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Host range of *Exserohilum monoceras* 1125, a potential biological control agent for *Echinochloa* spp.

(Pelbagai perumah untuk *Exserohilum monoceras* 1125, agen kawalan biologi yang berpotensi untuk kawalan *Echinochloa* spp.)

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Keywords: *Exserohilum monoceras*, host range, *Echinochloa* spp., bioherbicide

Abstract

A total of 54 plant species, including varieties and breeding lines from 14 families and 33 genera were screened against *Exserohilum monoceras,* a fungal pathogen with potential as biocontrol agent for barnyard grass, *Echinochloa crus-galli*. The selected plant species were treated with spore suspensions at about 2.5 x 10^7 spores/ml concentration in glasshouse conditions supplemented with 24 h dew. The ability of the fungus to infect the plants was evaluated by disease incidence and disease severity which translated into disease index (DI). The disease index of *Echinochloa crus-galli* var. *crus-galli, E. crus-galli* var. *formosensis* and *E. stagnina* were 4 (dead) while those for *E. colona* and *E. oryzicola* were 3 (severely damage). Among the economic plants tested, only *Zea mays* was infected with a disease index of 2 (tolerant). Young *Imperata cylindrica* was dead (DI = 4), however the older plant showed hypersensitive reaction (DI = 2). Other plants that were infected were *Cymbopogon* sp. (DI = 1), *Paspalum* sp. (DI = 3) and *Rhynchelytrum repen* (DI = 3). *Exserohilum monoceras* did not infect selected varieties of rice in Malaysia, such as MRQ 50, MRQ 74, MR 219, MR 220 and MR 84. Vegetables and medicinal plants used in the test were also not infected.

Introduction

The genus *Echinochloa* composed of about 53 species (Micheal 1973). The most cosmopolitan and economically important member of the genus is the taxon of *E. crus-galli* complex commonly known as barnyard grass. Azmi (1998) reported that there are five important species of *Echinochloa* in rice granary areas in Peninsular Malaysia namely *E. crus-galli (E. crus-galli* var. *crus-galli and E. crus-galli* var. *formosensis)*, *E. oryzicola, E. colona*, *E. stagnina* and *E. picta*. *Echinochloa colona* and *E. stagnina* are perennials while others are annual species. *Echinochloa crus-galli* complex, locally known as *rumput sambau*, consist of *E. crus-galli* var. *crus-galli* and *E. crus-galli* var. *formosensis*. *Echinochloa crus-galli* var. *crus-galli, E. crus-galli* var. *formosensis* and *E. oryzicola* closely resemble each other,

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thus they were grouped under the *E. crus-galli* complex. The significant characteristic differentiating the varieties are short awn or awnless with open spikelets (*E. crus-galli* var. *formosensis*) and long awn with closed or compact panicles (*E. crus-galli* var. *crus-galli*) (Azmi 1998). *Echinochloa oryzicola* closely resemble *E. crus-galli* var *formosensis* but it has bigger seeds. Tasrif et al. (2004) reported that there are 10 ecotypes of *E. crus-galli* complex in Malaysia and they vary genetically.

In Peninsular Malaysia, *E. crus-galli* complex is found in all rice granaries, namely Muda (Kedah), Kemubu (Kelantan), Kerian (Perak), Projek Barat Laut (Selangor), Seberang Perak and Sungai Manik (Perak), Besut (Terengganu), Seberang Perai (Pulau Pinang) and Kemasin Semarak (Kelantan). Azmi (1992) reported that only 10 plants of *E. crus-galli* per m^2 can cause yield reduction of approximately 18.7% of rice (representing 1 t/ha).

Research on biological control agents against *E. crus-galli* have been intensified in Asia since early 1990s. Among the fungal pathogens that have been suggested as biocontrol agents were *Cochliobolus lunatus* (Tsukamoto et al. 1997) and *Exserohilum monoceras* (*Septosphaeria monoceras*) (Zhang et al.1996). Tsukamoto et al. (1997) reported an approximate 80% reduction in dry matter was achieved when different botanical varieties (subspecies) of *E. crus-galli* was treated with virulent isolates of *E. monoceras* and *C. lunatus*.

Besides *E. monoceras*, *Bipolaris sacchari*, *Curvularia geniculata*, *C. lunata* var. *aeria*, *Dactylaria dimorhospora* and *Exserohilum oryzae* were also isolated from *E. crus-galli* in the Philippines (Zhang et al. 1996). In Korea, *Colletotrichum graminocola* showed strong pathogenicity in a wide range of growth stages *of E. crus-galli* var. *praticola* and *E. crus-galli* var. *caudata* (Yang et al. 2000). *Exserohilum monoceras* was also reported as potential bioherbicide and not pathogenic on planted rice varieties in China (Huang et al. 2001) and in Vietnam (Khanh 2002). In Malaysia, *E. longirostarum* has good control on *Rottboellia cochichinensis* (itch grass) (Kadir et al. 2003) and *E. crus-galli* (Ng 2007).

Host range evaluation is an important step in the determination of the safety of fungal pathogens considered for use as bioherbicides. The pathogens selected for biocontrol use should not infect crop species and other non-target plants grown in the area where the pathogens intended to be used to control target weeds.

Surveys carried out in 2004–2005 in Malaysian granaries (Tosiah et al. 2009*)* found that *E. monoceras* was a promising fungal biocontrol agent isolated from barnyard grass. However, reports on host range of *E. monoceras* are limited. In Malaysia particularly, no report of *E. monoceras* associated with plant disease has been published. Thus, the objective of this study was to determine the host range of *E. monoceras* isolate 1125, the indigenous fungus isolated from *E. crus-galli* as part of its development as bioherbicide.

Materials and methods

Inoculum production

Small pieces of *E. monoceras* mycelium from working culture kept in MARDI were aseptically transferred to petri dishes (9 cm diameter) containing fresh Potato Dextrose Agar (PDA). The plates were sealed with parafilm and kept in dark incubator at 28 ± 2 °C for 7 days. Agar plugs (5 mm diameter) from the margin of the colonies were used as seed inoculum. V8 juice agar (200 ml V8 juice Campbell's ®, 18 g of granulated agar and add up to 1 litre using distilled water) was used as a medium for conidia production. The fungus was incubated at 28 ± 2 °C light/dark photoperiod. Conidia were harvested 14 days after the incubation by flooding the plates with 10 ml distilled water and scraping the surface with plastic spatula. Resulting suspension was adjusted to the preferred concentration using a hemacytometer.

Target host inoculation

In this experiment, different species of *Echinochloa* at 4-leaf stage were inoculated with spore suspension at about 2.5 x 10^7 spores/ml concentrations. The spore suspensions were prepared as simple emulsion by adding 1% corn oil (Lam Soon Edible Oil Sdn. Bhd.) and 0.2% of maxi green (Saleswide Sdn. Bhd.), a commercial non-ionic surfactant and sticker. The 20 ml of spore suspensions was sprayed onto the plant using hand sprayer. The sprayer nozzle was held at about 30 cm away from the target plant to form an even spreading and sprayed until run off. Treatments were done in three replications. The plants were placed in the glasshouse at a temperature range of 28–38 ± 2 °C and covered with plastic bags over night to maintain humidity.

Host range determination

Host range evaluation was done based on Wapshere (1974). The species that were chosen for the host range test included several grasses that are closely related to *Echinochloa* (Family Poaceae), various modern rice varieties developed by MARDI, other problematic weeds, crop plant of economic importance in Malaysia and crops reported as host to *Bipolaris, Curvularia*, *Dreschlera* and *Exserohilum* (*Table 1*).

A total of 54 plant species, including varieties and breeding lines from 14 families and 33 genera were evaluated. The number of plants and development stage tested varied depending on the growth habit of the species. Each pot or polybag was planted with one to five plants, depending on the plant type. Basically, the plant species were evaluated based on their physiological age at 4 to 6-leaf stage. *Echinochloa crus-galli* was included as a control in each batch of plants tested. All plant species were sprayed with 20 ml spore suspensions at 10^7 spores/ml concentration and covered with plastic bags to maintain humidity and left for 24 h.

Table 1. Reaction of *Exserohilum monoceras* 1125 to the plant tested

Family/order	Species	Variety	Disease incidence	Disease index
A). Plants taxonomically related to *Echinochloa* species				
Poaceae				
Panicoideae	*Echinochloa crus-galli* var *formosensis*		100	4
	Echinochloa crus-gall var *crus-galli*		100	4
	Echinochloa orizycola		100	3
	Echinochloa colona		100	3
	Echinochloa stagnina		100	4
	Zea mays	Suwan 1,	100	2
		Mas madu	100	2
	Digitaria sp.		100	0
	Paspalum sp.		100	3
	Imperata cylindrica		100	4
Other poaceae	*Cynodon* sp.		0	0
(Turf grasses)	*Azonopus* sp.		0	0
	Zoysia zenith sp.		0	0
	Axonopus affinis		0	0
	Axonopus compressus		0	0
Other poaceae	*Brachiaria* sp.		100	2
	Rhynchelytrum repen		100	3
Cyperaceae	*Cyperus* sp.		0	0

(cont.)

Table 1. *(cont.)*

Family/order	Species	Variety	Disease incidence	Disease index
Oryzoideae	*Oryza sativa* L. (modern rice varieties of Malaysia)	MR 185	0	0
		MR 84	0	0
		MRQ 50	0	0
		MRQ 74	0	0
		MR 220	0	0
		MR 219	0	0
		MR 159	0	0
		MR 167	0	0
		MR 81	0	0
B). Plants reportedly infected by *Bipolaris, Curvularia, Dreshslera, Exserohilum* and *Helminthosporium* and their teleomorphs in Malaysia				
Poaceae	*Cymbopogon* sp.		100	1
Zingiberaceae	*Kaemperia galanga*		0	0
C). Vegetable				
Brassicaceae	*Brassica oleracea*	Cabbage (KK-Cross)	0	0
		Cauliflower	0	0
		Broccolli	0	0
	Brassica juncea	Unknown	0	0
	Brassica chinensis	Unknown	0	0
	Lactuca sativa	Unknown	0	0
Cucurbitacae	*Cucurmis sativa*	Unknown	0	0
	Luffa acutangular	Unknown	0	0
	Benincasa hispida	Unknown	0	0
Fabaceae	*Vigna* sp.	MKP 5	0	0
Malvaceace	*Abelmoschus esculantus* (L.) Moench	Unknown	0	0
Solanaceae	*Capsicum annum*	Long chilli (MC 12)	0	0
		Bell pepper (Bluestar)	0	0
	Solanum melongena	MT11	0	0
	Lycopersicum esculentum	Unknown	0	0
Amaranthaceae	*Amaranthus viridis*	Unknown	0	0
Convolvulaceae	*Ipomea aquatica*	Unknown	0	0
D). Potentially economic medicinal plants				
Acanthaceae	*Andrographis* sp.		0	0
Acanthaceae	*Asystasia intrusa*		0	0
Lamiaceae	*Pogostemon* sp.		0	0
Lamiaceae	*Orthosiphon* sp.		0	0
Mackinlayaceae	*Centella asiatica*		0	0
Phyllanthaceae	*Phyllanthus naruri*		0	0

Experimental design
All experiments were done in complete randomized design with three replications and repeated twice. Each replication consist of different plant that being tested with *E. crus-galli* as control. All experiments were done in a glasshouse structure in MARDI Serdang with temperature range from 26 °C (night) to 38 °C (noon).

Disease assessment
Disease incidence was recorded as the percentage of plants showing visible leaf symptoms. Disease index (DI) was used to evaluate the host range of the fungi. It was calculated using the equation below and the value of disease index was rounded to the whole number, which consist of 5 classes; 0 = immune, 1 = resistant, 2 = tolerant, 3 = severely damaged and 4 = dead.

$$\text{DI (disease index)} = \frac{\sum \text{(severity rating x number of plants in that rating)}}{\text{Total number of plants}}$$

Results and discussion
Effect on target host
All the *Echinochloa* spp. were infected by *E. monoceras* isolate 1125 but with different disease index (DI) value. *Echinochloa crus-galli* var *crus-galli* and *E. crus-galli* var *formosensis* had DI = 4, while *E. orizycola* and *E. colona* had DI = 3 (*Table 1A*).

Host range determination
All vegetable species and modern rice varieties tested were immune to the fungus *E. monoceras 1125* (*Table 1A and 1C*). No symptom of the disease developed when the fungus was inoculated to these plants. However, the fungus caused disease on *Zea mays, Imperata cylindrica, Rhynchelytrum repens*, *Brachiaria* sp. and *Paspalum* sp.. The disease index of *Zea mays* and *Brachiaria* was 2 and considered as tolerant to the fungus infection. The disease on *Zea mays* was observed mostly on the lower leaves and the plant recovered after the development of new shoots (*Table 1A*).

Disease index of *Paspalum* sp. and *Rhynchelytrum repens* was 3 and the plants were severely damage. Disease index of 4 was shown by *Imperata cylindrica* and died after 10 days of inoculation. However, when the fungus was inoculated on older plant of *I. cylindrica,* although all plants were infected, the disease index was only 2 and therefore considered to be tolerant to the disease. All the medicinal plants tested were immune (*Table 1D*) to the fungus except for *Cymbopogon* (*Table 1B*) which showed tolerant (DI = 2) to the fungus. *Cymbopogon* also has been reported as the host of *Curvularia* sp. (Sivanesan 1987; Tosiah et al. 2004).

Zhang et al. (1996) found that *E. monoceras* caused large necrotic lesions on *E. crus-galli, E. colona* and *E. glabrescens* but was not pathogenic to rice. They described the disease symptoms as necrotic fleck within 24 h and chlorosis and a diffuse, general, water-soaking reaction followed by rapid collapse and necrosis of the infected tissues 2 days after treatment (DAT). The latter symptom described was similar to our observations on *E. crus-galli* var. *crus-galli* and *E. crus-galli* var. *formosensis,* although in this case the total collapse of the plants was only observed 6 to 8 DAT which might be due to the difference of the physiological age of the plant used in the study, that is 4-leaf stage compared to 2-leaf stage used by Zhang et al. (1996). In addition, the plants used in this study were exposed to the dew for only 16 h.

Although the fungus was isolated from *E. crus-galli* var. *crus-galli*, it can also infect other *Echinochloa* spp. such as *E. colona*, *E. crus-galli* var. *formosensis*, and *E. orizycola*. Results showed that both *E. crus-galli* var. *crus-galli* and *E. crus-galli* var. *formosensis* were susceptible to *E. monoceras* compared to *E. colona* and *E. orizycola*. Zhang and Watson (1997) also found that *E. crus-galli* and *E. glabrescens* were more susceptible compared to *E. colona*. According to Azmi (1998), *E. glabrescens* is synonym to *E. crus-galli* var. *formosensis*.

The host range studies indicated that *E. monoceras* isolate 1125 was safe to all the rice varieties tested, suggesting that the rice plant is not a host of *E. monoceras*. This study is consistent with findings by Gohbara and Yamaguchi (1994), Zhang and Watson (1997), Huang et al. (2001) and Khanh (2002). The fungus was also safe to vegetable crops and medicinal plant tested in the studies.

Among the grasses tested, effect of *E. monoceras* was confined to *Imperata cylindrica* and *Rhynchelytrum repens*. This is the first report of *I. cylindica* and *R. repens* as hosts to *E. monoceras*. However, studies are needed to determine the efficacy of the fungus in controlling *I. cylindica* and *R. repens*. Both *Zea mays* cultivars tested in the studies, developed minor symptoms upon inoculation with the pathogen. However, these were restricted to the lower leaves and the new shoots were healthy and grew normally after the lower leaves senesced. This finding conformed to Hailmi (2006), who also found that maize is resistant to *E. monoceras*. Zhang and Watson (1997) demonstrated that although maize was infected by *E. monoceras* in the glasshouse, it did not infect the plant when applied in the field. The infection on maize might be due to simulated condition in the glasshouse which facilitated optimum condition for infection and disease development may artificially increase the perceived host range of the organism being tested.

Conclusion

Exserohilum monoceras isolate 1125 has the potential to be developed into bioherbicide. However, in our rice granaries there are other weeds that also play important roles in reducing rice yield, such as weedy rice, *Leptochloa chinensis* and *Monocchoria vaginalis*. Attempts to introduce host specific biocontrol agents may result in shifting these weeds to become more dominant. Although host specificity was an important character used in selecting a biological control agent, it is an advantage if the fungus also attacked *L. chinensis* sp. and *M. vaginalis*, or as an alternative the bioherbicide that will be developed contains other fungi with potential to control *L. chinensis* sp. and *M. vaginalis*. However, it must be remembered that biological control was introduced to fulfil the sustainable agriculture concept which is not to totally eradicate the weeds but to control it below the threshold level because these weeds can act as refuge for natural predators.

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Abstrak
Sebanyak 54 spesies tumbuhan, termasuk varieti dan titisan pembaikbakaan daripada 14 famili dan 33 genera telah disaring terhadap kulat *Exserohilum monoceras* yang merupakan kulat patogen yang berpotensi sebagai agen kawalan biologi rumput sambau (*Echinochloa crus-galli)*. Kajian di dalam rumah kaca dijalankan dengan semburan kulat pada kepekatan 2.5 x 10^7 spora/ml dengan tempoh lembap selama 24 jam. Kebolehan kulat ini untuk menjangkiti tumbuhan-tumbuhan tersebut dinilai melalui indeks penyakit (IP) yang dicerap daripada bacaan kejadian penyakit (disease incidence) dan keterukan penyakit (disease severity). Didapati indeks penyakit (IP) bagi *Echinochloa crus-galli* var. *crus-galli, E. crus-galli* var. *formosensis* dan *E. stagnina* ialah 4 (mati), manakala *E. colona* dan *E. oryzicola* ialah 3 (rosak teruk). Bagi tanaman ekonomi hanya *Zea mays* yang dijangkiti dengan indeks penyakit 2 (toleran). *Imperata cylindrica* yang muda mati (IP = 4), tetapi yang lebih tua menunjukkan reaksi hipersensitif (IP = 2). Tumbuhan lain yang dijangkiti ialah *Cymbopogon* sp. (IP = 1), *Paspalum* sp. (IP = 3), dan *Rhynchelytrum repen* (IP = 3). *Exserohilum monoceras* tidak menjangkiti varieti terpilih padi Malaysia seperti MRQ 50, MRQ 74, MR 219, MR 220 dan MR 84. Ia juga tidak menjangkiti sayuran-sayuran dan tanaman ubatan yang digunakan dalam kajian ini.

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