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Efficacy of *Exserohilum monoceras*, a potential fungi for biocontrol of *Echinochloa* species

(Keberkesanan *Exserohilum monoceras*, kulat yang berpotensi sebagai agen kawalan biologi spesies *Echinochloa*)

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Keywords: *Echinochloa*, *Exserohilum monoceras*, biocontrol agent, bioherbicide

Abstract

Indigenous *Exserohilum monoceras* isolate 1125 was evaluated for its efficacy on different *Echinochloa* species. *Exserohilum monoceras* spores, prepared at 10^3, 10^4, 10^5, 10^6 and 10^7 spores/ml concentrations and sprayed onto *E. crus-galli*, showed increased infectivity with increasing spore concentration. Disease progress of *E. crus-galli* at various leaf development stages revealed the highest area under the disease progress curve (AUDPC) was at the 4-leaf stage (535.69 unit^2), followed by the 3-leaf (446.75 unit^2), 2-leaf (377.22 unit^2) and 6-leaf stage (263.72 unit^2). The fungus was also tested on 4 species of *Echinochloa*. The results showed that the AUDPC was highest for *E. crus- galli* var *formosensis* (693.33 unit^2), followed by *E. crus-galli* var *crus-galli* (638.33 unit^2), *E. oryzicola* (470 unit^2) and *E. colona* (447 unit^2). The study illustrated that *E. monoceras* is a potential fungus for biological control that can be further developed into bioherbicide.

Introduction

Echinochloa spp. namely *E. crus-galli*, *E. oryzicola* and *E. colona* can be found throughout the world (Holm et al. 1977). The most widespread and economically important species from the genus is *E. crus-galli*, or barnyard grass (Maun and Barrett 1986) which has been reported to reduce the rice yield by 5–72% (Kuan et al. 1990). In Malaysia, the grass is mainly controlled by chemical herbicides which have contaminated the environment (Leong et al. 2007), affected the microorganism communities (Chen et al. 2009) and led to resistant biotypes of the weed (Sangakkara et al. 2004). Biological control has, therefore, been identified as an alternative method to avoid the negative impacts of chemical herbicides.

Research in biocontrol of *E. crus-galli* has been intensified in Asia in the early 1990s. From these studies, among the fungal pathogens that have been suggested as biocontrol agents were *Cochliobolus lunatus* and *Exserohilum monoceras* [*Septosphaeria monoceras*] (Zhang and Watson 1997a, b, c; Kadir et al. 2008).

Generally, control efficacy increased with increasing inoculum density or concentration. The concentration of

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inoculum that was reported to be effective varies among pathogen species. Zhang (1996) found that *E. monoceras,* at a rate of 10^7 spores/ml, killed *E. crus-galli*. Under glasshouse conditions, Kadir et al. (2000) found the rate of 10^6 spores/ml produced highest infection on *Dactylaria higginsii,* while Ortiz-Ribbing and William (2006) found that *Amaranthus* was controlled by *Phomopsis amaranthicola* and *Microsphaeropsis amaranthus* at a rate of 10^7 spores/ml and 10^6 spores/ml respectively.

Recently, surveys and evaluations of various fungi species and isolates associated with disease of *Echinocloa* spp., from 5 granaries in Peninsular Malaysia showed that *E. monoceras* 1125 was the most potential isolate (Tosiah et al. 2009). The isolate was evaluated for its pathogenicity against *Echinochloa* under glasshouse conditions. The objectives of the study were: 1) to determine the optimal dosage of *E. monoceras* 1125 to control *Echinochloa*, 2) to examine the effect of the selected inoculum levels on different leaf stages of *Echinochloa*, and 3) to evaluate the efficacy of the fungus as a biocontrol agent against different *Echinochloa* species.

Materials and methods

Inoculum production

Mycelium from a stock culture of *E. monoceras* 1125 was aseptically transferred to a fresh plate of potato dextrose agar (PDA), sealed with parafilm and incubated at 28 ± 2 °C for 7 days. Agar plugs (5 mm diameter) from the margin of the colony were used as seed inoculum on V-8 juice agar medium for conidia production. An agar plug was placed in the centre of each petri dish, sealed with parafilm and incubated in the dark at 28 ± 2 °C. Conidia were harvested after 14 days by swirling the plates with 10 ml distilled water and scraping the surface with a plastic spatula. The resulting suspension was adjusted to the desired concentrations using a haemocytometer.

Plant production

Seeds of *E. crus-galli* var *crus-galli*, *E. crus-galli* var *formosensis*, *E. colona* and *E. oryzicola* were collected from the fields in Alor Setar, Kedah and Seberang Perai area. The *Echinochloa* seeds were soaked in water for 4 days until incipient germination (coleoptile and radicle emerging). Five germinated seeds were planted in a 10-cm diameter plastic pot filled with clay soil (collected from a paddy field in Tanjung Karang, Selangor). All the pots were placed in a glasshouse at 28–35 °C night/day temperature with 12 h photoperiod.

Application of spore concentrations on Echinochloa crus-galli

Echinochloa crus-galli plants at the 4-leaf stage were used. Spore solutions were made to 10^3, 10^4, 10^5, 10^6 and 10^7 spores/ml concentration. A stock of 10^7 spores/ml was prepared, then diluted 10x to the required concentrations. Maxigreen (a commercial non-ionic two-in-one surfactant) was added at 0.02% (v/v) (Sales Wide Sdn. Bhd.) to prevent clumping of the spores which were sprayed on the plants using a hand sprayer. Twenty (20) mls of spore suspension was used to spray 3 pots containing 5 plants each, the sprayer held 30 cm from the foliage. Immediately after spraying, the plants were covered with a plastic bag for 24 h to maintain the humidity. Control plants were sprayed with sterile distilled water. The plants were maintained in the glasshouse as described earlier. The experiments were repeated twice in a Completely Randomized Design with three replications.

Application of inoculum on different plant growth stages

Echinochloa crus-galli var *crus-galli* plants at the 2, 3, 4 and 6-leaf stages were used. The plants were treated with 10^3, 10^4 10^5, 10^6 and 10^7 spores/ml and applied as described in the earlier experiment.

Application of inoculum on different Echinochloa species

Echinochloa crus-galli var *crus-galli*, *E. crus-galli* var *formosensis*, *E. colona* and *E. oryzicola* at the 4-leaf stage were used as test plants. The experiment was conducted as previously described and a concentration of 10^7 spores/ml was used.

Disease assessment

The disease incidence and severity were assessed every 2 days for 14 days after inoculation. The disease incidence was the number of plants infected by *E. monoceras*, expressed as a percentage of total number of plants inoculated (Horsfall and Cowling 1978; Kranz 1988). The disease severity was assessed on the individual plants by visually estimating the percentage of diseased (necrotic) leaves. The disease severity was rated in 10 classes, viz., 0 = No disease, 1 = <1% diseased leaves, 2 = 1–3%, 3 = 4–5%, 4 = 6–10%, 5 = 11–15%, 6 = 16–25%, 7 = 26–50%, 8 = 51–75% and 9 = 76–100%. A completely collapsed seedling was considered as dead. The disease severity was assessed daily for 14 days, starting from the second day after inoculation.

Data analysis

All percentage data were arc sine-transformed before analysis (Gomez and Gomez 1984) using the SAS 9.1 program (SAS Inst. 2007). Results from two trials of each experiment were pooled if homogeneity of variance was confirmed by the Bartlett's test (Gomez and Gomez 1984).

Disease progress curves were plotted for each treatment, and the area under the disease progress curve (AUDPC) was calculated according to Campbell and Madden (1990). The apparent infection rates (r_L) were calculated by transforming the disease severity data using a logistic model (ln(x/1-x)) as described by Campbell and Madden (1990).

Results and discussion

Infection of spore concentrations on Echinochloa crus-galli var crus-galli

The study showed control plants were not infected, and the disease infections increased with increase in the spore concentrations used. The lowest infection was recorded at 10^3 spores/ml (5%). The initial infection was slow but later accelerated at 10^6 spores/ml (83.05%) and 10^7 spores/ml (84.68%) to reach a plateau (*Figure 1*). Thus, the optimum spore concentration for disease infection was at 10^6 spores/ml.

The AUDPC of *E. crus-galli* also increased with the spore concentrations. It was lowest at 10^3 spores/ml (44.96 unit^2) and highest at 10^7 spores/ml (889.59 unit^2). The AUDPC at 10^6 spores/ml and 10^7 spores/ml were not significantly different (*Table 1*).

The fastest apparent infection rate was observed at 10^7 spores/ml (r_L= 0.43 logit/day) and the slowest at 10^3 spores/ml (r_L= 0.13 logit/day). Apparent infection rate at both concentration levels of 10^5 spores/ml and 10^6 spores/ml was r_L= 0.42 logit/day and was not significantly lower than 10^7 spores/ml. Overall, the disease severity, disease progress and apparent infection

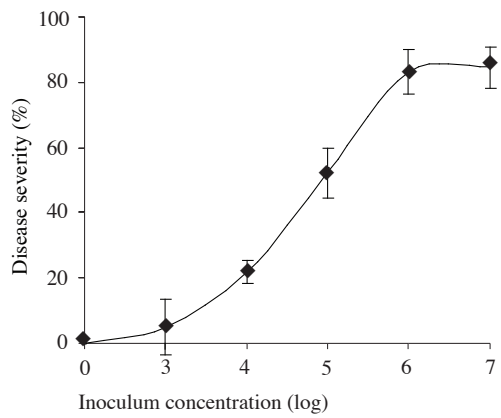

Figure 1. Disease severity on ***Echinochloa crus-galli*** *var* ***crus-galli*** *infected by* ***Exserohilum monoceras*** *at the 4-leaf stage. Data from two trials were pooled because the variance was homogenous. Each point represented means of six replicates. Vertical bars indicate standard errors*

rate had increased with the increase in spore concentrations. The differences were observed as in *Plate 1*.

Increasing the pathogen inoculum density was found to improve the control of *Echinochloa* spp. *Exserohilum monoceras* 1125 inoculum suspension was effective against *E. crus-galli* at10^6 spores/ml. Kadir et al. (2003) observed significantly higher mortality of itch grass (*R. cochinchinensis*) when treated with *E. longirostratum* inoculum suspension at 3.5 x 10^5 spores/ ml. Zhang and Watson (1997a) reported that *E. monoceras* caused 100% mortality of barnyard grass and *E. glabrescens* when applied at 2.5 x 10^7 spores/ml to the plants at the 1.5-leaf stage. However, in this study, a higher dosage (10^7 spores/ml) of *E. monoceras* 1125 did not increase disease development. This may be due to variability in the inocula at the infection sites. This suggests that a lower concentration (10^6 spores/ml) is sufficed to cause severe disease to *Echinochloa*.

The infection rate of *E. monoceras* was observed to increase with spore concentration. However at the higher concentration (10^5 to 10^7 spores/ml), the apparent infection rate was not significantly increased (*Table 1)*. Kadir et al. (2008) found the infection rate of *E. monoceras* at a concentration of 10^5 spores/ml was r_L = 0.48 logit/day while the current finding was r_L = 0.42 logit/day. At the 4-leaf stage the rate was r_L = 0.50 logit/day (*Table 2*). This may due to the different ecotypes (Tasrif 2005) or environmental factors involved during the study.

Effect of spore concentrations on different plant growth stages

Echinochloa at the 2, 3, 4 and 6-leaf stages sprayed with *E. monoceras* inoculum at different concentrations (0, 10^3, 10^4, 10^5,

Table 1. Area under disease progress curve (AUDPC) and apparent infection rate (r_L) of *Echinochloa crus-galli* at different spore concentrations. Data represented means of six replicates

Spore concentration (spores/ml)	Mean AUDPC (unit^2)	Apparent infection rate (r_L) logit/day
0	0d	0b
10^3	44.96 ± 07.69d	0.13 ± 0.02b
10^4	184.08 ± 26.38c	0.29 ± 0.06a
10^5	466.04 ± 53.89b	0.42 ± 0.07a
10^6	850.42 ± 42.39a	0.42 ± 0.07a
10^7	889.58 ± 37.04a	0.43 ± 0.08a

Mean values in the same column with different letters are significantly different (p <0.05) according to Tukey's HSD test

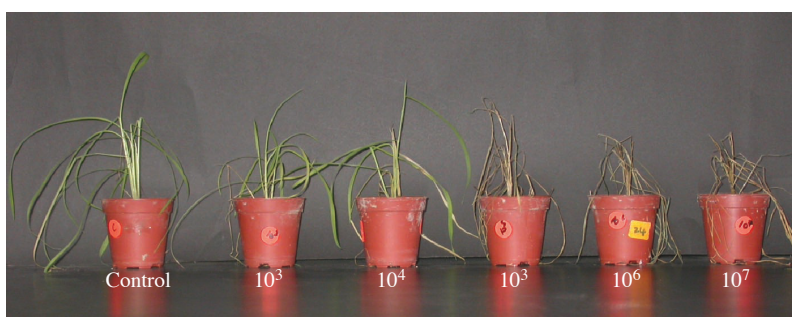

Plate 1. ***Echinochloa crus-galli*** *treated with* ***Exserohilum monoceras*** *at different spore concentrations (spores/ml) 10 days after inoculation*

10^6 and 10^7 spores/ml) showed more severe infection at higher spore concentrations. However, the most susceptible stage was the 4-leaf stage, followed by 2-leaf, 3-leaf and 6-leaf stage (*Figure 2*).

Echinochloa at the 4-leaf stage produced the highest AUDPC (535.69 unit^2) followed by the 3-leaf (446.75 unit^2), 2-leaf (377.22 unit^2) and, finally, 6-leaf stage (263.72 unit^2). The oldest leaf stage showed the lowest AUDPC and appeared to show some resistance to the fungus (*Table 2*).

The apparent infection rate was also influenced by the plant growth stage. The infection rate was fastest at 4-leaf stage (r_L = 0.50 logit/day) and slowest at 6-leaf stage (r_L = 0.05 logit/day). Overall, this study showed that the 4-leaf stage was most susceptible to the disease.

Table 2. Area under disease progress curve (AUDPC) and the apparent infection rate (r_L) for different growth stages of *Echinochloa crus-galli*. Data represented means of six replicates

Plant stage	Mean AUDPC (unit^2)	Apparent infection rate (r_L) logit/day
2-leaf	377.22 ± 71.86c	0.29 ± 0.06b
3-leaf	446.75 ± 68.83b	0.28 ± 0.05b
4-leaf	535.69 ± 72.44a	0.50 ± 0.06a
6-leaf	263.72 ± 39.49d	0.05 ± 0.02c

Mean values in the same column with different letters are significantly different (p <0.05) according to Tukey's HSD test

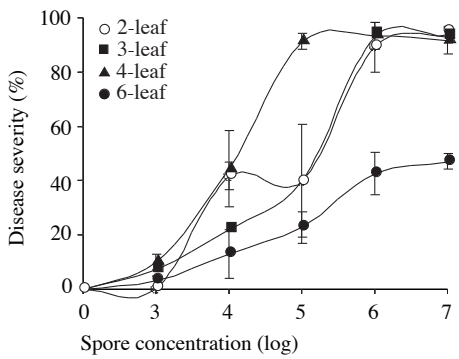

Figure 2. Disease severity on different stages of ***Echinochloa*** *at different spore concentrations. Data from two trials were pooled because the variance was homogenous. Each point represented means of six replicates. Vertical bars indicated standard errors*

Younger plants were reported to be more sensitive to infection (Zhang and Watson 1997a). However, the results indicated that the 4-leaf stage plants were more sensitive than the plants at the 2- and 3-leaf stages. Observations of plant development during this study showed that *Echinochloa* plants at the 2-leaf stage were small and the leaf blades were a bit folded. This offered a smaller landing area for the inocula. Thus only a small amount of inocula was expected to reach the leaves.

In irrigated rice fields, *Echinochloa* reached its peak emergence in about 7–10 days after sowing of rice, and reached the 5-leaf stage in about 15 days after (Azmi 1998). Thus timing the application of herbicides before the critical period of competition between *E. crus-galli* and rice is very important. Applying herbicides beyond the time could drastically reduce rice yield. With proper formulation and timing of application, *E. monoceras* has the potential to be exploited as an alternative bioherbicide for *Echinochloa*.

Effect of inoculum on different Echinochloa species

The fungus infected all the 4 species of *Echinochloa* tested (*Plate 2*). The AUDPC was highest for *E. crus-galli* var *formosensis* (693.33 unit^2), followed by *E. crus-galli* var *crus-galli* (638.33 unit^2), *E. oryzicola* (470 unit^2) and *E. colona* (447 unit^2) (*Table 3*). The AUDPC values for the two *E. crus-galli* species were not significantly different, but were significantly higher than for *E. oryzicola* and *E. colona*.

The disease progress on *E. crus-galli* var *formosensis* was the highest (r_L = 0.61 logit/day), followed by *E. crus-galli* var *crus-galli* (r_L = 0.44 logit/day), *E. colona* (r_L = 0.33 logit/day) and *E. oryzicola* (r_L = 0.28 logit/day) (*Table 3*). Although the fungus infected *E. colona* faster than *E. oryzicola*, the AUDPC of *E. oryzicola* was later found to be higher (*Table 3*).

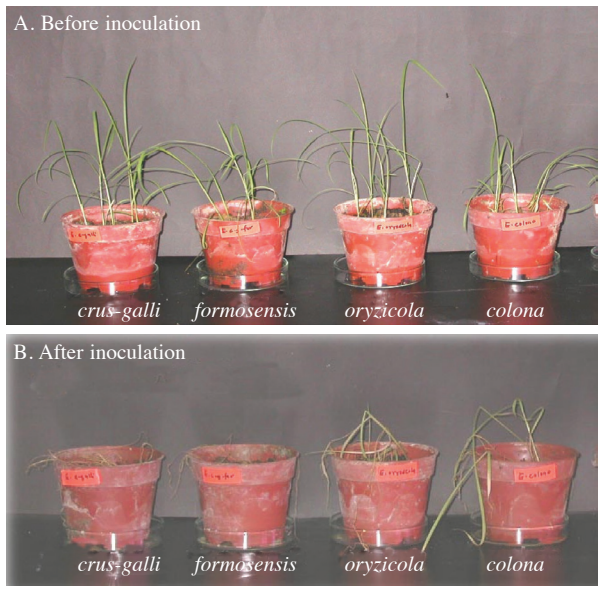

Plate 2. Effects of inoculating ***Exserohilum monoceras*** *at 2.5 x 10^7/ml on different species of* ***Echinochloa*** ***(E. crus- galli*** *var* ***crus galli, E. crus-galli*** *var* ***formosensis, E. crus-galli*** *var* ***oryzicola*** *and* ***E. colona****. A) before inoculation, B) 10 days after inoculation*

Table 3. Apparent infection rate and area under disease progress curve (AUDPC) of different *Echinochloa* species. Data represented means of six replicates

Species	Disease progress rate (r_L)	Mean AUDPC (Unit^2)
E. colona	y = – 3.40 + 0.33x (R^2 = 0.92)	447b
E. crus-galli var *crus-galli*	y = – 2.34 + 0.44x (R^2 = 0.96)	638.33ab
E. crus-galli var *formosensis*	y = – 4.08 + 0.61x (R^2 = 0.84)	693.33a
E. oryzicola	y = – 3.01 + 0.28x (R^2 = 0.94)	470bc

Mean values in the same column with different letters are significantly different (p <0.05) according to Tukey's HSD test. R^2 is the goodness of fit

The study also indicated that *E. colona* and *E. orizycola* were less susceptible to *E. monoceras* infection.

Conclusion

Generally, *E. monoceras* 1125 has the potential to be used as biopesticide for controlling barnyard grass in paddy fields. *Exserohilum monoceras* 1125 spore suspension was effective against *E. crus-galli* at 10^6 spores/ml. A higher dosage of *E. monoceras* 1125 did not increase the disease development. The infection rate of *E. monoceras* was also increased with spore concentration. However, at higher concentration (10^5 to 10^7 spores/ml) the infection rate was not significantly increased due to differences in the ecotypes used or environmental factors such as humidity and the surrounding temperature occurring during the study.

The 4-leaf stage plants were found to be more sensitive to infection of *E. monoceras* since at this stage the plants have significantly larger leaves that offer good landing areas for the inoculum. Thus, to get effective eradication of *E. crus-galli*, the herbicide should be applied at this stage. Applying herbicides beyond this stage may drastically reduce the rice yield.

With proper formulation and timing of application, *E. monoceras* has great potential to be exploited as an alternative bioherbicide for *Echinochloa*. *Exserohilum monoceras* 1125 was also found to infect other *Echinochloa* spp. such as *E. colona, and E. oryzicola* although they were less susceptible than the two *E. crus- galli* varieties. Further studies should be conducted to investigate its effects on other plants or weeds so as to identify other potential hosts of *E. monoceras*.

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Abstrak

Keberkesanan kulat *Exserohilum monoceras* 1125 pencilan tempatan telah dinilai terhadap spesies *Echinochloa* yang berbeza. *Exserohilum monoceras* dengan kepekatan 10^3, 10^4, 10^5, 10^6 dan 10^7 spora/ml yang telah disediakan dan disembur kepada *E. crus-galli* menunjukkan jangkitan bertambah dengan meningkatnya kepekatan spora yang digunakan. Perebakan penyakit pada *E. crus-galli* di pelbagai peringkat menunjukkan peringkat 4-daun memberi kesan tertinggi di bawah keluk perebakan penyakit (AUDPC) (535.69 unit^2) diikuti oleh peringkat 3-daun (446.75 unit^2), 2-daun (377.22 unit^2) dan 6-daun (263.72 unit^2). Kulat ini juga diuji terhadap empat spesies *Echinochloa*. Keputusan menunjukkan AUDPC adalah tertinggi pada spesies *E. crus-galli* var *formosensis* (693.33 unit^2) diikuti oleh *E. crus-galli* var *crus-galli* (638.33 unit^2), *E. oryzicola* (470 unit^2) and *E. colona* (447 unit^2). Keputusan kajian ini menunjukkan *E. monoceras* ialah kulat yang berpotensi untuk kawalan biologi yang boleh dibangunkan sebagai bioherbisid.

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