Rangsit Journal of Arts and Sciences, January-June 2013 RJAS Vol. 3 No. 1, pp. 39-43

ISSN 2229-063X (Print)/ISSN 2392-554X (Online)
DOI : 10.14456/rjas.2012.20

Diversity of causal fungi in weed diseases and potential use as a biological weed control for vegetable plots in Thailand

Duangporn Suwanagul[1*], Jitra Kokaew[2], and Anawat Suwanagul[2]

[1]Faculty of Biotechnology, Rangsit University, Patumthani 12000, Thailand
[2]Thailand Institute of Scientific and Technological Research, Patumthani 12120, Thailand

*Corresponding author: E-mail: dsuwanagul@rsu.ac.th

Submitted 6 January 2013; accepted in final form 31 May 2013

Abstract

The study was aimed to isolate and identify the causal microorganism of leaf spot and blight disease of weeds. Seven species of weeds showing leaf spot and leaf blight diseases were collected from vegetable-producing areas in Central Thailand. These were *Cyperus rotundus* (Cyperaceae), *Cyperus brevifolius* (Cyperaceae), *Brachiaria mutica* (Poaceae), *Eleusine indica* (Poaceae), *Dactyloctenium aegyptium* (Poaceae), *Pennisetum polystachyon* (Poaceae), and *Oryza sativa* f. *spontanea* (Poaceae). The causal microorganisms were isolated from leaf symptoms by tissue transplanting and moist chamber techniques. Identification was based on their morphological features and examined under stereo and light binocular microscopes. From this study, a total of 25 isolates of causal fungi comprised of 10 genera with 15 species identified, were found. These were *Alternaria alternata*, *Bipolaris bicolor*, *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, *Colletotrichum graminicola*, *Colletotrichum musae*, *Curvularia lunata*, *Curvularia pallescens*, *Drechslera holmii*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, *Myrothecium cinctum*, *Nigrospora oryzae*, *Pestalotiopsis guepinii*, *Phomopsis* sp. A pathogenicity test of *D. holmii* inoculums consisting of spore suspensions of $1x10^6$ spores/ml of *D. holmii*, were performed onto healthy weeds, *D. aegyptium* and *Brassica alboglaba*. It was shown that only *D. holmii* caused severe damage on their weed hosts, whereas disease symptom occurred on *B. alboglaba*. This can be concluded that specificity of *D. holmii* on *D. aegyptium* showed great potential utilization of fungus as a biological control agent for weed control in *B. alboglaba* vegetable plots.

Keywords: *weed, fungi, biological control, blight disease*

บทคัดย่อ

การทดลองนี้มีวัตถุประสงค์เพื่อแยกและจำแนกชนิดเชื้อราสาเหตุโรคใบจุดและใบไหม้ของวัชพืชในแหล่งปลูกผักในจังหวัดภาคกลางของประเทศไทย วัชพืชที่แสดงอาการโรคใบจุด ใบไหม้ 7 ชนิด ได้แก่ แห้วหมู *Cyperus rotundus* (Cyperaceae) กกตุ้มหู *Cyperus brevifolius* (Cyperaceae) หญ้าขน *Brachiaria mutica* (Poaceae) หญ้าตีนกา *Eleusine indica* (Poaceae) หญ้าปากควาย *Dactyloctenium aegyptium* (Poaceae) หญ้าขจรจบ *Pennisetum polystachyon* (Poaceae) และ หญ้าข้าวดีด *Oryza sativa* f. *spontanea* (Poaceae) ใบที่แสดงอาการโรคใบจุดของวัชพืชทั้ง 7 ชนิดดังกล่าวข้างต้นได้ถูกเก็บรวบรวมและนำมาแยกราสาเหตุโรคโดยวิธี Tissue transplanting และ moist chamber การจำแนกชนิดราทำโดยตรวจดูลักษณะทางสัณฐานวิทยา ใต้กล้องจุลทรรศน์สองตาและกล้องจุลทรรศน์สเตริโอ จากการศึกษานี้สามารถจำแนกราสาเหตุโรควัชพืชได้ทั้งหมด 25 สายพันธุ์ ซึ่งสามารถจำแนกเป็น 10 สกุล (genera) 15 ชนิด (species) ได้ดังนี้ *Alternaria alternate*, *Bipolaris bicolor*, *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, *Colletotrichum graminicola*, *Colletotrichum musae*, *Curvularia lunata*, *Curvularia pallescens*, *Drechslera holmii*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, *Myrothecium cinctum*, *Nigrospora oryzae*, *Pestalotiopsis guepinii*, และ *Phomopsis spp.*

การทดสอบความสามารถในการก่อโรคของรา *D. holmii* ที่เตรียมไว้ในรูปสเปรย์ฉีดพ่นที่มีความเข้มข้นของจำนวนสปอร์ที่ระดับ $1x10^6$ สปอร์ต่อมิลลิลิตรของสเปรย์ที่มีต่อหญ้าปากควาย (*D. aegyptium*) และคะน้า (*Brassica alboglaba*) พบว่ารา *D. holmii* ทำให้เกิดโรคใบจุดกับหญ้าปากควาย ในขณะที่ราชนิดนี้ที่ระดับความเข้มข้นของจำนวนสปอร์ที่เท่าเทียมกันไม่สามารถทำให้เกิดโรคกับคะน้า จึงมีความเป็นไปได้สูงในการที่จะนำรา *D. holmii* มาใช้ในการกำจัดวัชพืชหญ้าปากควายในแปลงปลูกผักคะน้า

คำสำคัญ: *วัชพืช, เชื้อรา, การควบคุมทางชีวภาพ, โรคใบไหม้*

1. Introduction

The investigation on fungi causing weed diseases has been reported by many researchers (Auld & Mc Rae, 1999; Babu, Sajeena, & Seetharaman, 2003). Biological weed control is an approach to utilize living organisms to control or reduce the population of weeds. Daniel, Templeton, Smith, and Fox (1973) introduced the concept of bioherbicide and demonstrated that an endemic pathogen might be rendered completely destructive to its weed host by applying a massive dose of inoculum at a particular vulnerable stage of weed growth. The use of biological control agent is an alternative method for weed control, where an organism is applied to achieve reduction in weed population. Among the new and possible environment-friendly strategies, the use of *Drechslera gigantea*, *Exserohilum rostratum* and *Exserohilum longirostratum*, have previously proved to be promising bioherbicide agents against several grass weeds in field trials (Casella, Charudattan, & Vurro, 2010). In particular, *Bipolaris setariae can be used to control* for goose grass (Figliola, Camper, & Ridings, 1988); *B. halepense, B. sorghicola* and *E. turcicum* for Johnson grass (Chiang, Van Dyke, & Chilton, 1989); *E.monoceras* for *Echinochloa* spp. (Zhang & Watson, 1997); and *Exserohilum longirostratum* for *Dactyloctenium aegyptium* (Ninsuwan Chuenchit & Petcharat, 2010), respectively. The exploitation of fungal plant pathogens as biological weed control agents has gained considerable importance. The purposes of this study were to isolate and identify fungal species from diseased weeds as well as their pathogenicity test for biological weed control.

2. Materials and methods

Weed disease samples used for isolation and identification of weed pathogen were collected from the Central part of Thailand as presented in Table 1.

2.1 Isolation of Fungi

2.1.1 Moist chamber method (Jeamjitt, 2007)

Each weed sample was placed in a moist chamber consisting of a glass bowl or plastic box lined with damp cotton or tissue-paper and placed by the window. They were incubated for 2-7 days or longer at 28 ^{o}C, and observation was made under a stereomicroscope. A needle was used to transfer fungal spores or fruiting structures from injury tissue onto a slide mounted with a drop of distilled water, then covered with cover slip and examined under light microscope with Normaski Interference Contrast.

2.1.2 Tissue transplanting method (Manoch, 2002)

Weed leaves showing spot and blight symptoms were cut into small pieces (2x2 mm^2) each with surface sterilized in 1.5% (w/v) sodium hypochlorite for 3 - 5 minutes, then rinsed 3 times in sterile distilled water for 30 seconds, and dried with sterile absorbent paper towels. Each piece of leaf was placed on water agar (WA) with 25 mg/L streptomycin. The plates were incubated for 3 - 5 days at 28ºC. Subsequently, the hyphal tips of each isolate were transferred to potato dextrose agar (PDA). After 7 days, the pure culture was obtained and used for identification.

2.2 Identification of fungi

Macroscopic features were studied including colony growth pattern, color, and texture on different agar media. Fungal growth rate was measured on PDA, CMA, CZA and MEA. Microscopic characters were observed on a slide preparation using sterile distilled water and lectophenol as mounting media and examined under a light microscope (Olympus BH-2 with Normaski Interference Contrast). Photomicrographs of fungal structure were taken under stereo and light microscopes.

2.3 Pathogenicity test (Kokaew, 2005)

The four-week-old tested plant species included the weed, *Dactylotenium aegyptium* and vegetable host, *Brassica alboglaba*, were sprayed with inoculums that consisted of spore suspensions of 1x10^6 spores/ml of *Drechslera holmii*. Non-inoculated control plants were sprayed with distilled water. There were 3 replications with 10 plants on each replication, growing tested plants in a 10 inch diameter pot. Inoculated plants were then covered with polyethylene bags for 24 hours to maintain humidity. The plants were observed for disease development over a two-week period.

3. Results and discussion

The results revealed that 25 isolates of micro fungi, comprising 10 genera with 15 identified species, were found on the leaf spot and leaf blight disease of weeds collected in Chanthaburi, Patum Thani, and Bangkok areas (Table 1, Figure 1). Most species of micro fungi

were similar to those previous reported (Thomas, Muller-Stover, Ziegler, Bedi, & Kroschel, 1998; Babu et al., 2003; Sandrin, TeBeest, & Weidemann, 2003; Kokaew, 2005; Kokaew, Manoch, Visarathanon, & Suwanagul, 2006). The most common species were: *Alternaria alternata*, *Bipolaris bicolor*, *Colletotrichum capsici*, *C. gloeosporioides*, *C. graminicola*, *C. musae*, *Curvularia lunata*, *C. pallescens*, *Drechslera holmii*, *Fusarium oxysporum*, *F.semitectum*, *F. solani*, *Myrothecium cinctum*, *Nigrospora oryzae*, *Pestalotiopsis guepinii*, *Phomopsis* sp. (Table 2 and Figure 2).

Table 1 Weeds species, Family and locations

No.	Weeds species	Family	Location
1	*Cyperus rotundus*	Cyperaceae	Bangkok
2	*Cyperus brevifolius*	Cyperaceae	Chanthaburi
3	*Brachiaria mutica*	Poaceae	Chanthaburi
4	*Eleusine indica*	Poaceae	Pathum Thani
5	*Dactyloctenium aegyptium*	Poaceae	Pathum Thani
6	*Pennisetum polystachyon*	Poaceae	Pathum Thani
7	*Oryza sativa* f. *spontanea*	Poaceae	Bangkok

Table 2 Micro fungi isolated from weed diseases at different host plants

Fungi	Class	Host plant
Alternaria alternate	Hyphomycetes	1,2*
Bipolaris bicolor	Hyphomycetes	1,3
Colletotrichum capsici	Coelomycetes	4
Colletotrichum gloeosporioides	Coelomycetes	2,4,5
Colletotrichum graminicola	Coelomycetes	4
Colletotrichum musae	Coelomycetes	4,5
Curvularia lunata	Hyphomycetes	7
Curvularia pallescens	Hyphomycetes	1,2,4,6
Drechslera holmii	Hyphomycetes	2,3,5
Fusarium oxysporum	Hyphomycetes	2,3,6
Fusarium semitectum	Hyphomycetes	1,2,5
Fusarium solani	Hyphomycetes	3,4,5
Myrothecium cinctum	Hyphomycetes	2,3,4
Nigrospora oryzae	Hyphomycetes	1,2,3
Pestalotiopsis guepinii	Coelomycetes	2,4,6
Phomopsis sp.	Coelomycetes	3,4,6

***** 1; *Cyperus rotundus*, 2; *Cyperus brevifolius*, 3; *Brachiaria mutica*, 4; *Eleusine indica*, 5; *Dactyloctenium aegyptium*, 6; *Pennisetum polystachyon*, 7; *Oryza sativa* f. *spontanea*

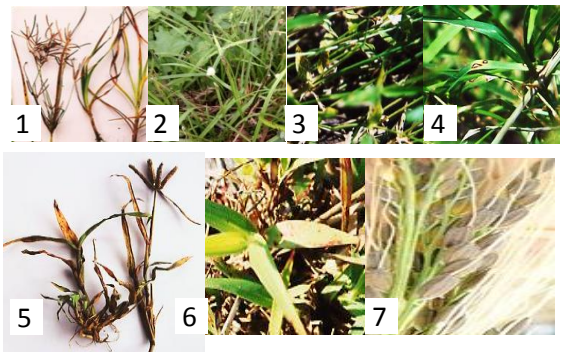

Figure 1 Diseased weeds 1; *Cyperus rotundus*, 2; *Cyperus brevifolius*, 3; *Brachiaria mutica*, 4; *Eleusine indica*, 5; *Dactyloctenium aegyptium*, 6; *Pennisetum polystachyon* and 7; *Oryza sativa* f. *spontanea.*

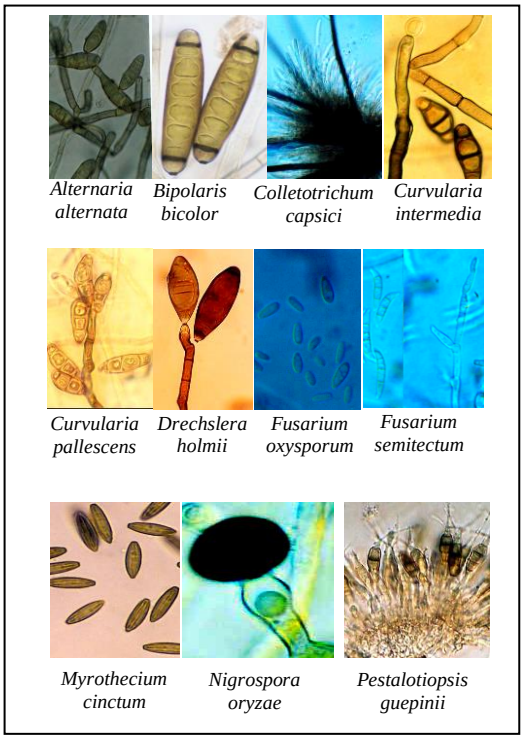

Figure 2 Morphological characteristic of fungal pathogens from diseased weeds

A number of previous studies proved that fungi have high potential for biological control agents of weeds. For example, *Alternaria alternata* was studied and evaluated for potential use as a mycoherbicide to control *Lantana camara* (Saxena & Pandy, 2002). *Curvularia intermedia* was also isolated from diseased crabgrass (*Digitaria* sp.) and the potential use as mycoherbicide to control

large crabgrass, *D. sanguinalis*, was reported (Tilley & Walker, 2002). Meanwhile, Kokaew (2005) isolated *C. pallescens* from diseased leaves of *Cyperus rotundus*, *Dactylotenium aegyptium*, *Echinochloa colona*, *Eleusine indica*, *Digitaria ciliaris*, *Brachairia reptans*, *Rottboellia cochinchinensis* and *Pennisetum polystachyon*.

The application of *Bipolaris bicolor* was reported to control Johnson grass (*Sorghum helepense*) (Bonilla, Lopez, Mena, Rodriguez, Perez, & Tomas, 1999). Moreover, Chandramohan and Charudattan (2001) successfully applied *Exserohilum rostratum* as foliar spray to control seven weedy grasses in Citrus orchard. Application of *Fusarium oxysporum* as a mycoherbicide to control *Striga hermonthica* was used in Western Africa (Clotala, Watson, & Hallett, 1995). Boyett, Walker, and Abbas (2002) reported the application of *Myrothecium verrucaria* to control Kudzu (*Pueraria lobata*). Secondary metabolites such as Verrucaria A,B,C,D,E,F,G,J; Roridins A,B,D,E,H; Muconomycin; Coprogen B and Gliotoxin also have antifungal properties.

For this study, we obtained 25 fungal isolates, but there was only one isolate, *Drechslera holmii*, which caused weed disease. Therefore, *D. holmii* was investigated for potential use as a biological weed control agent in the vegetable fields.

Figure 3 *Dactyloctenium aegyptium*, control (A); Sprayed with spore suspension at 10^6 spores/ml of *Drechslera holmii* (B)

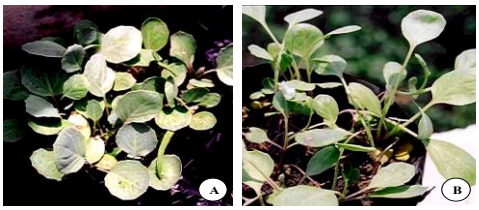

Figure 4 *Brassica alboglaba*, control (A), sprayed with spore suspension at 10^6 spores/ml of *Drechslera holmii* (B)

A pathogenicity test of fungi isolated from weed was conducted using *Drechslera holmii*. It was inoculated in the greenhouse to four healthy plant species; *Cyperus brevifolius*, *Brachiaria mutica*, *Dactyloctenium aegyptium* and vegetable *Brassica alboglaba.* Spore suspension at 10^6 spores/ml of *D. holmii* was sprayed on artificial wounded leaves of the 4 week-old healthy seedlings. It caused severe damage only on *D. aegyptium*. Disease symptoms were found after 5 days of inoculation. This fungus in USA was also reported to cause leaf spot disease of *D. aegyptium* but there were no further tests on any crops (Ellis, 1971). Therefore, the potential use *of D. holmii* as a biological control on vegetable plots is worth further investigating.

4. Conclusion

A total of 25 isolates of fungi were found on leaf spot and leaf blight of weed disease from Bangkok, Pathum Thani and Chanthaburi provinces. These isolates were common plant pathogens (*Alternaria alternate*, *Bipolaris bicolor*, *Curvularia lunata*, *C. pallescens*, *Drechslera halodes*, *Myrothecium cinctum*, and *Nigrospora oryzae*) causing leaf spot and leaf blight diseases.

Drechslera holmii caused severe damage only on the weed *Dactyloctenium aegyptium* after 5 days of inoculation, but no symptoms occurred on *Cyperus brevifolius*, *Brachiaria mutica*, or the vegetable *Brassica alboglaba*. This considered a host range specific of *Drechslera holmii* on the weed species. However, further study is needed to investigate development of a bioherbicide, as well as a biological weed control for vegetables.

5. Acknowledgement

We would like to thank the authorities of Faculty of Biotechnology, Rangsit University and Thailand Institute of Scientific and Technological Research for extending facilities on this research.

6. References

Auld, B. A., & Mc Rae, C. (1999). Emerging technologies in plant protection-bioherbicides. In: *Proceedings of New Zealand Plant Protection Society (NZPPS)*, New Zealand, pp. 1–4.

Babu, R. M., Sajeena, A., & Seetharaman, K. (2003). Bioassay of the potentiality of *Alternaria alternate* (Fr.) Keissler as a bioherbicide to control water hyacinth and

other aquatic weeds. *Crop Protection. 22*(8), 1005-1013.

Bonilla, T., Lopez, M. O., Mena, J., Rodriguez, K., Perez, E. & Tomas, Y., (1999). Mycobiota of *Sorghum halepense* (L.) Pers. and evaluation of capability of some species for biological control. *Revista de Protection Vegetal. 14*(1), 65-68.

Boyette, C. D., Walker, H. L., & Abbas, H. K. (2002). Biological control of Kudzu (*Pueraria lobata*) with and isolate of *Myrothecium verrucaria*. *Biologicalcontrol Science and Technology. 12*, 75-82.

Casella, F., Charudattan, R., & Vurro, M. (2010). Effictiveness and technological feasibility of bioherbicide candidates for biocontrol of green foxtail (*Setaria viridis*). *Biocontrol Science and Technology*. *20*(9/10), 1027-1045.

Chandramohan, S., & Charudattan, R. (2001). Control of seven grasses with a mixture of three fungal pathogens with restricted host ranges. *Biological Control. 22*, 246-255.

Chiang, M., Van Dyke, C. G., & Chilton, W. S. (1989). Four foliar pathogenic fungi for controlling seeding Johnson grass (*Sorghum halepense*). *Weed Science. 37*, 802-809.

Clotola, M., Watson, A. K., & Hallett, S. G. (1995). Discovery of an isolate of Fusarium oxysporum with potential to control Striga hermonthica in Africa. *Weed Research. 35*, 303-309.

Daniel, J. T., Templeton, G. E., Smith Jr., R. J., & Fox, W. T., (1973). Biological control of northern joint vetch in rice with an endemic fungal disease. *Weed Science. 21*, 303–307.

Ellis, M. B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey. 608 pp.

Figliola, S. S., Camper, N. D., & Ridings, W. H. (1988). Potential biological control agents for goosegrass (*Eleusine indica*). *Weed Science 36*, 830-835.

Jeamjitt, O. 2007. Diversity of coprophilos fungi, antagonism against plant pathogenic fungi, and secondary metabolites of Asodesmis macrospore and Sordaria fimicola. Ph.D. Thesis, Kasetsart University. 173 pp.

Kokaew, J. (2005). Diversity of fungi on major weed diseases in vegetable garden plots and their potential uses as biological weed control. M.S. thesis. Kasetsart University. 150 pp.

Kokaew, J., Manoch, L., Visarathanon N., & Suwanagul. D. (2006). Diseases of Weed in Vegetable Garden Plots and Their Potential Use for Their Biological Weed Control. *In* Abstracts Book of the 8th International Mycological congress. Cairns Convention Centre Queensland, Australia. 21-25 August 2006.

Manoch, L., Jeamjitt, O., Jaroenthai K., & Srigiew, C. 2002. Plant pathogenic fungi from rice kernels and other host plant. p. 99. *In* Abstracts of the 1st International Conference on Tropical and Subtropical Plant Disease. Chiang Mai, Thailand.

Ninsuwan, N., Chuenchit, S., & Petcharat., V. (2010). Evaluation of *Exserohilum longirostratum* (Subram.) Sivan. for biological control of *Dactyloctenium aegyptum* (L.) Beauv. *Journal of Agricultural Technology. 7*(2), 463-473.

Sandrin T. R., D. O. TeBeest, & Weidemann, G. J. (2003). Soybean and sunflower oils increase the infectivity of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* to Northern Jointvetch. *Biological Control. 26*(3), 244-252.

Saxena, S., & Pandy, A. K. (2002). Evolution of indigenous isolate of Alternaria alternate (LC≠508) for use as a mycoherbicide for *Lantana camara* L. *Crop Protection. 21*(1), 71-73.

Thomas, J. S., Muller-Stover, D., Ziegler, A., Bedi, J. S., & Kroschel, J. (1998). The Potential of *Fusarium oxysporum* f.sp. *orthocerasas* a Biological Control Agent for *Orobanche cumanain* Sunflower. *Biological Control. 13*(1), 41-48

Tilley, A. M., & Walker, H. L. (2002). Evaluation of *Curvularia intermedia* (*Cochiobolus intermedius*) as a potential microbial herbicide for large crabgrass (*Digitaria sanguinalis*). Biological and Chamical Diversity. *Pure. Appl. Chem. 70* (11).

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