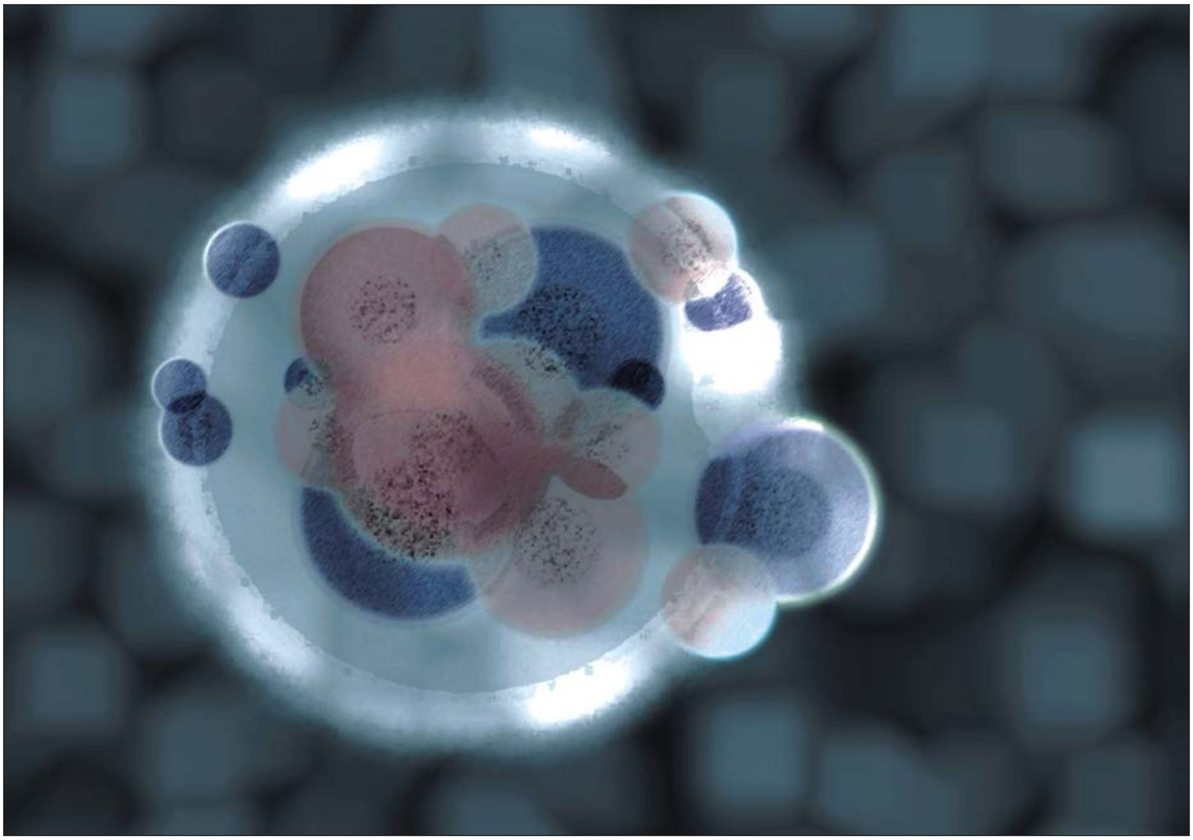

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Field Efficacy of *Exserohilum prolatum*-A Potential Mycoherbicide for Biological Control of Itchgrass (*Rottboellia cochinchinensis*)

[1]H. Alloub, [1]A.S. Juraimi, [1]J. Kadir, [2]S. Sastroutomo and [3]M. Begum
[1]Department of Crop Science, Faculty of Agriculture,
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]CAB International, South East Regional Centre, 43400 Serdang, Selangor, Malaysia
[3]Fellow Researcher, Institute of Tropical Agriculture,
Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia

Abstract: The experiments were conducted to evaluate the potential of the *Exserohilum prolatum* as a mycoherbicide for the itchgrass (*Rottboellia cochinchinensis*) control under natural conditions in two field experiments in a non crop situation and in association with a maize crop. Three doses were (single, double and triple) applied to the main plots with 2 week intervals. The sub-plot treatments were distilled water solution (check), the *Exserohilum prolatum* conidial concentration of 2×10^7, 2×10^8 and 2×10^9 conidial mL^{-1} or glyphosate (N-(phosphonomethyl) glycine) herbicide as control check. Disease severity was rated at 5 day intervals after application and the area under disease progress curve (AUDPC) was calculated for each treatment. The above ground parts of the itchgrass and maize were recorded at the end of the experiment. The results showed that application frequency and inoculum concentration greatly influence the itchgrass control. Significantly higher percentage control of the itchgrass were recorded from triple application frequencies (90.4%) compared to single (62.4%) and double (70.4%) applications. Within the three frequencies of application, increasing inoculum concentration increased the itchgrass control from between 53 to 90.4% compared to untreated check. The results indicate that the *E. prolatum* has a good potential as a biocontrol agent for the itchgrass.

Key words: Bioherbicide, fungi, *Exserohilum prolatum*, the itchgrass (*Rottboellia cochinchinensis*), maize, weed control

INTRODUCTION

The itchgrass (*Rottboellia cochinchinensis* (Lour) Clayton), is a noxious and widely distributed annual grassy weed. It poses serious problems in annual and perennial crops in the tropical and subtropical regions of the world. The weed is of South East Asian origin with one biotype believed to have originated in East Africa (Holm *et al*., 1977; Reeder and Ellison, 1999). The direct competition of the itchgrass, reduced corn yield 85% in the Philippines (Fisher *et al*., 1985) and by 81% in Honduras (Sharma and Zelaya, 1986). Sugar cane yield reduction was 43% in Louisiana (Lencse and Griffin, 1991) and 64% in Sudan (Makkawi *et al*., 2001). The itchgrass control is mainly through regular cutting in areas with low infestations and extensive application of herbicides in areas with heavy infestation (Anwar, 1990). Hand weeding is effective when the plants are young, but it is labor intensive and expensive. Several chemical herbicides can effectively control the weed. However, negative impacts of herbicides on the environment and development of herbicide resistance has been widely reported by Terry (1991) and Garazon and Lazo (1996). The use of biological weed control would reduce over dependency to the herbicides, the development of herbicide resistant weed populations and reduce the risk of herbicide contamination in the environment. Many weeds have been successfully controlled by plant pathogenic fungi (Juraimi *et al*., 2006; Charudattan, 1991; Kadir *et al*., 2000). Many fungi have been found associated with the itchgrass, but only a few were reported as having potential as mycoherbicides for the itchgrass control such as *Sporisorium ophiuri* (Reeder and Ellison, 1999), *Colletotrichum* sp. near *graminicola* (Ellison and Evans, 1995), *Fusarium moniliforme* and two strains of another *Fusarium* sp. (Zuniga *et al*., 2000). Preliminary investigations on the use of mycoherbicides for the control of the itchgrass in Malaysia were reported by Ahmed *et al*. (2002), Alloub *et al*. (2004) and Kadir *et al*. (2003). Reports on the use of indigenous fungi as

Corresponding Author: Mahfuza Begum, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia

bio-herbicides under field conditions have been mixed. Successful examples include the use of *Microsphaeropsis amaranthi* for the control of *Amaranthus albus* under controlled conditions as well as under field conditions (Mintz *et al*., 1992). *Puccinia carduorum* on the other hand, effectively controlled musk thistle (*Carduus theormeri*) in the greenhouse, but in the field the pathogen only accelerated senescence and reduced seed production (Baudoin *et al*., 1993). Little data exists on the use of indigenous fungi for control of the itchgrass under the field conditions. The fungus *Colletotrichum* sp. near *graminicola* was found to be sufficiently virulent under greenhouse conditions, but when tested in the field the results were variable (Ellison and Evans, 1995). *Fusarium* sp., demonstrated to have potential as bio-control agent of the itchgrass under the greenhouse conditions, however did not completely kill the weed when evaluated under field conditions (Zuniga *et al*., 2000). The results of greenhouse experiments clearly demonstrated control efficacy and safety of the *Exserohilum prolatum* isolated from the itchgrass (Alloub *et al*., 2004). However, the effectiveness of *E. prolatum* in the field is unknown. Therefore, aim of this study is to evaluate the efficacy of *Exserohilum prolatum* as a mycoherbicide to control the itchgrass under field conditions associated with crop and non crop situation.

MATERIALS AND METHODS

The experimental materials such as seeds of the itchgrass and inoculum of *Exserohilum prolatum* were multiplied in the greenhouse and laboratory, respectively during the period November 2003 and March 2004 and subsequently two field experiments were conducted during April to June 2004 at the Universiti of Putra, Malaysia.

Fungal source: An isolate of the *E. prolatum* was obtained from previous experiment (Alloub *et al*., 2004). The fungus was isolated from diseased the itch grass (*Rottboelllia cochinchinensis*) and has identified based on the colony morphology, conidia morphology and pattern of conidia germination (Kadir *et al*., 2003).

Seed sources: Two biotypes of the itchgrass selected and used. Pre-germinated seeds of both biotypes were sown in clay pots (25 cm diameter), containing a soil mixture (3:1:1, soil: sand: Kosas peat). One week after the emergence, plantlets were thinned as to be 2 seedlings per pot. Mature maize plants were harvested and they were used in this experiment. Maize seeds (Putra J-58 cultivar) were obtained from the Prof. Ghizan Saleh and seed collection that located at UPM, Serdang, Selangor, Malaysia.

Inoculum production: The inoculum of *E. prolatum* was produced on V8 juice agar (200 g of V8 juice (Campbell Soup Company, Camden, NJ, USA), 18 g granulated agar and 1 L of distilled water), using the method described by Kadir and Charudattan (2000). The conidia were collected by gently scraping the surface of the media with a rubber spatula and then placing them into sterile water. The conidial suspensions were adjusted to required conidial concentrations using a hemocytometer (Reichert Scientific Instruments, Buffalo, NY, USA).

Effect of *Exserohilum prolatum* on the itchgrass under field condition: Two experiments were conducted at the field 10B Universiti Putra Malaysia, Serdang (3 02' N, 101 42' E and asl 31 m). Mean maximum temperature was 33.9°C, mean minimum temperature was 23.8°C and mean relative humidity value was 94.1%. Mean annual rainfall was 15.8 mm with most of the rain falling in April (Table 1). The meteorological data were taken from the nearby university meteorological station. Metolachlor (2 chloro-N-(2 ethyl-6-methyl phenyl)-N-(2 methoxy-1-methylethyl) acetamide) a herbicide under the family chloroacetanilide, with different mode of action of elongase inhibition and inhibition of geranylgeranyl pyrophosphate (GGPP) cyclisation enzymes, part of the gibberellin pathway was applied at 1.8 kg a.i ha^{-1} as pre-emergence herbicide to control weeds. Subsequent weeding was done manually when required. Irrigation was provided as required by overhead sprinklers.

The first experiment was conducted in a site frequently cultivated with annual crops. The soil was a sandy loam with 4% organic matter; 0.19% total nitrogen; 28.8 $\mu g\ g^{-1}$ phosphorous; 91 $\mu g\ g^{-1}$ potassium and pH 4.74. Forty pre-germinated seeds of itchgrass Biotype 1 were planted of which 20 itchgrass plants were kept after thinning in each sub-plot. Each sub-plot was separated by 0.5×0.5 m border area. Treatments were applied at the 2-4 leaf stage (Zhang and Watson, 1997). The second experiment was conducted in a site previously cultivated with maize. The soil was a sandy loam with 4.1% organic matter; 0.14% total nitrogen; 31.1 $\mu g\ g^{-1}$ phosphorous; 88 $\mu g\ g^{-1}$ potassium and pH 5.79. Basal fertilizer (15:15:15) at the rate of 100 kg N ha^{-1} was applied one week before planting. Ten pre-germinated maize (Putra J-58) seeds were planted in sub-plots. One week later 10 itchgrass

Table 1: Meteorological data for the period April-June 2004 in Serdang, Selangor

Month	RH (%)	Max. temp. (°C)	Min. temp. (°C)	Rainfall (mm)
April	94.2	34.5	23.7	15.8
May	93.5	33.9	24	4.3
June	94.6	33.3	23.9	1.5

Source: Universiti Putra Malaysia (UPM) Meteorological Station, Serdang (biotype 2) was also planted in the same sub-plots. Treatments were applied when the itchgrass plants were at 2-4 leaf stage while maize plants at 6 to 7 leaf stage.

The experimental design was a split plot in a randomized complete block design with 3 replications and 3 spraying doses (single, double or triple) were applied to the main plots with 2 week intervals. The sub-plot treatments were the inoculum concentration and glyphosate spray. The itchgrass plants were inoculated with 200 mL aqueous suspension of inoculum containing 2×10^7, 2×10^8 and 2×10^9 conidia mL^{-1} suspension plus 0.5% Tween 20 (Cayman Chemical, Ann Arbor, MI, USA) using 1 L hand sprayer. Uninoculated control plants were sprayed with 0.5% Tween 20 or glyphosate (N-(phosphonomethyl) glycine) at the rate of 1.0 kg a.i. ha^{-1}. The herbicide was applied with CP15 backpack sprayer. The disease severity was observed and recorded at 5 days interval for 40 days after the inoculation (Kadir *et al*., 1999; Masangkay *et al*., 1990). At the end of the experiment, parts of the itchgrass and maize were harvested, dried in paper bags for 48 h at 70°C and weighed with digital balance.

Statistical analysis: Percentage data were arcsine transformed and dry weight data were log (x+1) transformed. Data were subjected to ANOVA using GLM procedure and differences between means were determined using Tukey's test at the 5% level of significance (Gomez and Gomez, 1984). The effect of *E. prolatum* on the itchgrass was assessed based on the area under disease progress curve (AUDPC) and the AUDPC values were calculated as described by Campbell and Madden (1990).

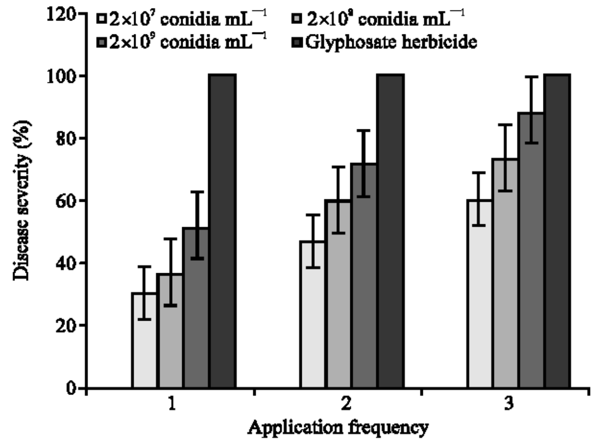

Fig. 1: Effect of *E. prolatum* on disease severity on the itchgrass at 35 days after treatment

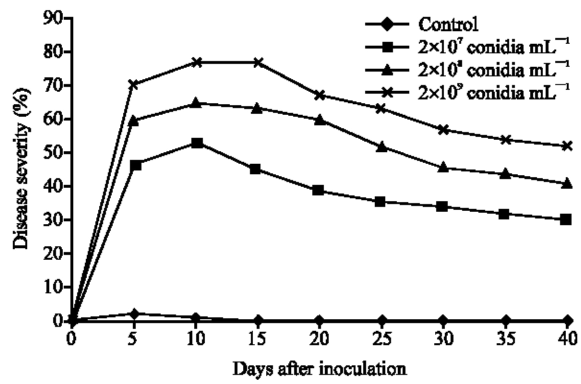

Fig. 2: Disease progress curve on the itchgrass receiving single application of *E. prolatum* at different concentrations

RESULTS

Efficacy of *E. prolatum* on the itchgrass in a non-crop situation: Results of *E. prolatum* disease severity showed significant differences between the three application frequencies (Fig. 1). In general increasing application frequency increased disease severity. Plots receiving triple application of the *E. prolatum* showed higher disease severity than plots receiving double application and the lowest disease severity was obtained in plots receiving single application. Within each application frequency treatments also showed significant differences. Increasing concentration of the *E. prolatum* provided higher percentage disease severity within each application frequency (Fig. 1, 2).

Disease progress of the *E. prolatum* on the itchgrass receiving single application is shown in Fig. 2. With 2×10^7 conidia mL^{-1}, disease severity increased to 53.33% at 10 days after application and then decreased gradually to 30% at 40 days after application. With 2×10^8 conidia mL^{-1}, disease severity increased to 64.33% at 10 days after application and then decreased gradually to 40.67% at 40 days after application. With 2×10^9 conidia mL^{-1}, disease severity increased to 76.67% at 15 days after application and then decreased gradually to 51.67% at 40 days after application.

Plots receiving a second application of the *E. prolatum* at 15 days after the first application showed similar responses in disease progress as in plots that received a single application (Fig. 3). However, at 5 days after the second application (20 days after the first application) disease severity with 2×10^7 and 2×10^8 conidia mL^{-1} increased to 55 and 76.67%, respectively. With 2×10^9 conidia mL^{-1} disease severity continued to increase until it reached 83.33% at 10 days after the second application. Subsequently at 15 days after the

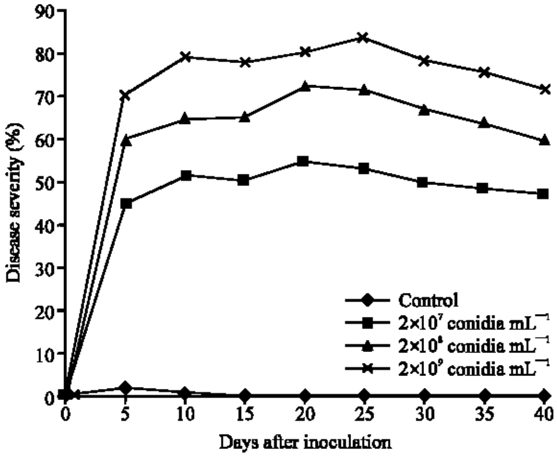

Fig. 3: Disease progress curve on the itchgrass receiving double application of *E. prolatum* at different concentrations

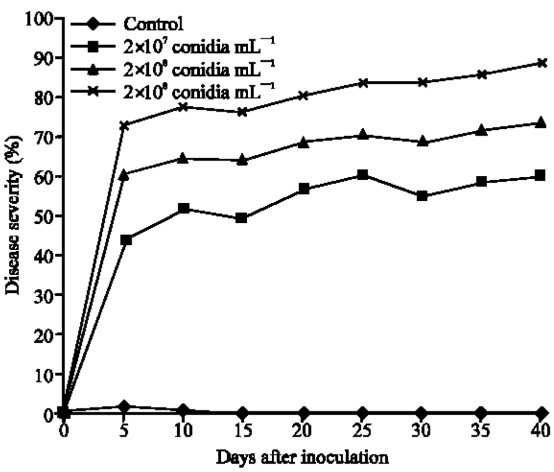

Fig. 4: Disease progress curve on the itchgrass receiving three applications of the different concentrations of *E. prolatum*

second application disease severity decreased to 46.67, 60 and 71.33% with 2×10^7, 2×10^8 and 2×10^9 conidia mL^{-1}, respectively.

Figure 4 shows disease progress with the *E. prolatum* in plots receiving a third application at 15 days after the second application (30 days after the first application). Disease severity showed similar responses in plots receiving two applications and at 10 days after the third application disease severity increased to 60, 73 and 88.3% with 2×10^7, 2×10^8 and 2×10^9 conidia mL^{-1}, respectively.

AUDPC showed similar responses as disease severity. The results differed significantly between application frequencies (Table 2). AUDPC also showed significant differences within each frequency. AUDPC increased with increasing inoculum concentration. At 40

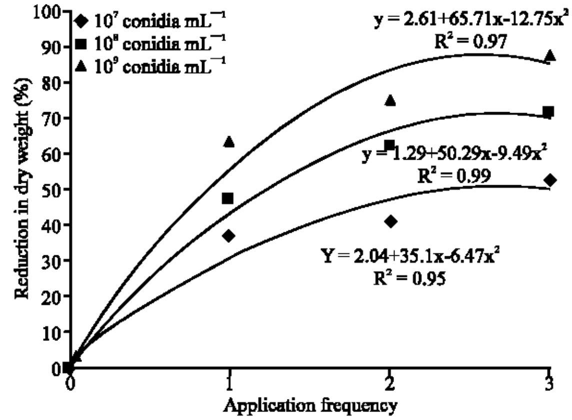

Fig. 5: Effect of application frequency on percent reduction in dry weight of the itchgrass sprayed with three concentrations of *E. prolatum*

Table 2: Effect of *E. prolatum* and glyphosate on the itchgrass Biotype 1 dry weight and AUDPC at 40 days after application

Frequency	Treatments	Dry weight (g m^{-1})	AUDPC
F_1	Control	331.10^{a}	0.00
	2×10^7 conidia mL^{-1}	207.87^{b}	1200.00
	2×10^8 conidia mL^{-1}	195.20^{c}	1626.80
	2×10^9 conidia mL^{-1}	158.03^{d}	2066.80
	Glyphosate	0.00^{e}	-
F_2	Control	345.40^{a}	0.00
	2×10^7 conidia mL^{-1}	182.80^{b}	1866.80
	2×10^8 conidia mL^{-1}	130.63^{c}	2400.00
	2×10^9 conidia mL^{-1}	98.63^{d}	2853.20
	Glyphosate	0.00^{e}	-
F_3	Control	320.00^{a}	0.00
	2×10^7 conidia mL^{-1}	117.07^{b}	2400.00
	2×10^8 conidia mL^{-1}	79.47^{c}	2933.20
	2×10^9 conidia mL^{-1}	39.67^{d}	3533.20
	Glyphosate	0.00^{e}	-

F_1: Frequency 1; F_2: Frequency 2; F_3: Frequency 3; AUDPC: Area under disease progress curve. Values in columns within the same frequency with same superscripts are not significantly different at p = 0.05 based on Tukey's test

days after application the highest AUDPC value (3533.20) was obtained with triple application of 2×10^9 conidia mL^{-1} and the lowest value (1200) was obtained with single application of 2×10^7 conidia mL^{-1} (Table 2).

Results of the itchgrass dry weights with single, double and triple application frequencies sampled at 40 days after treatments (Table 2) showed similar responses as with disease severity and AUDPC. All treatments significantly reduced the itchgrass dry weight. Results with glyphosate herbicide showed 100% reduction in dry weight. There were significant differences between application frequencies and within each frequency and in all cases 2×10^9 conidia mL^{-1} gave the lowest weed dry weight compared to 2×10^8 and 2×10^7 conidia mL^{-1}. Compared to the control, 2×10^9 conidia mL^{-1} resulted in 87.6, 71.4 and 52.3% reduction in above ground dry biomass with triple application, double and single application, respectively (Fig. 5).

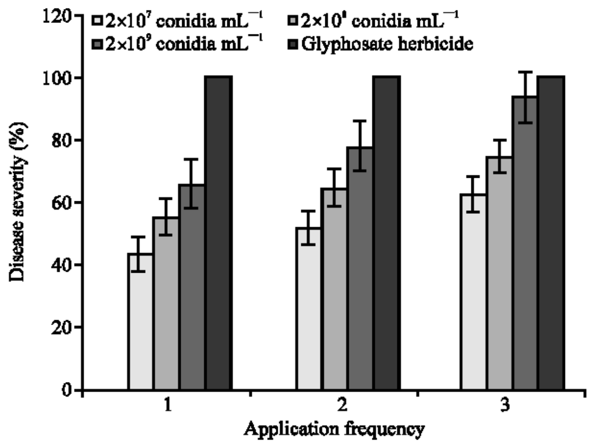

Fig. 6: Effect of *E. prolatum* on disease severity in the itchgrass at 35 days after application

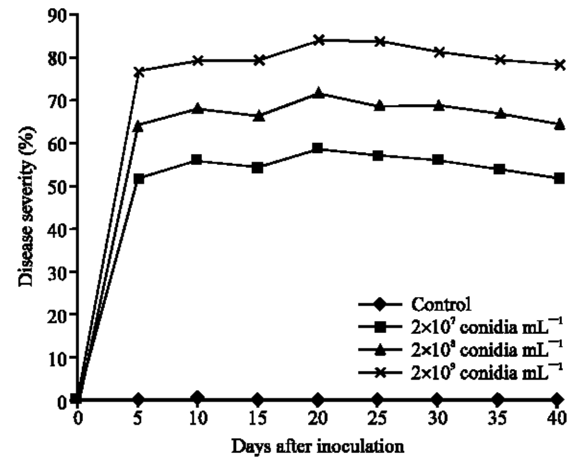

Fig. 8: Disease progress on the itchgrass with double application of *E. prolatum* at three concentrations

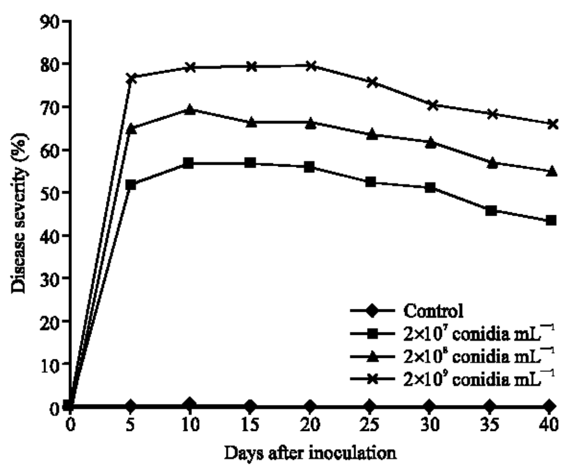

Fig. 7: Disease progress on the itchgrass with single application of *E. prolatum* at three concentrations

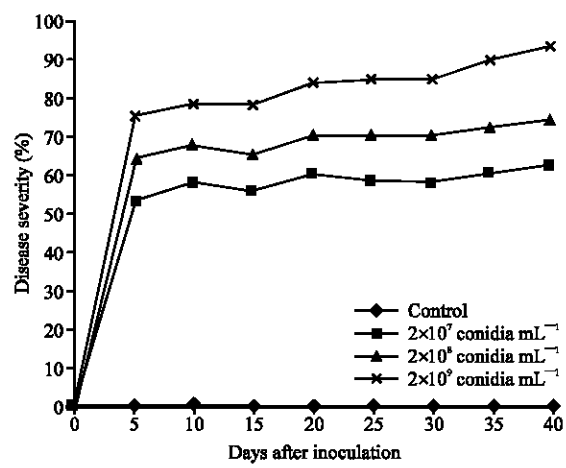

Fig. 9: Disease progress on the itchgrass with triple application of *E. prolatum* at three concentrations

Efficacy of *E. prolatum* on the itchgrass in maize: Disease severity varied greatly between and within application frequencies (Fig. 6). Triple application of the *E. prolatum* gave higher disease severity compared to double or single application and the control. Increasing concentration of the *E. prolatum* provided higher percentage disease severity within each application frequency.

Figure 7 shows disease progress in plots receiving single application of the *E. prolatum*. With 2×10^7 conidia mL^{-1}, disease severity was 56.66% at 15 days after application and then decreased gradually to 43.30% at 40 days after application. With 10^8 conidia mL^{-1} concentration, disease severity were 69.33% at 10 days after application and then decreased gradually to 55% at 40 days after application, while with 2×10^9 conidia mL^{-1} disease severity were 79.67% at 20 days after application and then decreased gradually to 65.67% at 40 days after application.

Disease progress in plots receiving a second application of the *E. prolatum* at 15 days after the first application showed similar responses as plots receiving a single application (Fig. 8). However, at 5 days after the second application (20 days after the first application) disease severity increased to 58.67, 71.33 and 83.67% with 2×10^7, 2×10^8 and 2×10^9 conidia mL^{-1}, respectively. Subsequently disease severity decreased slightly to 51.67, 64.33 and 77.67%, at 25 days after the second application, with 2×10^7, 2×10^8 and 2×10^9 conidia mL^{-1}, respectively.

Figure 9 shows disease progress of the *E. prolatum* in plots receiving a third application 15 days after the second application (30 days after the first application).

Table 3: Effect of *E. prolatum* and glyphosate on the itchgrass Biotype 2 and maize dry weights and AUDPC at 40 days after treatment

		The itchgrass		Maize	
Frequency	Treatments	Dry weight (g m^{-1})	AUDPC	Dry weight (g m^{-1})	AUDPC
F_1	Control	197.90^{a}	0.00	1451.33^{a}	0.00
	2×10^{7} conidia mL^{-1}	109.97^{b}	1732.00	1483.30^{a}	0.00
	2×10^{8} conidia mL^{-1}	94.33^{c}	2200.00	1533.33^{a}	0.00
	2×10^{9} conidia mL^{-1}	74.47^{d}	2626.80	1531.30^{a}	0.00
	Glyphosate	0.00^{e}	-	0.00^{b}	-
F_2	Control	206.07^{a}	0.00	1497.33^{a}	0.00
	2×10^{7} conidia mL^{-1}	100.27^{b}	2066.80	1448.30^{a}	0.00
	2×10^{8} conidia mL^{-1}	85.10^{c}	2573.20	1482.67^{a}	0.00
	2×10^{9} conidia mL^{-1}	60.46^{d}	3106.80	1485.00^{a}	0.00
	Glyphosate	0.00^{e}	-	0.00^{b}	-
F_3	Control	218.27^{a}	0.00	1523.33^{a}	0.00
	2×10^{7} conidia mL^{-1}	87.36^{b}	2493.20	1461.67^{a}	0.00
	2×10^{8} conidia mL^{-1}	61.47^{c}	2973.20	1491.67^{a}	0.00
	2×10^{9} conidia mL^{-1}	20.87^{d}	3733.20	1527.67^{a}	0.00
	Glyphosate	0.00^{e}	-	0.00^{b}	-

F_1: Frequency 1; F_2: Frequency 2; F_3: Frequency 3; AUDPC: Area under disease progress curve. Values in columns within frequencies with same superscripts are not significantly different at p = 0.05 based on Tukey's test

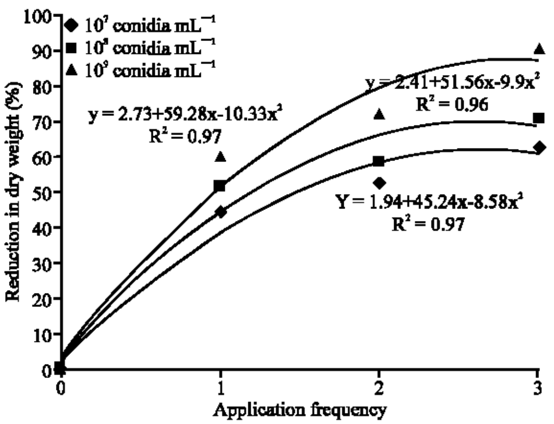

Fig. 10: Effect of application frequency on percent reduction in dry weight of the itchgrass sprayed with three concentrations of *E. prolatum*

Disease severity showed responses similar to plots receiving two applications and at 10 days after the third application disease severity increased to 62.33, 74.3 and 93.3% with 2×10^{7}, 2×10^{8} and 2×10^{9} conidia mL^{-1}, respectively.

The results on AUDPC showed similar responses as disease severity. Significant differences were observed between and within application frequencies (Table 3). AUDPC increased with increasing inoculum concentration up to 40 days after application, where triple application with 2×10^{9}conidia mL^{-1} gave the highest AUDPC value (3733.20) and a single application of 2×10^{7} conidia mL^{-1} gave the lowest AUDPC value (1732). No disease symptoms developed on the maize crop and AUDPC was zero for all treatments (Table 3).

The itchgrass dry weight at 40 days after treatment showed significant differences between and within application frequencies (Table 3). Plots receiving triple application gave lower dry weight compared to double or single application and the control. On the other hand, maize dry weight at 40 days after treatment showed no significant differences between and within application frequencies (Table 3). Glyphosate herbicide resulted in 100% reduction in the itchgrass as well as maize dry weight. Within each frequency 2×10^{9} conidia mL^{-1} gave the lowest itchgrass dry weight. Reduction in above ground dry biomass with triple application was 90.4%, 70.4% with double application and only 52.3% with single application compared to the control (Fig. 10).

DISCUSSION

Obtained results demonstrated the effectiveness of *E. prolatum* to control itchgrass under the field conditions. Data on disease severity, AUDPC and dry weight values were showed that *E. prolatum* was significantly reduced the itchgrass up to 40 days after application compared to the untreated control. Triple applications were gave the highest percentage control compared to double and single applications over the 6 weeks. Within frequencies greater increase in disease severity and AUDPC and reduction in weed dry weight values were obtained at the higher inoculum concentration. The findings indicate that the application frequency and inoculum concentration of *E. prolatum* has greatly affected the itchgrass control under field conditions. Other researchers working with different weed species (such as *Cyperus rotundus*, *Echinochloa* sp.) have also reported increasing weed control efficacy with increasing application frequency and inoculum concentration (Zhang and Watson, 1997; Kadir and Charudattan, 2000; Chandramohan *et al*., 2002). Single application of glyphosate herbicide completely killed the itchgrass at 3 to 4 leaf stage; however, it also completely killed 7 to 8 leaf stage of maize plants. This was expected because glyphosate is a broad spectrum, non-selective herbicide that can kill or suppress many annual and perennial plants. In terms of relative reduction in weed dry weight, the single, double and triple applications with 2×10^9 conidia mL^{-1} gave 53, 72 and 88% reduction, respectively, in the first experiment and 62.4, 70.4 and 90.4% in the second experiment as compared to the 100% reduction obtained with glyphosate.

In this study, although *E. prolatum* did not completely kill the itchgrass under field conditions, reduction in above ground dry biomass was significant. Similarly, Dewey *et al.* (1997) opined that weed control studies have shown that a 100% weed kill is not needed for maximum yields. Weed dry weight reductions of 70% generally provided acceptable levels of weed control and weed reductions in excess of 80% are generally considered good. Complete kill of the target weed is not necessary for a mycoherbicide since suppression or depletion may achieve the desired level of control (Templeton and Heiny, 1990; Watson and Wymore, 1989). Absence of disease development on maize plants indicates that the fungus is safe to be used in the cropping system. However, studies in combination with other control methods could provide more options for sustainable management of the itchgrass. The findings indicate that at both sites, application frequency and inoculum concentration greatly influence the itchgrass control. Better controls were observed on the itchgrass plants received three applications with high concentration of *E. prolatum*. Single application of 2×10^9 conidia mL^{-1} of the *E. prolatum* conferred reduce control of the itchgrass. This may be due to the rapid growth of the itchgrass under favourable conditions and to its ability to re-growth by developing tillers and secondary branches from the axillary buds. Double applications of 2×10^9 conidia mL^{-1} *E. prolatum* gave acceptable levels of the itchgrass control and if integrated with other control methods such as cultural, mechanical or application of reduced rates of chemical herbicide before or after application of the pathogen, could provide excellent control of the weed. Other researchers have reported good control of the itchgrass when combined pathogen application with other control methods (Ellison and Evans, 1995; Zuniga *et al.*, 2000). Glyphosate herbicide provided complete control to the itchgrass, however the results indicate that it is not safe to be used in maize. Additionally, increasing concerns of environmental pollution due to chemical herbicides and good control of the itchgrass obtained by three applications 2×10^9 conidia mL^{-1} of the *E. prolatum* make it the right sustainable control method for the itchgrass.

Early weed control is important to minimize competition and subsequent yield losses. Only 2 weeks of the itchgrass interference after planting reduced sugarcane yield and a 10 weeks weed free period after planting is needed to maximized sugarcane yield (Dalisay and Mercado, 1985). Critical period of competition of the itchgrass to maize occurred within the first 20 to 45 days and up to 60 days after planting (Rojas *et al.*, 1993). Bridgemohan *et al.* (1992) also noted the critical period of the itchgrass interference on maize was from 0-63 days after emergence and this resulted in 50% reduction yield. Results of the our study indicate that the *E. prolatum* effectively control the itchgrass under field condition in non-crop situation and with maize. Additionally, three application of the *E. prolatum* at concentration of 2×10^9 conidia mL^{-1} effectively control the itchgrass plants up to 6 weeks. This weed free time is essential for corn for maximum yield.

CONCLUSION

Obtained results demonstrate that *E. prolatum* effectively control of the itchgrass under the field conditions. A high disease level and significant weed dry weight reduction were observed in both field experiments. Frequency of application and inoculum concentration were important factors in determining efficacy of the *E. prolatum* under field conditions. Three application of the pathogen gave the highest percentage control compared to double and single applications and within the frequencies, 2×10^9 conidia mL^{-1} resulted in maximum control followed by 2×10^8 and 2×10^7 conidia mL^{-1} compared to the untreated control over the 6 week study period. The prevailing environmental conditions were apparently favourable for the *E. prolatum* to grow and cause disease.

As expected glyphosate herbicide completely killed the itchgrass, but was non-selective on the maize. The *Exserohilum prolatum* can effectively control the itchgrass in non-crop situations and without injury in the maize. However, two to three applications would be necessary to provide acceptable level of the itchgrass control during the critical period of competition to the maize crop. The high degree of weed control under the field conditions and excellent safety to crops indicate that the *E. prolatum* is a highly promising bio-control agent control of the itchgrass in Peninsular Malaysia. Further research is required for possible integrating of the *E. prolatum* with chemical, cultural and mechanical methods for more effective control of the itchgrass while ensuring prevention of ecological shifts in weed populations.

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