J. Agrobiotech. **Vol. 4**, 2013, p. 43-56.

ISSN 1985-5133 (Press)
ISSN 2180-1983 (Online)

Effect of Inoculum Form of *Exserohilum longirostratum* on Disease Development

Hailmi M. S.[1], Kadir J.[2], Ng S. C.[2] and Rahim A. A.[3]

[1]Department of Agricultural Science, Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, 21300 Kuala Terengganu, Terengganu Darul Iman, MALAYSIA.
[2]Department of Plant Protection, [3]Department of Soil Science, Universiti Putra Malaysia (UPM), 43400 UPM Serdang, Selangor Darul Ehsan, MALAYSIA.

***Corresponding author; E-mail: mhailmi@unisza.edu.my**

ABSTRACT

Two types of inoculum (spore suspension and mycelium suspension) of *Exserohilum longirostratum* were tested on barnyard grass (*Echinochloa cruss-galli*) to compare the efficacy of mycelial base-suspension and spore-base suspension as two different substrata on the disease severity. The best result of both suspensions was selected to investigate an easy, rapid and inexpensive treatment as inoculum type used. The results showed that a concentration of 10^5 spores/mL in 30% oil of *E. longirostratum* incited higher disease severity compared to 10^7 and 10^8 spores/mL. However, no significant difference was observed between treatments of 10^5 and 10^6 spores/mL. In a mycelium base study, 20% oil of 5 day-old mycelial-shake cultures presented a better result compared to 3 day-old and 7 day-old cultures. Moreover, 1:5 ratio of 5 day-old mycelium suspension showed significantly higher disease severity than ratios of 1:3, 1:4 and 1:6.

Keywords: *Exserohilum longirostratum*, *Echinochloa crus-galli*, mycelium suspension, spore suspension

ABSTRAK

Dua jenis inokulum *Exserohilum longirostratum* (ampaian spora dan ampaian miselium) telah diuji kepada rumput sambau (*Echinochloa crus-galli*) untuk membandingkan kemujaraban kedua-dua inokulum berasaskan miselium dan berasaskan spora sebagai dua substrat berbeza terhadap keterukan penyakit. Keputusan terbaik dari kedua-dua jenis inokulum tersebut telah dipilih untuk menguji tahap mudah, pantas dan kos efektif inokulum tersebut. Hasil kajian menunjukkan kepekatan 10^5 spora/mL pada 30% minyak *E. longirostratum* telah

menyebabkan tahap keterukan penyakit yang lebih tinggi berbanding dengan 10^7 dan 10^8 spora/mL. Walaubagaimanapun, tiada perbezaan bermakna di antara rawatan pada 10^5 dan 10^6 spora/mL. Pada kajian inokulum berasaskan miselium, 20% minyak pada kultur miselium-goncang yang berusia 5 hari menunjukkan keputusan yang lebih baik berbanding dengan kultur yang berusia 3 hari dan 7 hari. Tambahan lagi, nisbah 1:5 pada miselium yang berusia 5 hari menunjukkan keterukan penyakit yang lebih tinggi berbanding dengan nisbah 1:3, 1:4 dan 1:6.

Kata kunci: *Exserohilum longirostratum*, *Echinochloa crus-galli*, ampaian miselium, ampaian spora

INTRODUCTION

The production of fungus inoculum is a lengthy and laborious process. An easy and rapid technique is required to reduce the cost of producing bioherbicides. Although spore suspensions were initially used in weed control, the mycelium has also been used (Merwe, 1992; Shabana *et al.,* 2001; Neumann and Boland, 2002). It is easier to produce mycelium as liquid culture to be used, and it can be harvested earlier than conidia. In the quest to use fresh mycelium as inoculum, several new methods for mass production have been developed (Garbayc, 1991; Durand, 1995). Although both conidia and mycelium are effective in causing infection, the requirement for leaf wetness has been a constraint in their use against weeds. A delay in the onset of dew after applying the inoculum can result in greatly lower infectiveness, although this constraint can be overcome by formulating the bioherbicide in an oil emulsion (Amsellem *et al.,* 1991). Ng (2004) reported that applying *Exserohilum longirostratum* spores in an oil emulsion caused more severe disease on barnyard grass than with an aqueous suspension. Shabana (2005) reported that the mycelium of *Alternaria eichhorniae* in oil emulsion caused greater disease incidence and severity on water hyacinth (*Eichhornia crassipes*) than with an aqueous carrier.

Although the spores of *Exserohilum longirostratum* have been reported effective in controlling barnyard grass (Ahmad 2004), the use of the mycelium has yet to be documented. Therefore, this study was initiated to evaluate the different inocula (spores and mycelium) of *E. longirostratum* in infecting barnyard grass, and their effectiveness in controlling the weed.

MATERIALS AND METHODS

Plant Production

Seedlings of barnyard grass were taken from Tanjung Karang, Selangor, Malaysia, and were produced in flats in the glasshouse, then transplanted at the cotyledon stage into round pots (10 cm diameter × 10.6 cm height; 5 seedlings/pot) containing a potting medium (3:2:1 top soil: sand: organic matter). The plants were watered twice daily and allowed to grow until the 3- to 4-leaf stage (LS).

Conidia Production

A small mycelia plug from a stock culture was aseptically transferred to fresh modified V8 agar. The plates were incubated for 1-2 days (25 °C, 12 h light/dark, 35 $\mu E/m^2/s$) until adequate colony growth was observed. Mycelia plugs from the margins of a growing young colony were transferred to fresh plates of V8 agar (5 plugs per plate). The plates were incubated as before for two weeks. To harvest the conidia, the agar plates were flooded with 10 mL distilled water and the conidia were scraped off the agar surface with a rubber spatula. The resulting conidial suspensions were passed through a single layer of cheesecloth. The concentration of conidia was determined with a Haemocytometer, and the conidia suspension was adjusted to the desired concentration by dilution in water.

Mycelium Production

Five mycelium plugs from the margin of a young growing colony were transferred to three 1 L flasks containing 300 mL V8 broth with 20% palm oil (V8 broth:oil v/v) (Ng, 2004) and 0.05% Maxigreen, a non-ionic surfactant Maxigreen (Sales Wide Sdn. Bhd.). The flasks were shaken at 100 rpm for 3, 5 and 7 days at 28 °C on a rotary shaker to obtain mycelium of different maturity. The mycelial pads were drained and blended in a Waring Blender for 15 seconds before separating into different ratios of mycelium weight and V8 juice (1:3, 1:4, 1:5 and 1:6 w/v).

Effect of Conidia on Disease Development

To study the effect of inoculum spore concentration, barnyard grass at the 3- to 4-LS were inoculated with four concentrations of spore suspension (10^5, 10^6, 10^7 and 10^8 spores/mL) in three oil concentrations (10%, 20% and 30%) with 0.05% Maxigreen. Spraying was done with approximately 15 mL of the suspensions. The control was a suspension containing only 0.05% Maxigreen. There were four replicates. The disease severity was assessed 24 h after inoculation by using the scale of Kadir *et al.* (2000).

Effect of Mycelium on Disease Development

Mycelium of different maturity (3, 5 and 7 days) was tested on the seedlings in four ratios of mycelium weight and V8 broth (1:3, 1:4, 1:5 and 1:6 w/v). Spraying was done with approximately 15 mL of the suspensions. The control was a suspension containing only 0.05% Maxigreen. There were four replicates. The disease severity was assessed 24 h after inoculation by using the scale of Kadir *et al.* (2000).

Data Analysis

All experiments were done twice with the treatments in each experiment replicated four times. The experiments were carried out in a completely randomized design. All the percentages were arcsine-transformed for analysis (Gomez and Gomez, 1984). Since the data for the two trials have equal variance, they were pooled. The statistical analysis was by the standard Statistical Analysis System (SAS Institute, Cary, NC, 2001). Mean separation was done if the treatments showed significant differences by Tukey's HSD test.

RESULTS

Disease Symptoms

Disease symptoms were observed just 24 h after inoculation. All the seedlings were infected, whether sprayed with spores or mycelium. The initial symptoms were 'water-soaked' lesions starting off at the tips of the leaf blades as necrotic specks which then coalesced into discrete lesions. The lesions then spread throughout the plants causing blight. The tips and edges of infected leaves turned dark green and brown and eventually dried up (Figure 1). Seedling mortality occurred within a week in the treatment with 30% oil concentration. The control plants sprayed only with surfactant were not affected at all and remained healthy throughout the experiment.

Effect of Conidia on Disease Development

The seedlings of all the barnyard grass inoculated with conidia initially developed watery spots on the leaves which then coalesced into whole necrotic areas. The Area Under Disease Progress Curve (AUDPC) values of all the spore concentrations in 20% oil and 30% oil did not differ significantly except for 10^8 spores/mL. At high oil concentration, the amount of infective inoculum used did not appear as a factor affecting its efficacy. This suggests that barnyard grass can be controlled with rather lower conidia concentrations than most of those reported in the literature (Zhang and Watson, 1997). All the spore suspensions in 30% oil caused high disease severity and faster disease progress as indicated by the high

values of their epidemic rates (r_L = 2.50, 2.73, 2.30 and 1.98 logit/day, respectively) (Table 1). The oil emulsion improved the effectiveness of the pathogen applied. However, at a lower oil concentration of 20%, the spore concentration was a factor in the infection. The disease severity was not significantly different between the treatments inoculated with 10^5, 10^6 and 10^7spores/mL in 20% oil, but was lower with 10^8 spores/mL. Substrate exhaustion and the accumulation of auto-inhibitory substrances as a consequence of overcrowding (Cooke and Whipps, 1993), or the run-off of conidia may act as the primary factors involved.

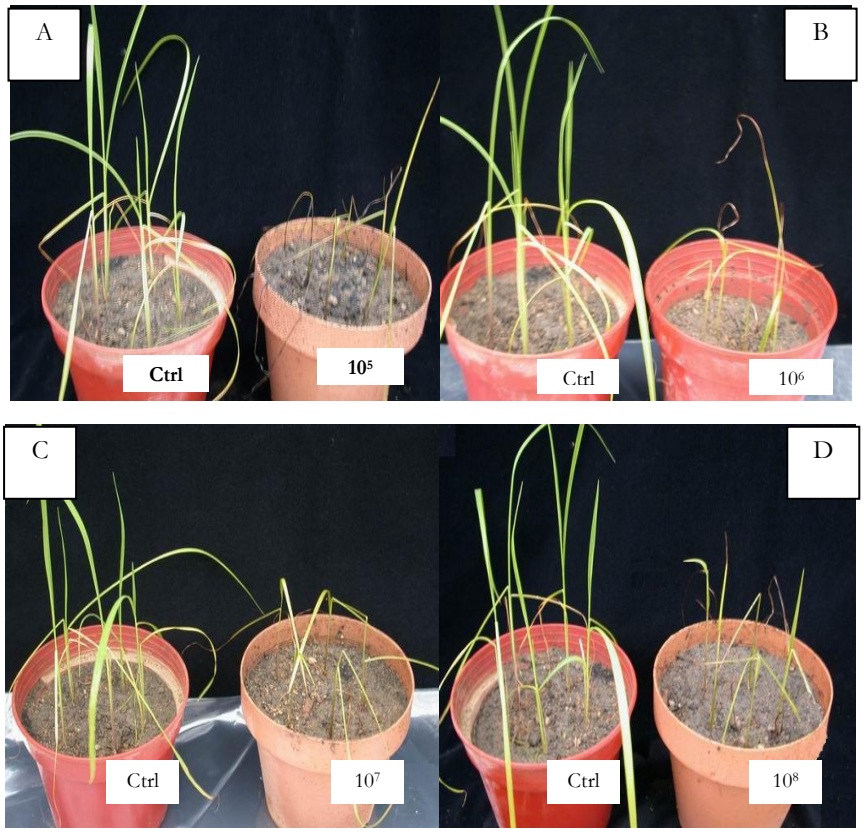

Fig. 1. Effect of different spore concentrations on disease development of 3- to 4-LS barnyard grass; 10^5 spores/mL (A), 10^6 spores/mL (B), 10^7 spores/mL (C), 10^8 spores/mL (D) and non-inoculated controls (Ctrl)

Table 1. Effects of different concentrations of oil and spore on disease severity [as represented by the Area Under Disease Progress Curve (ADUPC)]. Each figure is the mean of percentage disease severity readings taken.

Spore concentration (per mL)	Oil concentration (%)	AUDPC (mean)*		Apparent disease progress rate (logit/day)*	
10^5	0	7.5	d	0.47	cd
	10	138.13	bc	1.33	bcd
	20	221.25	a	1.44	abcd
	30	243.75	a	2.50	ab
		201.04	A	1.76	A
10^6	0	7.5	d	0.47	cd
	10	116.88	bc	0.31	d
	20	213.75	a	1.52	abcd
	30	223.13	a	2.73	a
		184.58	A	1.52	A
10^7	0	7.5	d	0.47	cd
	10	96.88	c	0.52	d
	20	215.63	a	1.23	bcd
	30	230.63	a	2.30	ab
		181.04	A	1.35	A
10^8	0	7.5	g	0.47	cd
	10	121.88	e	0.78	cd
	20	153.13	d	0.82	cd
	30	237.50	ab	1.98	abc
		170.83	A	1.19	A

*Means with the same capital letter within the same column are not significantly different from each other ($P > 0.05$) according to Tukey's HSD test.

*Means with the same small letter within the same column are not significantly different from each other ($P > 0.05$) according to Tukey's HSD test.

Disease Progress

The disease progress on barnyard grass caused by conidia infection (assessed by the disease severity) is shown in Figure 2. It follows a typical sigmoid curve. The disease had a short incubation period (a few hours). Initially, the progress was slow, but picked up after just one day and reached the maximum severity (100%) in four days, eventually killing all the inoculated seedlings. However, in 10% oil, the disease declined after day 3 for all the spore concentrations with the exception of 10^8 spores/mL. Such progress is described by the logistic growth model (Fig. 2). The r_L values (representing the apparent epidemic rates) for 30% oil at all spore concentrations were higher than those for 10% and 20% oil. The disease progress was not significantly different between all the spore concentrations in 30% oil. The overall apparent infection rates are shown in Table 1. This fungus is not capable of causing secondary infection, so no disease was observed in the control plants placed close to the infected plants.

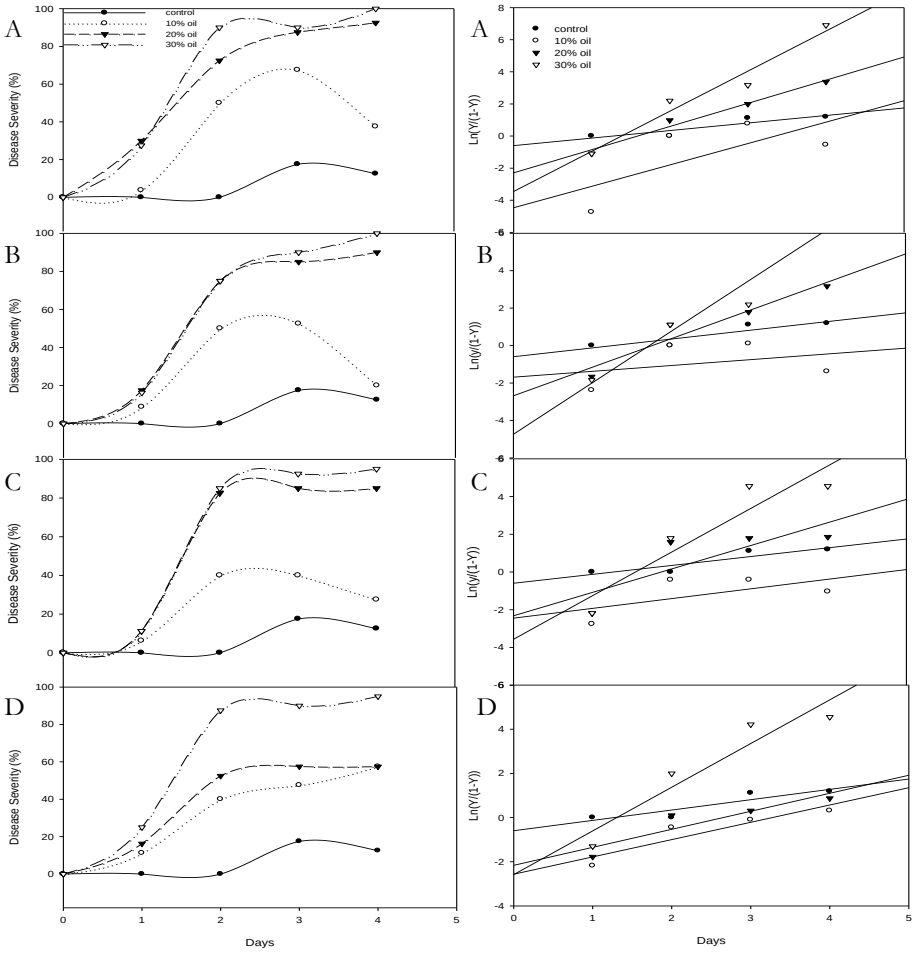

Fig. 2. Disease progress on barnyard grass caused by different spore concentrations of *E. longirostratum* in 10%, 20% and 30% oil. (A) 10^5, (B) 10^6, (C) 10^7 and (D)10^8 spores/mL. The graphs were plotted with the untransformed disease severity values (graphs on the left), but the regressions were fitted on the transformed disease severity values using the logistic model Ln (Y/(1-Y)) (graphs on the right).

Effect of Mycelium in Causing Disease

The age of the mycelium used did not affect the intensity of disease produced on barnyard grass. The use of mycelium as the inoculum produced similar symptoms as with conidia (Figure 3). The effect of 3-DO, 5-DO, and 7-DO mycelium at 1:4, 1:5 and 1:6 (wt:vol) were not significantly different in their ability to cause severe

disease. The amount of disease that was produced was similar for all ages of mycelium (Table 2).

Table 2. Effect of the age and ratio of the mycelium suspension on mean disease severity as represented by the Area Under Disease Progress Curves (AUDPC) and disease progress rates of *E. longirostratum* as represented by the slopes of the regression lines.

Mycelium age (DO)	Mycelium ratio (g/mL)	AUDPC (mean)*	Disease progress rates (logit/day)*
3	0	5 e	0.69 b
	1:3	178.75 cd	0.49 b
	1:4	238.75 ab	0.46 b
	1:5	223.75 ab	0.47 b
	1:6	227.50 ab	0.57 b
		217.19 A	0.43 B
5	0	5 e	0.69 b
	1:3	171.25 d	0.38 b
	1:4	218.75 abc	0.54 b
	1:5	247.5 a	2.27 a
	1:6	223.75 ab	0.49 b
		215.31 A	0.92 A
7	0	5 e	0.69 b
	1:3	202.50 bcd	1.04 b
	1:4	203.75 abcd	1.02 b
	1:5	227.50 ab	1.10 b
	1:6	168.75 d	1.10 b
		200.63 A	0.06 A

*Means with the same capital letter within the same column are not significantly different from each other ($P > 0.05$) according to Tukey's HSD test.

*Means with the same small letter within the same column are not significantly different from each other ($P > 0.05$) according to Tukey's HSD test.

The amount of mycelium used did influence the severity of disease caused. At higher mycelium ratios (1:4, 1:5 and 1:6 w/v), the mycelium caused significantly higher severity than the lower ratio. The 1:5 mycelium ratio seemed ideal as it caused severe infection with all the different-aged mycelium tested (Table 2) although the 5-DO mycelium caused the fastest disease progress as indicated by its higher disease epidemic rate (r_L) (Table 2) over those of the others. Therefore, a 5-DO mycelium suspension with 1:5 ratio (wt:vol) which produced a faster disease progress rate apparently is effective as the inoculum for the barnyard grass control.

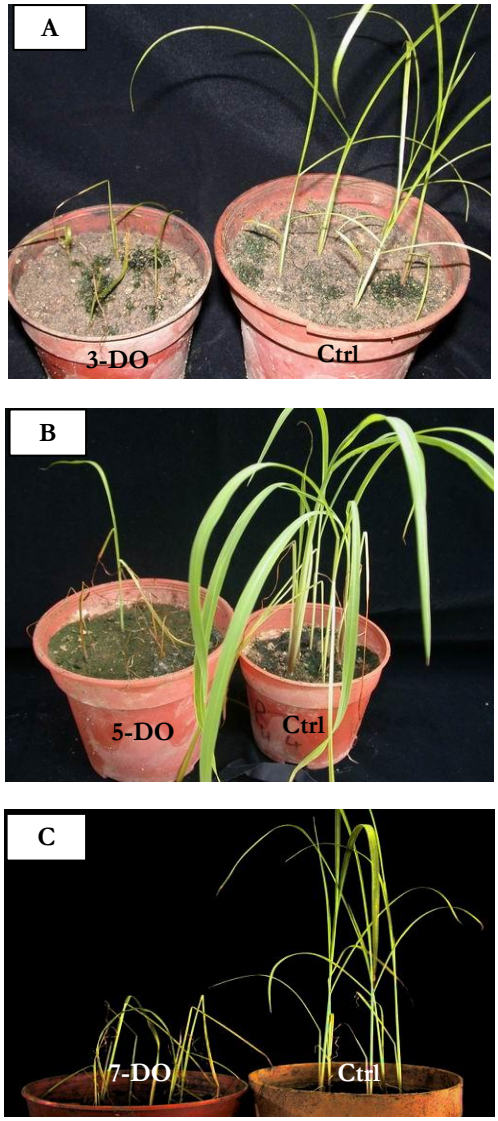

Fig. 3. Effects of culture age of the mycelium [3 day-old (A), 5 day-old (B) and 7 day-old (C)] on disease development of the 3- to 4-LS barnyard grass as compared to the control (Ctrl) after 4 days of inoculation.

Disease Progress

Disease progress caused by *E. longirostratum* mycelium is shown in Figure 4. There was no incubation period for the 3-DO and 5-DO mycelium; the disease started straight from infection with increasing severity until the maximum 100% severity in

four days with 5-DO mycelium, killing all the seedlings. The disease caused by both 3-DO and 5-DO mycelium both showed logarithmic growth. However, the disease caused by 7-DO mycelium progressed by a sigmoid growth with an initial short incubation period (Figure 4), and is best described by the logistic growth model (Figure 4).

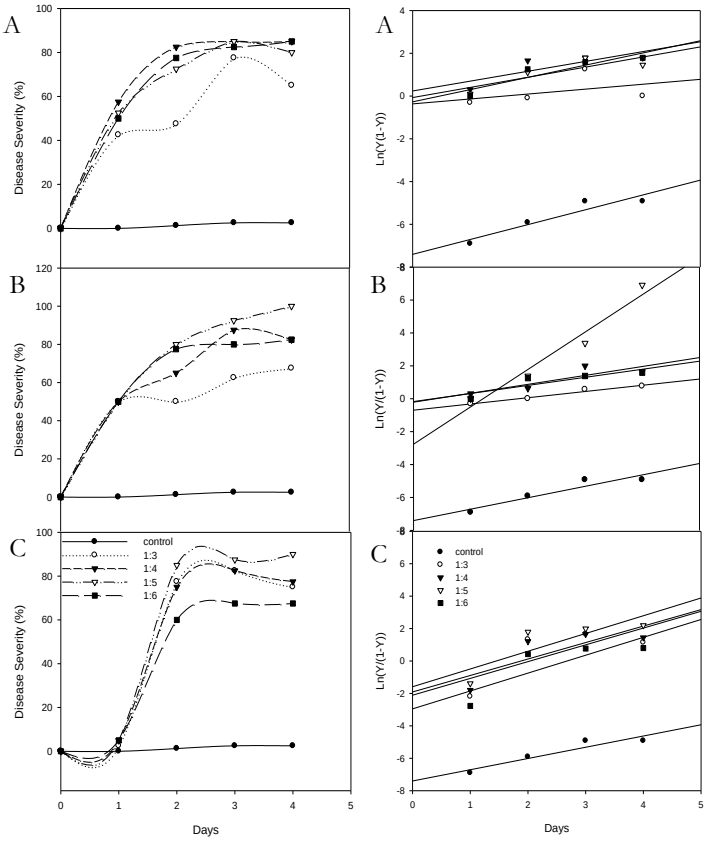

Fig. 4. Disease progress on barnyard grass caused by *E. longirostratum* mycelium in different ratios of mycelium weight:V8 broth: 1:3, 1:4, 1:5 and 1:6. (A) 3 day-old, (B) 5 day-old, and (C) 7 day-old mycelium age. The graphs are plotted using the untransformed disease severity values (graphs on the left) while the regressions were drawn through the transformed disease severity values using the logistic model Ln (Y/(1-Y)) (graphs on the right).

The apparent infection rates of all mycelium at different ages are shown in Table 2.

Comparison of Spore versus Mycelium Suspension

Both the conidia and mycelium of *E. longirostratum* have proven to be pathogenic to barnyard grass. Their efficacies are now compared. A suspension of 10^5 spores/mL in 30% oil concentration and 5-DO mycelium in 1:5 ratio of mycelium weight:V8 broth were used to compare their weed control efficacy. Both these suspensions had the highest AUDPC values in the previous assessments of conidia and mycelium.

The disease progress for both inoculum types was best described by the logistic growth model (Figure 5). The overall apparent infection rate caused by the conidia was r_L = 2.50 logit/day (r^2 = 0.96) while that by the mycelium was r_L = 2.27 logit/day (r^2 = 0.96). Regressions of the disease progress in time to reach 50% severity are shown in Table 4.5. It took 1.4 days for the conidia and 1.2 days for the mycelium to reach 50% disease severity, and were not significantly different from each other, thus they had similar effectiveness in controlling barnyard grass. However, the time and materials required for production favor the use of mycelium as inoculum.

DISCUSSION

The number of viable inoculum is often linked to good biocontrol of weeds (Charudattan, 2000). If the inoculum concentration is too low, infection may be inadequate giving poor control. There have been some estimates that the inoculum concentration should be higher than 10^6 spores/mL in order to maintain effective control, because spore germination is often low due to auto-inhibition between the spores (Vanderplank, 1975; Heiny and Templeton, 1991; Makowski, 1993). However, in this study, there was no instance of very low spore concentration of *E. longirostratum* causing in adequate infection on barnyard grass. Spraying the conidia in a suspension of 30% oil at all the concentrations tested caused severe disease.

In this study, 10^5 spores/mL of *E. longirostratum* was sufficient for excellent control of barnyard grass in the glasshouse. Nonetheless, it may still be inadequate in the field without a prolonged dew period. This limiting factor can be circumvented by application of spores in an oil emulsion. Simple vegetable oil emulsions have shown promise in reducing dew dependence (Auld *et al.,* 2003; Ng, 2004). Less water loss can be expected from leaves covered with a thin film of oil as compared to that from untreated leaves (Shabana, 2005). Indeed, the use of an oil emulsion or invert emulsion (Amsselem *et al.,* 1990; Boyette *et al.,* 1993; Womark *et al.,* 1996) of water-in-oil-in-water (WOW) (Auld, 2002) may not only reduce the dew dependence but also reduce the spore concentration requirement.

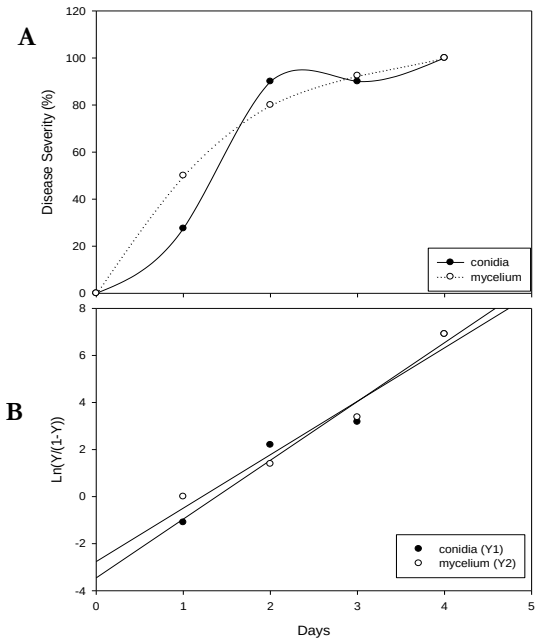

Fig. 5. Disease progress on barnyard grass caused by the conidia and mycelium of *E. longirostratum*. The graphs in (A) are plotted using the untransformed disease severity values, while in (B) the regressions were fitted using the transformed disease severity values in the logistic model Ln (Y/(1-Y)). The regressions obtained are: Y1 = -3.45 + 2.50X (r^2 = 0.96) (conidia) and Y2 = -2.76 + 2.27X (r^2 = 0.96) (mycelium).

The oil itself can be toxic to the weed seedlings predisposing the seedlings to the activity of the inoculum (Egley *et al.,* 1993). In the development of the disease, another mechanism may be involved in increasing the infectivity on barnyard grass. One of the important enzymes in the infection process, cutinase, has been reported to be induced by a fatty acid monomer of cutin (Woloshuk and Kolattukudy, 1986). The fatty acid constituent of the oil may have induced production of cutinase, establishing the infection without the need for a long dew period.

The pathogenic variables that may influence the quality and quantity of a fungal inoculum are its type, age and concentration. The age of the mycelium is one of the most important. Neumann and Boland (2002) reported that 5-DO mycelium of *Phoma herbarum* incited higher disease severity on *Taraxacum officinale* as compared to 3-, 7-, 9-, 11- or 13-DO culture. In this glasshouse study, 5-DO mycelium was the best inoculum, used in the ratio of 1:5 (w/v).

Historically, the active ingredients in bioherbicides have been spores despite the difficult mass production of infective conidia. But mycelium has been

tried and generally found to be as infective (Shabana *et al,* 2001; Neumann and Boland, 2002). Amsellem *et al.* (1999) stated that highly virulent bioherbicides have been formulated with the mycelia of *Fusarium oxysporum* Schlecht, *Fusarium aegyptiaca* Pers, *Orobanche ramosa* L. and *Orobanche cernua* Loeftl. Mycelium formulations may, therefore, be preferable, especially if its infectivity is adequate, for it is easier to mass produce than conidia. Mycelium can be propagated in liquid, with less media requirement, whereas conidia have to be produced in solid media. The mycelium of *E. longirostratum* can therefore be used in lieu of its conidia as inoculum for barnyard grass control.

ACKNOWLEDGEMENTS

This research was supported by fund provided by the Ministry of Science, Technology and Innovation (MOSTI) of Malaysia, Grant No.: 01-02-04-0504.

REFERENCES

Ahmad, A. 2004. Potential of *Exserohilum longirostratum* bioherbicide for *Rottboellia cochinchinensis*. M.Sc. Thesis, Serdang, Malaysia: Universiti Putra Malaysia. 171 pp.

Amsellem, Z., Sharon, A. & Gressel, J. 1991. Abolition of selectivity of two mycoherbicidal organisms and enhanced virulence of a virulent fungi by an invert emulsion. *Phytopathology* **81**: 985-988.

Amsellem, Z., Sharon, A., Gressel, J. & Quimby Jr., P. C. 1990. Complete abolition of high inoculum threshold of two mycoherbicides (*Alternaria cassiae* and *A. crassa*) when applied in invert emulsion. *Phytopathology* **80**: 925-929.

Amsellem, Z., Zidack, N. K., Quimby Jr., P. C. & Gressel, J. 1999. Long-term dry preservation of viable mycelia of two mycoherbicidal organisms. *Crop Protection* **18**: 643-649.

Auld, B. A. 2002. Bioherbicidal formulations. **Australian Provisional Patent Application 2002952094.** Patent Office, IP Australia, Canberra.

Auld, B. A., Hetherington, S. D. & Smith, H. E. 2003. Advances in bioherbicide formulation. *Biology and Management* **3:** 61-67.

Boyette, C. D., Quimby Jr., P.C., Bryson, C. T., Egley, G. T. & Fulgham, F. E. 1993. Biological control of hemp sesbania (*Sesbania exaltata*) under field conditions with *Colletotrichum truncatum* formulated in an invert emulsion. *Weed Science* **41**: 497-500.

Charudattan, R. 2000. Current status of biological control of weeds. In *Emerging Technologies for Integrated Pest Management: Concepts, Research, and Implementation.* Kennedy, C. G. and Sutton, T. B. (eds.). APS Press, St. Paul, MN. p. 269-288.

Cooke, R. C. & Whipps, J. M. 1993. *Ecophysiology of Fungi.* Blackwell Scientific Publications, Oxford, U.K. 337 pp.

Durand, A. 1995. The INRA-Dijon reactions: Design and applications. *Abstracts of 2nd International Symposium on Solid State Fermentation*. Montpellier, France.

Egley, G. H., Hank, J. E. & Boyette, C. D. 1993. Invert emulsion droplet size and mycoherbicidal activity of *Colletotrichum truncatum*. *Weed Technology* **7**: 417-424.

Garbayc, J. 1991. Utilisation des mycorhizes on sylviculture. In *Les Mycohizesdes Arbreset Plantes Cultivees*. P. G. Trullu (ed.). Lavoisier, Paris, France.

Gomez, K. A. & Gomez, A. A. 1984. *Statistical Procedures for Agricultural Research*. John Wiley & Sons, New York. 407 pp.

Heiny, D. K. & Templeton, G. E. 1991. Effects of spore concentration, temperature and dew period on disease of field bindweed caused by *Phoma proboscis*. *Phytopathology* **81**: 905-909.

Kadir, J., Charudattan, R. & Begger, R. D. 2000. Effects of some epidermiological factors on levels of disease caused by *Dactylaria higginsii* on *Cyperus rotundus*. *Weed Science* **48**: 61-68.

Makowski, R. M. D. 1993. Effect of inoculum concentration, temperature, dew period, and plant growth stage on disease of round-leaved mellow and velvet leaf by *Colletotrichum gloeosporioides* f. sp. *malvae*. *Phytopathology* **83**: 1229-1234.

Merwe, M. D. V. A. D. 1992. An improved method to evaluate Avocado root stocks for resistance to *Phytophthora cinnamomi*. *Proceedings of the Second World Avocado Congress*. p. 101-104.

Neumann, S. & Boland, G. J. 2002. Influence of host and pathogen variables on the efficacy of *Phoma herbarum,* a potential biological control agent of *Taraxacum officinale*. *Canadian Journal of Botany* **80:** 425-429.

Ng, L. C. 2004 Enhancing control efficacy of *Echinochloa crus-galli* by *Exserohilum longirostratum* using oil emulsion. *Bac. Sc. Thesis*. Universiti Putra Malaysia, Malaysia.

SAS Institute. 2001. *Statistical Analysis Systems, Version 8.02*. Cary, NC.

Shabana, Y. M. 2005. The use of oil emulsions for improving the efficacy of *Alternaria eichhorniae* as a mycoherbicide for water hyacinth (*Eichhornia crassipes*). *Biological Control* **32**: 78-89.

Shabana, Y. M., Elwakil, M. A. & Charudattan, R. 2001. Biological control of water hyacinth by a mycoherbicide in Egypt. In *Biological and Integrated Control of Water Hyacinth, Eichhornia crassipes. ACIAR Proceedings 102*. Julien, M. H., Hill, M. P., Center, T. D. and Jianqing, D. (eds.). p. 53-56.

Vanderplank, J. E. 1975. *Principles of Plant Infection*. Academic Press, London. 216 pp.

Woloshuk, C. P. & Kollattukudy, P. E. 1986. Mechanism by which contact with plant cuticle triggers cutinase gene expression in the spores of *Fusarium solani* f. sp. *pisi*. *Proceedings of the National Academy of Sciences USA* **83**: 1704-1708.

Womark, J. G., Eccleston, G. M. & Burge, M. N. 1996. A vegetable oil-base invert emulsion for mycoherbicide delivery. *Biological Control* **6**: 23-28.

Zhang, W. M. & Watson, A. K. 1997. Effect of dew period and temperature on the ability of *Exserohilum monoceras* to cause seedling mortality of *Echinochlo*a species. *Plant Disease* **81**: 629-634.