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Evaluation of *Exserohilum longirostratum* (Subram.) Sivan. for biological control of *Dactyloctenium aegyptium* (L.) Beauv.

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The objective of this study was to search for the indigenous plant pathogen to control crowfoot grass (*Dactyloctenium aegyptium* (L.) Beauv. leaf blight and leaf spot caused by *Helminthosporium* – complexs (*Bipolaris* spp., *Drechslera* spp. and *Exserohilum* spp.) were collected and isolated for the fungi. *Exserohilum longirostratum* which caused severe disease on *Dactyloctenium aegyptium* was selected and evaluated for its potential use as a bioherbicide. The results showed that *E. longirostratum* produced high concentration of spore on V_8A at 25 ^{O}C in dark condition. For the efficacy of *E. longirostratum* control crowfoot grass, *E. longirostratum* caused more than 80% mortality of *Dactyloctenium aegyptium* was obtained at the concentration of spore suspension greater than 10^5 spore/ml at 7 day-old growth stage.

Key words: *Exserohilum longirostratum*, *Dactyloctenium aegyptium*, crowfoot grass, bioherbicide

Introduction

Dactyloctenium aegyptium (L.) Beauv. (Crowfoot grass) is one of the serious weed in Thailand. It occurred in gardens, cultivated land, open waste and along roadside. It is an important weed of sugarcane, rice fields, rubber fields and horticultural crops. Crowfoot grass can be controlled through various management strategies, including hand – weeding, mechanical cultivation, cultural practices or chemical herbicides can control this weed. (Chandramohan and Charudatttan, 2001) However, use of physical or chemical methods of weed control alone is not sufficient in every situation. The use of biological control agent as an alternative for weed control, where an organism is applied to achieve reduction in weed population. Several fungal pathogen have been reported as potential candidates for weed control are: *Bipolaris setariae* for

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goose grass (Figliola *et al*., 1988) *B. halepense, B. sorghicola* and *E. turcicum* for Johnson grass (Chiang *et al*., 1989) and *E.monoceras* for *Echinocola* spp. (Zhang and Watson , 1997a, b) On crowfoot grass, numerous fungal pathogen were found to cause disease on leaves including *Drechslera gigantean, E. longirostratum* and *E. rostratum.* Among these fungi, *E. longirostratum* has been reported to be a potential biocontrol agent to suppress growth of weeds such as barnyard grass (*Echinochloa cruss-galli* var.*crus-galli*) (Abdul *et al*., 2006) and Itchgrass (*Rottboellia cochinchnensis* (Lour. W.D.Clayton)). (Kadir *et al*., 2007) Therefore, the experiment was conducted to determine the optimum condition for growth and sporulation of *E. longirostratum* and to evaluate the use of this pathogen as bioherbicides for controlling *Dactyloctenium aegyptium*.

Materials and methods

Pathogen collection and isolation

Diseased leaves of crowfoot grass were collected from various location in Southern Thailand. The specimens were incubated in moisture plates and determined for the associated fungus on disease tissues. The fungus was isolated into pure culture by single spore isolation. Single – conidium isolated of fungi were kept on PDA slants at 4 ^{O}C in the dark as stock cultures.

Identification

The fungus was directly taken from the disease tissues and mounted on the slide using lactophenal as a mounting medium. Details of morphological characters were observed under an Olympus microscope (BH2). Thirty conidia and conidiophores were measured using micrometer. The identification followed the key of Ellis (1971, 1976) and Sivanesan (1987).

Growth and conidia production on standard agar media

Effect of nutrient media on mycelial growth and conidia production: Growth and conidia production of *E. longirostratum* were observed on different agar media. The media tested were cornmeal agar (CMA), Czapek – Dox agar (CDA), Malt extract agar (MEA), Potato dextrose agar (PDA), ½ PDA and V-8 juice agar (V_8A). Five replicate dishes of each medium were incubated for 15 days at 28^{O}C in continuous darkness in an incubation chamber. At the end of the growth study, plates were assessed for the production of conidia. Conidia were harvested with 15 ml of distilled water and scraping the surface of

colonies with a glass slide. The resulting conidia suspensions from five colonies/growth medium were counted individually in a haemacytometer.

Effect of temperature on mycelial growth conidia production: The effects of constant temperatures on linear growth and sporulation were determined on V_8A in the dark. Radial mycelial growth and conidia production were measured for five temperature between 5 and 40^OC.

Effect of dark , light/dark and near – ultraviolet (NUV) light: The effects of dark, light/dark and NUV light on radial mycelial growth and conidia production were evaluated on V_8A following the procedure outlined above. Cultures were incubated on V_8A for 24h. at 28^OC in the dark, 12h. of alternating light/dark and near– ultraviolet (NUV) light.

Host- range determination

The host – range test included rice (*Oryza sativa* Linn), corn (*Zea mays* Linn.), sorghum (*Sorghum bicolor* (Linnaeus) Moench), goosegrass (*Eleusine indica* (L.) Gaertn.), barnyard grass (*Echinochloa cruss-galli*) and jungle rice (*Echinochloa colonum*). Twenty-five plants of a single crop were grown in each pot. The number of plants and age of the plants were 2 weeks old. The plants were sprayed with inoculums that consisted of spore suspensions of $1x10^7$ spore/ml. Non-inoculated control plants were sprayed with distilled water. Inoculated plants were then covered with polyethylene bags for 24 h. to maintain humidity. The bags were then removed. The plants were observed for any disease development over periods of 4 week.

Interaction between inoculum concentration and plant growth stage

Seedings at the 7, 14 and 15 days stages of crowfoot grass were inoculated at concentration of spore suspension 10^3, 10^4, 10^5, 10^6 and 10^7 spore/ml. Non-inoculate control plants were sprayed with distilled water. Inoculated plants were then covered with polyethylene bags for 24 h. to maintain humidity. The bags were then removed. Disease incidence and disease severity were recorded every day. Diseased leaves were collected and the fungus was re-isolated from symptomatic lesions to confirm Koch's postulate.

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 = 1 - 25 % disease severity
2 = 26 - 50% disease severity
3 = 51 - 75% disease severity
4 = over 76% disease severity

Data analysis

All experiments were used to model the effect of nutrient media on mycelial growth, effect of temperature on mycelial growth and effect of light. A completely randomized design five replicate was used for all experiments. Treatment means were separated using Duncan's multiple-range test. All analysis were conducted using SAS software.

Results

Pathogen collection, isolation and identification

Collection of leaf spots and leaf blights on crowfoot grass were collected in Southern Thailand. The associated fungal on disease leaves were identified to 3 *Bipolaris*, 1 *Drechslera* and 5 *Exserohilum*. (Table 1) However, only *E. longirostratum* of the fungus gave the high degree of disease damage to crowfoot grass. The conidia of *E. longirostratum* were olivaceous brown, broadest around the basal area, narrowing towards the apex into a long beak, end cell often cut off by dark, thick, septum, 8 – 13 pseudoseptate, 12 – 26 x 60 - 475 µm and basal cell often building a little hilum distinctly protuberant. The germination is bipolar. (Fig. 1) The conidiophore emerged through the single, straight, mid to dark brown, up to 200 µm long.

Effect of nutrient media on mycelial growth and conidia production

Significant difference in radial mycelial growth visible after 10 days at 28^{o}c in continuous darkness. The maximum radial mycelial growth occurred on V_8A however, this growth were not significant different from the growth rates on CDA. *E. longirostratum* produced greatest number of conidia on V_8A after 15 days incubation in dark condition. (Table 2)

Effect of temperature on mycelial growth conidia production

Significant difference in radial growth was observed between 5 to 40^{0}C. (Table 3) Fungus radial growth was inhibited at temperature below 20^{0}C or

above 35^0C and higher rates occurring at 25 to 30^0C. The maximum conidia production was observed at 25^0C**,** however *E. longirostratum* did not sporulation below 15^0C or above 35^0C**.** The predicted optimum temperatures for radial mycelial growth and conidia production were 28, 27, respectively.

Effect of dark , light/dark and near – ultraviolet (NUV) light

E. longirostratum on V_8A was no significant difference in mycelial growth among treatments with dark, light/dark 12 h. and near – ultraviolet (NUV) light. The maximum conidia production in continuous dark ($30.4x10^7$) was greater than that seen in alternating light/dark 12h. (Table 4).

Host- range determination

The respond of rice, corn, sorghum, goosegrass, barnyard grass and jungle grass seedings to *E. longirostratum* inoculum that consisted of spore suspensions of $1x10^7$ spore/ml did not causes disease on rice, sorghum, goosegrass and jungle rice leaves but caused disease on corn and barnyard grass leaves. *E. longirostratum* induce corn leaf blight, early symptoms are oval, water soaked spots on leaves were visible 4 days after inoculation. At 7 days, leaf blight are elliptical shaped lesion that are three to six inch long, gray – green lesions on the lower leaves

E. longirostratum induce leaf blight on barnyard grass. The extensive necrosis were visible 5 days after inoculation. At 9 days, Blight become distinct and enlarged to approximately 0.5 mm.

Interaction between inoculum concentration and plant growth stage

All growth stages of crowfoot grass responded to concentration of spore suspension. For each plant growth stage, the highest level of control was observed on 7 days-old seeding where as the lowest level of control was observed on 21 days-old seeding. For the inoculation concentration, it was found that over 80% mortality was obtained at the concentration of spore suspension greater than 10^5 spore/ml at 7 days-old growth stage. At the older stage the crowfoot grass was more resistant to *E. longirostratum.* (Table 5)

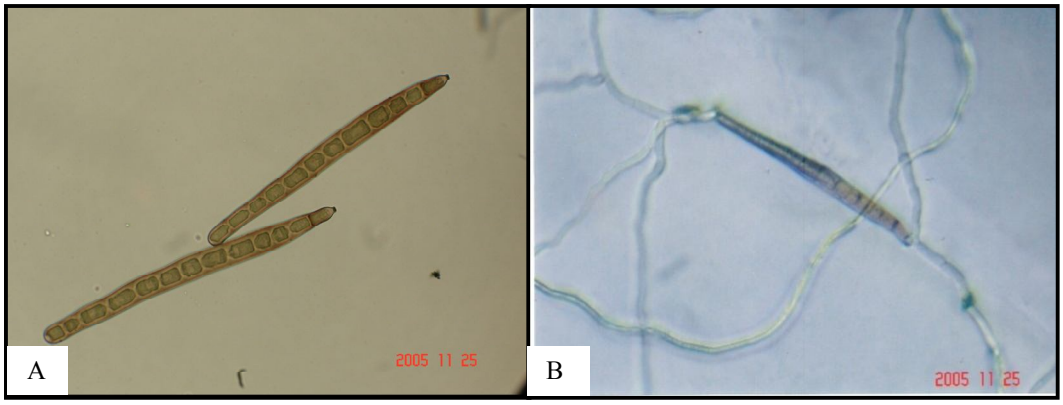

Fig. 1. Morphology of the conidia (A) and germination pattern of conidia (B) of *E. longirostratum*.

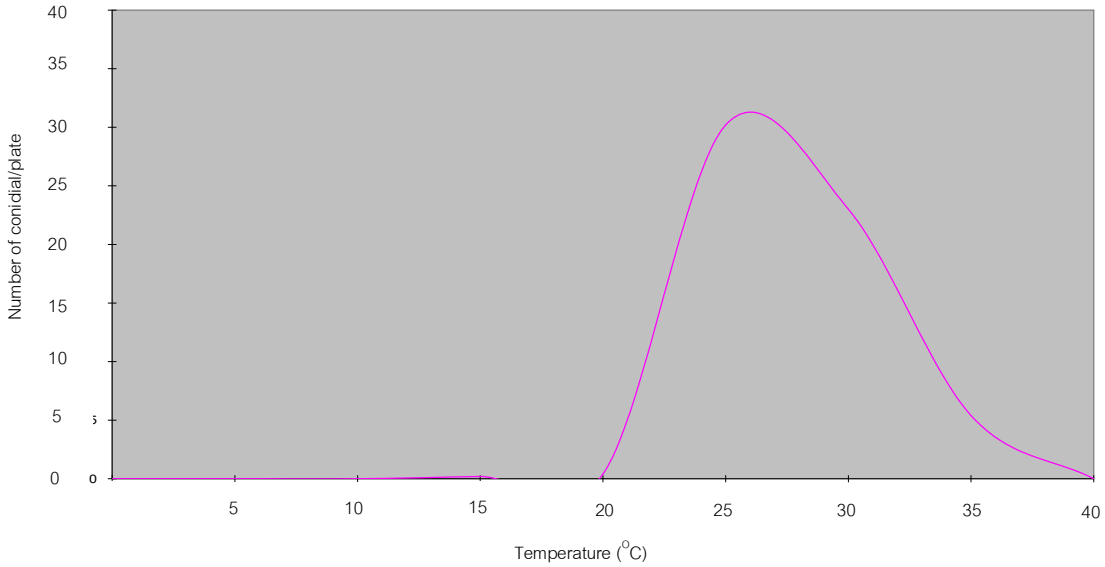

Fig. 2. Effect of temperature on conidia production of *E. longirostratum* grown on V_8A.

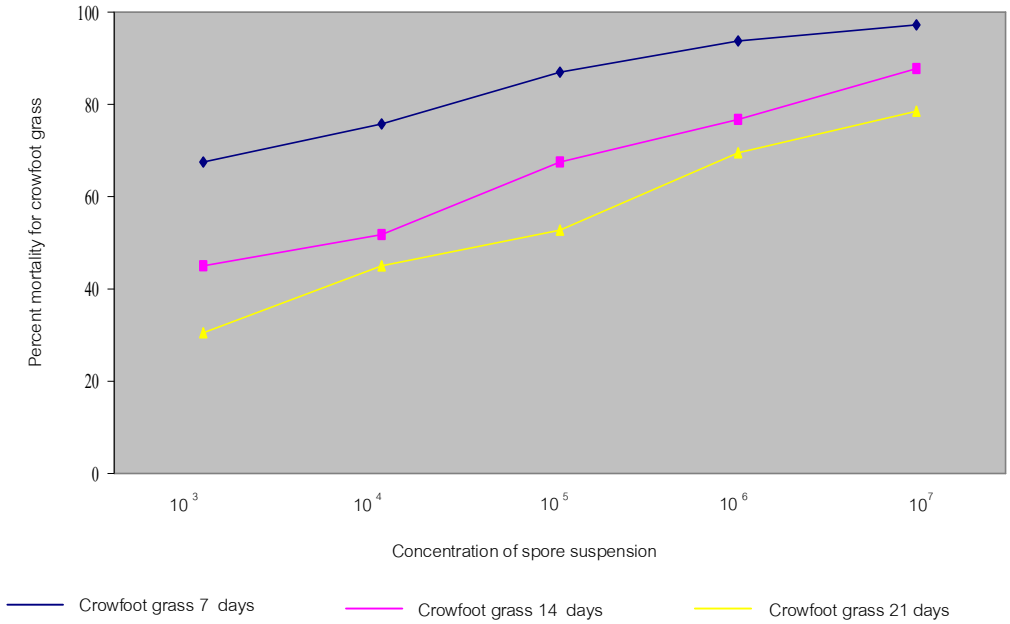

Fig. 3. Effect of *E. longirostratum* concentration of spore suspension and plant growth stage on control of crowfoot grass.

Table 1. Fungus isolated from disease tissue of crowfoot grass (*Dactyloctenium aegyptium*).

Fungal name	
Bipolaris hawaiiensis (Ellis) Uchida & Aragaki	*E. longirostratum* (Subram.)Sivan
B. sorokiniana (Sacc.) Shoem.	*E. khartoumensis* El Shafie & Webster
B. setariae (Saw.) Shoem	*E. minor* Alcorn.
Drechslera euphorbiae (Hansford) Ell	*E. paspali* Muchovej & Nesio
Exserohilum holmii (Luttr.) Leonard & Suggs.	*E. prolatum* Leonard & Suggs.

Table 2. Effect of nutrient media on *E. longirostratum* mycelial growth and conidia production.

Media	Radial mycelial[1] growth (cm)	Density of mycelial[1] growth	Number o f[2] conidial/plate 1 x 10^7
(CDA)Dox Agar –Czapek	8.60 a	++++	1.0 d
(CMA)Corn Meal Agar	8.20 b	++++	6.4 c
(MEA)Meal Extract Agar	7.20 c	+++	1.0 d
(PDA)Potato Dextrose Agar	7.50 c	+++	12.8 b
½ Potato Dextrose Agar	6.20 c	++	1.2 d
V-8 juice Agar	9.00 a	++++	30 a
F – test	**		**
C.V.	3.97		9.86

[1] Radial mycelial growth and Density of mycelial after 10 days incubation.
[2] Number of conidia after 15 days incubation.

Table 3. Effect of temperature a radial mycelia growth and conidia production of *E. longirostratum* grown on V_8A.

Temperature	Radial mycelial[1] growth (cm)	Density of mycelial[1] Growth	Number of[2] conidial/plate (1x10^7)
5	0	-	0 c
10	3.4	+	0 c
15	3.7	+	0.2 c
20	4.2	+	0.4 c
25	9	++++	30.2 a
30	9	++++	23.0 b
35	9	++++	5.0
40	0	-	0
F- test	**	-	**
C.V.	2.28	-	13.08

[1] Radial mycelial growth and density of mycelial after 10 days incubation.
[2] Number of conidia after 15 days incubation.

Table 4. Effect of dark, light/dark 12h., NUV – light on mycelial growth and conidia production of *E. longirostratum* grown on V_8A.

Treatment	Radial mycelia growth (cm)	Number of conidial/plate)(1x10^7)
Dark	8.94	30.4 a
Light/dark 12h.	8.92	1.4 b
NUV /Light	8.90	1.0 b
F – test	**	**
C.V.	1.21	8.51

[1] Radial mycelial growth and density of mycelial after 10 days incubation.
[2] Number of conidia after 15 days incubation.

Table 5. Disease severity of leaf blight caused by *Exserohilum longirostratum* on *Dactyloctenium aegyptium* at different growth stage and different spore concentration.

Age (days)	Concentration of spore suspension (spore/ml)					
	$^{3}10$	$^{4}10$	$^{5}10$	$^{6}10$	$^{7}10$	Mean
7	67.5	75.75	87	93.75	97.25	84.25 a **
14	45	51.75	67.5	76.75	87.75	65.75b **
21	30.5	45	52.75	69.5	78.5	55.25c**
Mean	47.66E **	57.50 D**	69.08 C **	80.00 B **	87.83 A**	
F test **						
C.V. 3.39						

Discussion

In this study under laboratory condition, the optimal radial mycelial growth, mycelial density and conidia production of *E. longirostratum* were observed on different nutrient media, temperature and light condition. Conidia production were best on V_8A.This result corresponding with Jackson (1990) and Shabana(1997) who, highlighted the importance of production medium and cultural conditions on quantity, viability and efficacy of propagates of several fungi investigated as biocontrol agents. They reported that fungal growth and conidia production were 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. However, they made cautionary statement that media supporting good radial mycelial growth may not be suitable for conidia production.

Light and Temperature had profound effect on radial mycelial growth and conidia production on V_8A. Cultures incubated in 24h. dark condition higher conidia compared to cultures incubated in 12h of alternation light/dark. NUV light did not stimulate sporulation of this pathogen on V_8A. This result corresponding with the other finding Leach (1967) and Sivanesan (1987) for dematiaceous fungi including *Exserohilum.* Zonation was observed when cultures were exposed to alternation dark and light periods. Conidia production were optimal in the range of 25^{O}C to 30^{O}C and did not sporulation below 15^{O}C, who reported that corresponding with Zhang and Watson (1997c), who reported

that the optimal temperature for both growth and sporulation was near 28^{O}C. At 35^{O}C, radial mycelial growth decreased greatly and conidia production ceased.

This result corroborated with the findings of Abdul et al., (2006) in which they reported that *E. longirostratum* potential as a bioherbicide of barnyard grass was confirmed in repeated greenhouse trials in which fungus exhibited lesions within 24 h. after inoculation. This pathogen caused 100% mortality of the young seeding. The blights of seeding may be associated with phytotoxins which may be involved in pathogenesis and rapid necrosis.

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