

Weeding Efficacy of Sulfonyleurea Resistance Weed, *Monochoria vaginalis* with Brown Leaf Blight Caused by BWC01-54

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A summer annual weed of monochoria (*Monochoria vaginalis*) grows in the edges of rice paddies, ditches, and moist upland throughout Korea. It is very difficult to control with herbicide because of its sulfonyleurea resistance. It is very competitive with fast growing pattern, that can cause reducing yields of rice. Brown leaf blight of monochoria (*Monochoria vaginalis*) occurred naturally in rice paddy, is first reported in Korea. The fungal isolate BWC01-54 was successfully isolated from the diseased leaves of monochoria. The fungus BWC 01-54 was grown well at 25-28°C, conidia of the greyish black brown mycelia were abundant produced on PDA at 15 days. The fungus was grown well in potato dextrose broth at 28°C and fully grown within 10 days in 250 ml of flask. In host and pathogenicity test, conidia suspension of BWC01-54 was the most effective to control of monochoria compare to others isolates. Typical symptoms having pin point brown lesions were formed on stem and leaf and which severely affected the whole plants were blighted within two weeks, respectively. Under paddies field condition, conidial suspension of the fungus BWC01-54 gave around 90% control. Therefore, we conclude that the fungus may have a potential as a biological control agent against sulfonyleurea resistance weed in rice paddy.

Keywords : brown leaf blight, BWC01-54, monochoria (*Monochoria vaginalis*), sulfonyleurea resistance weed

Monochoria vaginalis is distributed widely and has been caused weed problem in rice production area in Korea. Sulfonyleurea (SU) herbicides have been widely used in major cereal growing areas to control or suppress broad leaf weeds and sedges since their introduction in the early 1980s. With the repeated use of the same SU-based herbicides, several weed species have developed resistance to SU herbicides (Heap, 1997; Heap, 2003). Before 1990s, it is almost true to say that the weed was easily controlled with chemical herbicide, sulfonyleurea. There was a serious

problem which a sulfonyleurea resistance was occurred because of the only one sulfonyleurea was used for a long time. After that the control efficacy was very low and it was to become a serious weed variety to control with chemical control (Fig. 1). Recently in Japan, SU-resistant *Monochoria korsakowii* Regel et Maack. and *Monochoria vaginalis* Presl. were identified in rice in Hokkaido (Kohara et al., 1999). In addition, eleven species and one variety of SU-resistant biotypes are spreading and causing problems in all the areas of Japan. Among them, most serious herbicide resistant weeds in rice field are *Monochoria vaginalis* and *Scirpus juncooides* in Korea and Japan (Itoh, 1999; Park, 1999). Brown leaf blight of Monochoria (*M. vaginalis*) observed at naturally occurred in rice paddy, is first reported in Korea. The fungal isolate BWC01-54 was successfully isolated from the diseased leaves. Symptoms first appeared on leaf of *monochoria* sp. in July and the lesions rapidly elongated, expanded around the leaves, and blighted completely in August. In 2001, we could find out an isolate from diseased *Monochoria* sp. which was naturally occurred in field with dying the plants, was observed at the rice. The causal organism was identified as *Monosporascus* sp., and subsequently named as brown leaf blight of monochoria by authors (Hong et al., 2001). In a following study revealed that the fungus is locally distributed in Korea, and seems to be well adapted in rice

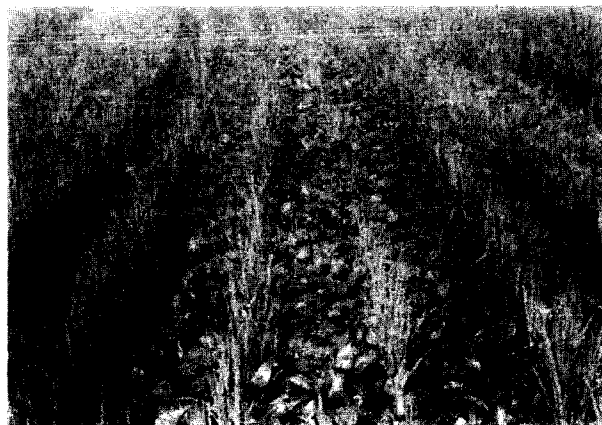


Fig. 1. Severely infected with monochoria (*Monochoria vaginalis*) in rice Paddies.

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fields in Korea. Infected *M. vaginalis* plants were blighted first and ultimately killed during the later season. The pathogen survived in infected stems of the plants from the previous year infection period and reinfected host plant (Hong et al., 2001, 2004a, 2004c). To use an organism as a biological control agent, selection of the promising isolate is most important (Auld et al., 1990; Hong et al., 1991, 1999, 2002a, 2002b); it should be highly pathogenic to target organisms but not pathogenic to other crop plants (Hong et al., 1991, 1996, 2002d). During the period of new infections due to unfavorable environmental conditions, effectiveness of mycoherbicide must result from dispersal and secondary infections of these pathogens which have a functional relationship with the increase of the pathogen population (Hong et al., 1997, 2002a, 2002c). Promising biological control agents of pests should be isolated and studied in areas where the target organisms were suppressed naturally (Hong et al., 1992, 2002e, 2003a, 2003b).

The purpose of this research was to isolate the fungus, to elucidate the host range of weed and weeding efficacy and to develop a successful biological control system of Monochoria (*M. vaginalis*), using fungal isolate BWC 01-54.

Materials and Methods

Isolation. Monochoria (*M. vaginalis*) plants showing typical symptoms with brown spot lesions were collected from rice paddies during the summer season from 2000 to 2001. Diseased leaves with brown lesions were cut into 2-3 mm pieces and surface-sterilized by submerging them in 70% ethyl alcohol for 1 minute and 2% sodium hypochloride for 1 min and then washed them with the sterilized water. The sterilized pieces were transferred aseptically to water agar containing 200 µg/l chloramphenicol, 88 µg/l ampicillin and 70 µg/l streptomycin. Hyphal tips of the colonies formed were transferred to acidified potato dextrose agar (PDA, pH 5.5) and cultured for 5 days at 28°C. A mycelial disc (d-5-mm) of the culture was preserved for future use in a -70°C deep freezer in Cryovial (Nunc, Inc., IL., USA), containing 1:1 mixture of 1.5 ml of 40% glycerol and 10% skim milk (Aoshima et al., 1983). Three isolates used in this study were selected by their characteristics on solid agar among 30 isolates collected from diseased monochoria from 12 regions of fields in Korea. Isolates were characterized by the mycelial color.

Inoculum production. For the production of spore by using as the inoculum, 5 pieces of agar disc with mycelia were cultured in a 250 ml Erlenmeyer Flask with 150 ml of PD broth (Difco, MI. USA). Shaking the culture on a rotary shaker (Jeiotech, Korea) at 120 rpm at 28°C for 7 days. The fully growth of spores were harvested and the conidia

suspension containing 10^5 spore/ml was adjusted with hemocytometer under 100X microscope.

Preparation of seedlings. Plants with 3 to 5 leaves were collected from the rice paddies in summer. The whole plants with rhizomes were transplanted in a plastic pot (21×17 cm) containing fertilized upland soil (N-P₂O₅-K₂O=15-15-15 g/m²). The seedlings were grown up to 7- to 10- leaf stage for pathogenicity and host range test.

Pathogenicity test to monochoria. Conidia suspension of isolates (3.2×10^5 cfu/ml containing 0.5% dextrose in distilled water) sprayed on the whole plant. The inoculated plants were incubated in a dew chamber controlled at 28°C for 16 h, then the pots were placed on a greenhouse bench at 25-32°C for the lesion develop. The progress of symptom was recorded after 5 days and a number of infected shoots were recorded. Where applicable, the data was processed by SAS GLM procedure (IRRI) and analyzed by Duncan's new multiple range test.

Host range test. To study the host range of this mycoherbicide agent, 7 weed species were inoculated with mycelia suspension of the isolate BWC01-54 as described in the pathogenicity test. Plants used in host range tests were grown in a plastic pot (21×17 cm) containing fertilized upland soil (N-P₂O₅-K₂O=15-15-15 g/m²). Each pot contained 30 seedlings and grew to the 7 to 10 leaf stage. The plant mortality was observed 15 days after sprayed with the conidia suspension. Non-treated plants sprayed with water were used as controls.

Experimental design. Field experiments were conducted at Yeongnam Agricultural Research Institute, Milyang. In 2001, 2 m×2 m plots, separated each other by 0.5 m border line were prepared and planted with 100 plants. Treatments were arranged in a completely randomized block design with four replications. Rice (Sangjubyeo) was cultivated on end of May and other weeds were hand weeded.

Assessment of the weeding efficacy. The number of diseased shoots and the mortality were counted within 50×50 cm of sub-sampling site in each plot on August 30.

Statistical analysis. Analysis of variance was done using the ANOVA procedure of Statistical Analysis System (SAS Software Co.). All data were analyzed statistically, and treatment means were separated by Duncan's new multiple range test for significance at $p = 0.05$.

Results and Discussion

Distribution of monochoria leaf blight disease in rice field. The disease occurred in all 18 counties surveyed for 2 years, and the causal fungus were isolated from Jangsoo, Jinan and Sangju. Frequency of the incidence, however, differed by rice area (Table 1). The disease incidence of plain area like Gimjae and Gimhae was relatively very low,

Table 1. Distribution of monochoria leaf blight disease in rice field between 2001 and 2002

Region	Disease Incidence (%)		Isolate No.
	2001	2002	
Cheolwon	62.5	49.3	
Wonju	40.4	45.2	
Boeun	50.3	38.5	
Jangsoo	78.2	69.4	BWC01-54 ^b
Jinan	69.7	70.1	BWC02-03
Sangju	78.5	75.3	BWC00-95
Echeon	10.3	23.9	
Yeoju	19.3	- ^a	
Cheonan	4.1	-	
Cheongju	3.6	-	
Gongju	13.1	21.1	
Gimjae	18.1	9.8	
Jangseong	8.4	-	
Jinyang	6.2	33.6	
Gimhae	5.9	15.2	
Milyang	11.5	3.4	
Changnyeong	9.1	20.1	
Youngdeok	15.3	3.2	

^aNot examined.^bHost specific microorganism selected.**Table 2.** Meteorological conditions of the two experimental fields, in 2001

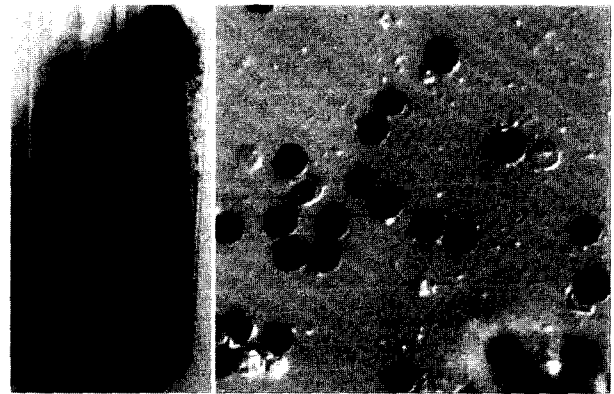
Experimental Fields	Temp. (°C) ^a		Dew duration (hr) Dew Period (hr)
	Max.	Min.	
Miryang	27.1	17.6	14.3
Sangju	24.1	17.4	16.1

^aMean temperature during July to August.

whereas Cheolwon, Jangsu and Sangju of mountain area was showed very high incidence of the disease. Brown leaf blight of monochoria occurred in less than 20% in Gimjae and Milyang, whereas in a mountainous area it occurred in about 40-80% such as Sangju, Jangsoo and Jinan. Thus different climatic conditions may result in a regional disparity showed this study. In Milyang area, mean temperature during July to August ranged between 17.6 and 27.1°C with 14.3 h of dew period, whereas in mountain area, Sangju, Gyeongbook that ranged between 17.4 and 24.1°C with 16.1 h of dew period (Table 2). Occurrence of the fungus over the wide areas suggests that the fungus has been locally adapted in rice fields in Korea. The other host specific mycoherbicide fungus was isolated from weed or monochoria which was fitted to the previous description of *E. nematosporus* (Hong et al., 1996; Suzuki, 1991). Mountain areas are known to have a higher temperature

Table 3. Incidence of brown leaf blight of monochoria caused by the fungal isolates

Isolates	Percent of infected plant ^a	Percent of plants mortality ^b	Location
BWC00-95	31.5 a ^c	21.3 b	Sangju
BWC01-54	90.7 b	72.6 c	Jangsoo
BWC02-03	36.7 a	25.3 b	Jinan
Control ^d		10.1 a	

^aThe number of diseased shoots in a plot measured by a three replication in each plot.^bPlants mortality were recorded on August 30 in 2001.^cNumbers in each column followed by the same letter are not significantly different by Duncan's new multiple range test ($p = 0.05$).^dThe herbicide for control the monochoria sp. (piperophos 4.4% ai.).**Fig. 2.** Typical mycelia and spores of *Monochoria vaginalis* were produced on PDA medium.

fluctuation between day and night, and also have longer dew period than that of plain areas (Auld et al., 1990; Hong et al., 2002d). Britton (1993) observed that anthracnose infection of dogwood seedlings in natural was very severe in the mountain areas than any other plain areas.

Pathogenicity. All of 3 isolates were developed brown leaf blight on *M. vaginalis* seedlings as observed in fields, thus were confirmed as the pathogen of weed (Table 3). When inoculated with conidia suspension, the rate of infection was 31.5% to 90.7% on plants among which BWC01-54 isolated from Jangsoo was the most virulent isolate. Other isolates, BWC00-95 and BWC02-03 were less virulent, showing weeding efficacy of 31.5% and 36.7%, respectively. Infected shoots were killed in 10 to 15 days. Symptoms first appeared on stems of the plants 4 to 5 days after inoculation in greenhouse conditions. Pin point brown speck lesions were enlarged with time, the lesions which rapidly expanded around the stem and leaves, the whole plant completely blighted within 10 to 15 days (Fig. 3) and thereon emerged abundant conidia (Fig. 2) which come to be an inoculum of next year's and can be suppressed new



Fig. 3. Typical symptom of brown leaf blight *Monochoria vaginalis* caused by the mycoherbicide fungus, BWC01-54.

plant emergence. By this, the effect of the fungus maintained control efficacy year around. Much differences were resulted from treatments with conidia suspension of isolate BWC01-54 and piperophos (4.4% ai.) (Fig. 4). The higher weeding efficacy occurred after treatment of conidia suspension, when treated with the piperophos (4.4% ai.) as

a control showed 11% of plant mortality. This was lower than the treatment of conidia suspension of BWC01-54 73% within 10 days. To effective control, we have to consider established shoots, reshoots, as well as the underground root formation (Hong et al., 1997, 2003a, 2004a). One of the difficulties encountered in attempting to kill weed is the weed's ability of regeneration (Hong et al., 2002e, 2003a). Therefore the shoots must be severely infected and die before regeneration occur. Ormeno-Nunez et al. (1988) observed regeneration in bindweed seedlings inoculated with *P. convolvulus*. Regeneration is particularly important because underground parts such as rhizomes collapse lastly. The best method to get a high weeding efficacy will be achieved when all the underground rhizomes were diseased with this fungus infected at the time of fungal application.

Host range. All tested crop plants seemed non-host to isolate BWC01-54; *M. vaginalis* was the only test plant on which typical brown leaf blight lesions were observed. No test plants produced a typical symptom, but some plants in genus of pontederiaceae family, like *M. korsakowii*. There were no differences in overall plant size, leaf number, or

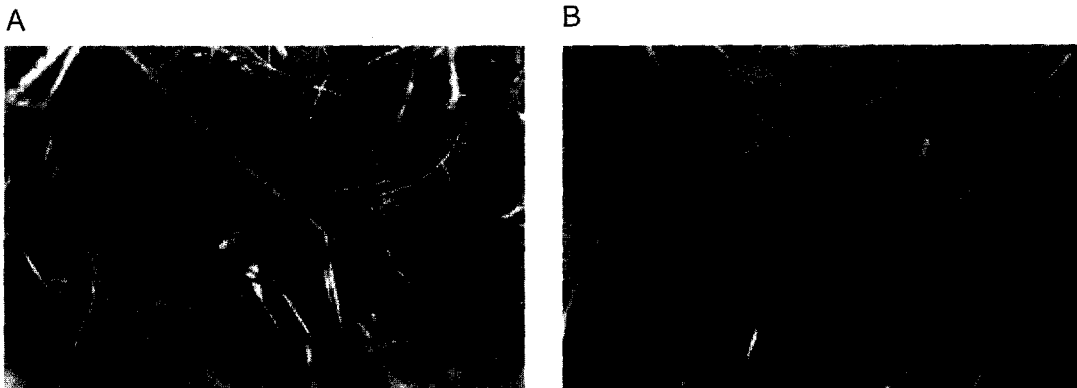


Fig. 4. The symptom appeared on the plant inoculated with the BWC01-54 (A) and the herbicide (B).



Fig. 5. The symptom of brown leaf blight disease of *Monochoria* 7 days (A) and 20 days (B) after inoculation with conidial suspension of the fungus, BWC01-54 in field condition.

Table 4. Host range of isolate BWC01-54 to seven species of weeds in paddy fields

Family	Host	Infection*
<i>Pontederiaceae</i>	<i>Monochoria korsakowii</i>	+
<i>Pontederiaceae</i>	<i>Monochoria vaginalis</i>	+
<i>Cyperaceae</i>	<i>Scirpus planiculmis</i>	-
<i>Cyperaceae</i>	<i>Scirpus juncooides</i>	-
<i>Cyperaceae</i>	<i>Cyperus difformis</i>	-
<i>Lythraceae</i>	<i>Rotala indica</i>	-
<i>Scrophuriaceae</i>	<i>Lindernia dubia</i>	-

*+ : Infected, -: Uninfected.

plant vigor between any of the inoculated test plants and the corresponding control plants in 15 days (Table 4). In contrast, *M. vaginalis* became heavily infected; evidence of infection was apparent within 4 to 6 days on whole plants. *Leguminosae* sp. and *Gramineae* species containing rice produced no disease symptom. The result obtained in this study and elsewhere indicated that isolate BWC 01-54 seemed highly pathogenic to monochoria but either non-pathogenic or very weakly pathogenic to other plant species. In the studies of biological control of weeds with pathogens, in general, most of weed species are hosts for many pathogens (Hong et al., 1996, 2002d, 2002f, 2004b; TeeBeest, 1985) has recently discovered that the candidate of mycoherbicide agent for *Leguminosae* sp. and others weed species, has much broader host range than that originally reported by authors. Daniel (1973) suggested that one of the highly desirable characters for biological weed control agents must be genetically stable and specific to the target weed.

Assessment of the weeding efficacy. Aerial temperature and dew period duration affected the disease development. As similarly have been reported in many mycoherbicide field tests (Elwakil et al., 1990; Hong et al., 1997, 1999, 2003a, 2004c; Kirkpatrick et al., 1982; Makowski et al., 1990; TeeBeest et al., 1985). These studies show that the infection can occur during the summer season when suitable temperature with over 12 h of dew period. Hong et al. (1996) reported that the first symptoms on water chestnut at paddy rice field in Korea appeared in late July or early August. 20 -30 days old of monochoria are the most heavily infected which usually occurs in mid-July. When hot and dry conditions are prevail after field application of mycoherbicide fungus, infection rates are low, and disease development is slow, giving poor control of the weed (Hong et al., 2002e, 2003a). Therefore applications should be properly timed to achieve optimum conditions in the field (Hong et al., 1997, 2003a; Makowski et al., 1989; Mortensen et al., 1988).

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