

Fig. 6: Effect of incubation temperatures on radial growth of *E. longirostratum* when cultured on CMA. Each point was represented by the average of ten replicates

number of conidia was produced on V8 juice agar compared to PDA and CMA, with the lowest conidia production on CMA (Table 3).

Effect of temperature on fungal radial growth and conidiation: No significant difference (p>0.05) in radial growth was observed between 25 to 35°C. Fungus radial growth was inhibited at temperature <25°C (Table 4). The radial growth of *E. longirostratum* was visible after 24 h at temperatures between 20 and 35°C with higher rates occurring at 25, 30 and 35°C (Fig. 3-5). Conidia were produced in all media at all temperatures tested; however, the numbers produced in each medium were dependent on temperatures. Maximum conidia production was observed at 25°C in all media; however the mean of number of conidia produced on CMA was not significantly (p>0.05) different at 25 and 35°C (Table 5). The fungus did not conidiate well in all media at temperatures below 25 and above 30°C (Fig. 6). Number of conidia produced differed significantly (p<0.05) between media tested (Fig. 7). V8 juice agar was the superior medium as it supported higher number of conidia followed PDA, whereas the lowest number of conidia was produced on CMA.

Table 5: Effect of incubation temperatures on conidia production of *E. longirostratum* when cultured on various media

	Mean number of conidia (conidia mL^{-1} medium)[x]		
	Media		
Temperature (°C)	PDA	V8	CMA
15	170150[c]	240000[c]	15000[c]
20	300000[c]	465000[c]	72000[b]
25	1452500[a]	3650000[a]	240750[a]
30	567500[b]	1372500[b]	280000[a]
35	230000[c]	270000[c]	91000[b]
Mean	544030[B]	1199500[A]	139750[C]

[x]Cultures were grown from standard-sized inoculum disc for 7 days. Mean number of conidia were average from two trials, each with ten replicates per temperature. Means within column denoted by different small letter(s) and means within rows denoted by different capital letter(s) are significantly different at p<0.05 according to Fisher's protected LSD

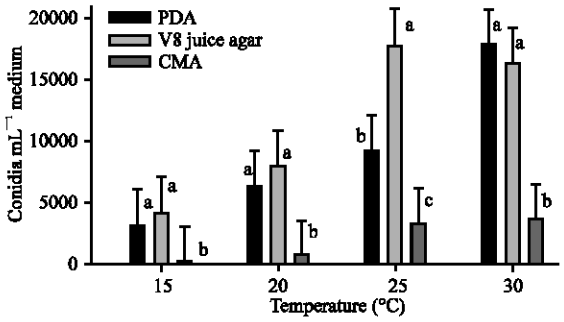

Fig. 7: Effect of different temperature regimes on conidia production of *E. longirostratum* when cultured on different culture media. Means within a vertical bars followed by the same letter are not significantly different at p<0.05 according to Fisher's protected LSD. Y bars indicate standard error of means

DISCUSSION

Currently, there is no bioherbicide commercially available to control *R. cochinchinensis*. However, a few fungal plant pathogens have been evaluated for their potential as bioherbicide for controlling this weed (Reeder and Ellison, 1999). The head smut pathogen (*Sporisorium ophiuri*) which significantly infect itchgrass during heading and virtually eliminated seed-set and eventually reduce weed seed bank over time has been intensively investigated. However, biotrophic fungus such as *S. ophiuri* is difficult to mass produce and therefore difficult to develop as bioherbicide.

E. longirostratum, an endemic fungal pathogen that was isolated from diseased itchgrass has been screen for its bioherbicide activity and has been found as a potential bioherbicide. Its potential as a bioherbicide of itchgrass was confirmed in repeated glass house trials in which fungus exhibited lesions within 24 h after inoculation. This pathogen caused 100% mortality of the young seedlings. It induced lesions typical of infection by *Drechslera* and *Exserohilum* species and resulted in blight symptom which appeared as a severe burnt appearance on the foliage of highly susceptible hosts. The blighting of the seedlings may be associated with phytotoxins which may be involved in pathogenesis and rapid necrosis. Species of *Helminthosporium* group of fungi have been reported to produce host specific toxins which are important in plant pathogenesis (Walton and Panacione, 1993).

The most important characteristics in a mycoherbicide is its ability to readily produced viable and virulent propagules (Trujillo, 1992). Jackson *et al.* (1996) and Shabana *et al.* (1997) highlighted the importance of production medium and cultural conditions on quantity, viability and efficacy of propagules of several fungi investigated as biocontrol agents. They reported that fungal growth and conidia production are strongly affected by the production media. Most fungi require carbon and nitrogen (both inorganic and organic) for growth. Carbon containing compounds are required to provide both sources of energy and also the basic molecules for biosynthesis. Nitrogen is essential for biosynthesis of complex molecules such as amino acids, protein, nucleic acids and some vitamins. Carbon concentration and carbon:nitrogen ratio are known to affect conidia yield and quantity, including germinability, pathogenicity and virulence (Jackson and Bothast, 1990).

In our study, media containing carbon: nitrogen ratio of 10:1 as in V8 juice agar and PDA produced more conidia compared to medium (CMA) containing carbon:nitrogen ratio of 40:1. This result corraborated with the findings of Wyess and Charudattan (2000), in which they reported that conidia production of *Dactylaria higginsii* was significantly improved when cultured on media containing low C:N ratio. It also reported that *D. higginsii* has highest growth rate on CMA compared to V8 juice and PDA. However, they made cautionary statement that media supporting good radial growth may not be suitable for conidial production.

Light and temperature had major effect on the number of conidia produce by *E. longirostratum*. Exposing the fungal culture to longer duration of light significantly increase conidia production. Cultures incubated in 24 h light formed higher conidia compared to cultures incubated in the dark. Contradictory to the report of Roger and Tivoli (1996), who obtained less spores of *Mycosphaerella pinodes* in continous light, but produced numerous ascospores in continous darkness. This result also support report of Hare and Walker (1944) who obtained fewer conidia in darkness, but produce more conidia when exposed to light. The lack of reproductive structures and/or sporulation in continous darkness is frequently observed for other fungi and it has been frequently reported that reproductive development and/or sporulation are light-induced (Roger and Tivoli, 1996; Halama and Lacoste, 1992). Conidia production was optimal at 25 to 30°C and were sparse at 15°C. Our study corroborate with Kadir *et al.* (2000), who reported that the optimal temperature for growth and sporulation of *D. higginsii* is in the range of 25 to 30°C.

The effect of different environmental conditions on conidia production of this fungus may have implications to disease development and should be subjected to further research, which should also include analysis of the effect of various carbon and nitrogen sources on accumulation of exogenous reserves in the conidium and how this reserves may affect viability and virulence of conidia.

ACKNOWLEDGMENT

This study is supported by the Intensified Research Priority Areas (IRPA) grant No: 01-02-04-0504 provided by the Ministry of Science and Technology and Innovation (MOSTI), Malaysia.

REFERENCES

Alcorn, J.L., 1988. The taxonomy of *Helminthosporium* species. Ann. Rev. Phyopathol., 26: 37-56.

Chan, L.G., S.O. Leong and S.T. Chen, 1990. *Rottboellia cochinchinensis*, a new weed in Malaysia. MAPPS Newlett., 14: 3.

Dinghra, O.D. and J.B. Sinclair, 1995. Basic Plant Pathology Methods. Lewis publisher, Boca Raton, Florida. pp: 363.

Ellison, C.A. and H.C. Evan, 1990. Preliminary Studies to Assess the Potential of Fungal Pathogens as Biological Control Agents for *Rottboellia Cochinchinensis* (Gramineae). Proceedings of the VII International Symposium on Biological Control of Weeds Delfose, E.S., (Ed.) Ministero dell' Agricolturae delle Foreste, Rome/ CSIRO, Melbourne., pp: 477-482.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn. J. Wiley, NY, pp: 680.

Halama, P. and L. Lacoste, 1992. Etudes des conditions optimales permentale la pycniogenese de *Phaeosphaeria nodorum* (hedj) agent de la septoriose. Agronomie, 12: 705-710.

Hare, W.W. and J. Walker, 1944. Ascochyta disease of caning peas. Wisconsin Res. Bull., 150: 1-8.

Holm, L.G., D.L. P lucknett, J.V. Pancho and J.P. Herbenger, 1977. The world's worst weeds. University Press of Hawaii, Hawaii, USA, pp: 609.

Horsefall, J.G. and E.B. Cowling, 1978. Pathometry: The Measurement of Plant Disease. In: Plant Disease: An Advance Treatise (Horsfall, J.G. and E.B. Cowling. Eds.), Academic Press, New York. 2: 119-136.

Jackson, M.A. and R.J. Bothast, 1990. Carbon concentration and carbon-to-nitrogen ratio influence submerged-culture conidiation by the potential bioherbicide. *Colletotrichum truncatum* NRRL 13737. Applied Environ. Microbiol., 56: 3435-3438.

Jackson, M.A., D.A. Schisler, P.J. Slininger, C.D. Boyette, R.W. Silman and R.J. Bothast, 1996. Fermentation strategies for improving the fitness of a bioherbicide. Weed Technol., 10: 645-650.

James, W.C., 1974. Assessment of plant disease and losses. Ann. Rev. Phytopathol., 12: 27-48.

Kadir, J.B. and R. Charudattan, 2000. *Dactylaria higginsii*, a bioherbicide agent for purplenutsedge (*Cyperus rotundus*). Biol. Control., 17: 113-124.

Kadir, J.B., R. Charudattan and R.D. Berger, 2000. Effects of some epidemiological factors on levels of disease caused by *Dactylaria higginsii* on *Cyperus rotundus*. Weed Sci., 48: 61-68.

Kranz, J., 1988. Measuring Plant Disease. Experimental Techniques in plant Disease Epidemiology. In: Kranz, J. and J. Rotems (Eds.). Springler, Berlin, pp: 35-50.

Reeder, R.H. and C.A. Ellison, 1999. Current status of research into the classical biological control of *Rottboellia cochinchinensis* with the smut *Sporisorium ophiuri*: Potential and risks. CATIE, Costa Rica, pp: 101-135.

Roger, C. and B. Tivoli, 1996. Effect of culture medium, light and temperature on sexual and asexual reproduction of four strains of *Mycosphaerelle pinodes*. Mycol. Res., 100: 304-306.

Shabana, Y.M., R. Charudattan, J.T. DeValerio and M.A. El Wakil, 1997. An evaluation of hydrophilic polymers for formulating the bioherbicide agents *Alternaria cassiae* and *A. eichhorniae*. Weed Technol., 11: 212-222.

Sivanesan, A., 1987. Graminicolous species of *Bipolaris*, *Curvulari*, *Drechslera*, *Exserohilum* and their telemorphs. Mycol. Pap., 158: 1-249.

Trujillo, E.E., 1992. Bioherbicides. In Frontiers in Industrial Mycology. Leatham, G.F. (Ed.). Chapman and Hall: London, pp: 197-211.

Walton, J.D. and D.G. Panaccione, 1993. Host-selective toxins and disease specificity: Perspectives and progress. Ann. Rev. Phytopathol., 31: 275-303.