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Anamorphs of *Cochliobolus* on disease plants in Southern Thailand

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The anamorphic stages of *Cochliobolus* spp. were studied in Songkhla province and nearby areas. The disease plant specimens were collected and isolated for the associated fungi. Twenty six species of *Bipolaris* and *Curvularia* were identified. The ability of these fungi to produced sexual organs were determined by mating the single conidial isolate of each species on Sach 's agar medium holding sterilized corn leaves. Only a pair of isolates 3 and 5 of *Bipolaris hawaiiensis* produced complete perithecium 45 days after mating while *B. ellisii*, *C. andropogoni*, *C. geniculata*, *C. pallescen* and *C. senegalensis* produced protothecium on the medium.

Key words: *Bipolaris*, *Cochliobolus*, *Curvularia*, taxonomy

Introduction

The fungus *Cochliobolus* is the teleomorph of *Bipolaris* and *Curvularia* which are the causal agents of a wide variety of an economic important crops and weeds. The teleomorphic stage of this fungus is extremely rare in nature and thus it is the anamorphic stage which causes infection in the fields. Some examples of severe diseases caused by *Cochliobolus* in Thailand are leaf blight of corn (*C. heterostrophus*) (Lapbanjob *et al*., nd), brown spot of rice (*C. oryzae – sativa*) black kernel of rice (*C. geniculatus*, *C. lunatus*) (Ou, 1985), bird 's eye leaf spot of para rubber (*C. heveicola*) etc.

Cochliobolus also effects weed growth, therefore many researchers tried to use them as biocontrol agent to control weeds. Some examples of weed control with *Cochliobolus* 's anamorphs were exist, such as *Digitaria sanguinalis* infected by *C. intermedia* (Tilley *et al*., 2002) *Eleusine indica*

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infected by *B. setariae* (Figliola *et al*., 1988) and *Sorghum halepense* infected by *B. halepense* (Chiang *et al*., 1989) etc.

The objective of this study was to identify the distribution of *Cochliobolus* spp. on disease plants through field surveys in Songkhla province and nearby areas. The ability of anamorph to produced the sexual organs in agar media was determined.

Meterials and methods

Collection and isolation

The surveys and collections of disease specimens were done in Songkhla province and nearby area during 2005–2007 (Table 1). The specimens were incubated in moisture plates and determined for the associated fungus on the diseased tissues. The fungus was isolates into pure culture by single spore isolation on PDA + 200 ppm of streptomycin sulfate.

Identification

The fungus was directly taken from the disease tissues and mounted on the slide using lactophenol as a mounting medium. Details of morphological characters were observed under an Olympus microscope (BH2). Thirty conidia and conidiophores were measured using micrometer. The identification followed the key of Ellis (1971, 1976) and Sivanesan (1987).

Mating tests

The medium used for teleomorph production in culture was Sach's agar holding sterilized corn leaves. After two strains of single conidial isolates were transferred to the same medium, the Petri dishes were incubated at 30°c in the dark condition. The ascostroma were examined under microscope after 2-8 weeks.

Results

Three hundred and fifty specimens of leaf blight and leaf spot of 5 crop plants and 13 weed plants were collected in Songkhla, Suratthani, Phatthalung, Phangnga, Phuket, Krabi and Nakhonsithammarat Provinces in southern Thailand during 2005 - 2007. Twenty six *Cochliobolus*'s anamorphs (13 *Bipolaris*, 13 *Curvularia*) were found associating on diseased tissues as shown on Table 1. All species were able to be isolated into pure culture on PDA. The

teleomorphic stage of *Cochliobolus* was never observed on disease tissues after incubation of the specimens in moisture for plates for up to 2 months.

After mating the different strains of each species on Sach's agar holding a fragments of sterilized corn leaves, only *B. hawaiiensis* (isolate 3 and 5 paring) produced complete sexual organs on the agar after 45 days incubation at 30°c and were identified as *Cochliobolus hawaiiensis*. The perithecia were globose, 400-450 um diam, dark brown color and with long ostiole neck up to 700 um long, 70-150 um wide. Inside the perithecium were asci, cylindrical, each asci contained 8 transparent filiform and hyaline ascospores, rather loosely coiled in the ascus and doubled back at the ends, 72 – 154 x 2 – 4 um. (Fig. 1).

B. ellisii, *C. andropogonis*, *C. geniculata*, *C. pallescen and C. senegalensis* produced incomplete perithecium called protothecium on agar medium after 3-8 weeks incubation. The protothecia were globose to irregular shaped, dark brown to black similar to perithecium but do not form asci and ascospores inside.

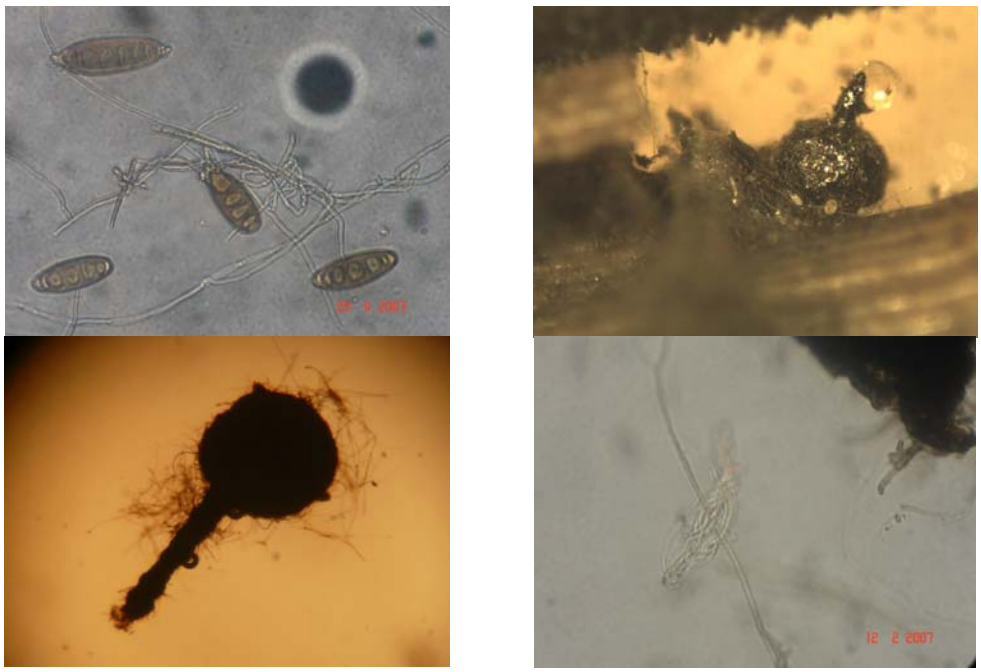

Fig. 1 *Cochliobolus hawaiiensis* A: conidial stage B: perithecium on corn leaves C: perithecium, D: ascus with ascospores.

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Discussion

The teleomorph stage of *Cochliobolus* spp. has never observed on disease plants in natural condition of southern Thailand, although the specimens were incubated in moist chamber upto two months. One limited condition maybe due to the high temperature. Most of *Cochliobolus* spp. have been reported to produce sexual organs at 20 – 26°c (Sivanesan, 1987) but an average temperature in southern Thailand is about 28–30°c. In this study, it was confirmed that *Cochliobolus hawaiiensis* is heterothallic fungi and *B. hawaiiensis* is the anamorphic stage of *C. hawaiiensis*.

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References

Chiang, M.Y., Van Dyke, C.G. and Chilton, W.S. (1989). Four foliar pathogenic fungi for controlling seedling Johnson grass (*Sorghum halepense*). Weed Sciences 37: 802 – 809.

Ellis, M.B. (1971). Dematiaceous Hyphomycetes. Cambrian News, Aberystwyth. UK.

Ellis, M.B. (1976). More Dematiaceous Hyphomycetes. Cambrian News, Aberystwyth. UK. Figliola, S.S., Camper, N.D. and Riding, W.H. (1988). Potentail biological control agent for goosegrass (*Eleusine indica*). Weed Sciences 36: 830 – 835.

Lapbanjob, S., Po-ngam, P., Singhbura U-dom, N. and Manupeerapan, T.(nd.) Genetic and pathogenicity variation among isolates of *Bipolaris maydis* (Nisik. and Miyake) in Thailand. Field Crop Research Institute, Department of Agriculture Bangkok. Thailand [online]. Available from http://210.246.186.28/fieldcrops/res/0744.HTM [2008 May 11]

Ou, S.H. (1985). Rice Diseases. CAB Publications, Central Sales, Farnham Royal, Slough, U.K. 2. nd. Ed. p. 380.

Sivanesan, A. (1987). "Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and their teleomorphs"CAB Int. Wallingford.

Tilley, A.M. and Walker, H.L. (2002). Evaluation of *Curvularia intermedia* (*Cochliobolus intermedius*) as a potential microbial herbicide for large crabgrass (*Digitaria sanguinalis*). Biological control 25: 12 – 21.

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Table 1. List of *Bipolaris* and *Curvularia* anamorphic stage of *Cochliobolus* and their hosts in southern Thailand**.**

Fungal name	Descriptions	Hosts
Bipolaris australiensis (Ellis) Tsuda & Ueyama	Conidiophores single, flexuous or geniculate, cylindrical, septate, smooth, pale brown to reddish brown, up to 250 um long, 2 – 8 um thick. Conidia straight, ellipsoidal, rounded at the ends, pale brown to mid brown, usually 3 distoseptate, 8 – 9 x 19 – 21um.	*Chloris barbala* (SW.) *Dactyloctenium aegyptium* L.P. Beauv *Digitaria ascenden* (HBK) Henr *Eleusine indica* (L.) Gaerth *Rhycholytrum repens* Willd *Zea mays L.*
Bipolaris australis Alcorn	Conidiophores single, branched, cylindrical, geniculate, septate, smooth, reddish brown to dark brown, upto 200 um long, 4.5 - 7.5 um thick. Conidia straight, cylidrical, 4 - 6 pseudoseptate, 7 - 10 x 11.5 - 38 um.	*Cenchus echinatus* L. *Cynodon dactylon* (L.) Pars
Bipolaris colocasiae (Tandan & Bhargava) Alcorn	Conidiophores single or in groups, geniculate near the apex, septate, smooth, brown to dark brown, upto 200 um long, 4 - 6 um thick. Conidia straight, fusoid, smooth, 4 - 9 pseudoseptate, pale brown to brown, 5 - 10 x 18 - 49 um.	*Chloris barbala* (SW.)

Table 1. Continued…

Fungal name	Descriptions	Hosts
Bipolaris cynodontis (Marignoni) Shoem.	Conidiophores single, flexuous, cylindrical, septate, smooth, dark brown, up to150 um long, 4 - 7 um thick. Conidia straight or cylindrical, slightly curved, smooth, 6 - 11 pseudoseptate, pale brown, 14 - 17 x 30 - 67 um.	*Chloris barbala* (SW.) *Cynodon dactylon* (L.) Pars *Dactyloctenium aegyptium* L.P. Beauv *Digitaria ascenden* (HBK) Henr *Eleusine indica* (L.) Gaerth *Heveae brasiliensis* Muell. Arg. *Penisetum polystachyon* Schult
Bipolaris ellisii (Danquah) Alcorn	Conidiophores single, straight to flexuous, branched, cylindrical, septate, smooth, mid brown to dark brown, up to 300 um long, 3 - 6 um thick. Conidia usually clavate, slightly curved, smooth, 3 - 5 pseudoseptate, mid brown to dark brown, 18 - 22 x 7.5 - 12 um.	*Brachiaria mutica* (Forsk.) Stapf *Dactyloctenium aegyptium* L.P. Beauv *Echinochloa colona (L.) Link.* *Hymenachne pseudointerrupta* C. Muell. *Imperata cylindrical* (L.) Beauv *Sorghum vulgare* Pers.
Bipolaris hawiiensis (Ellis) Uchida & Aragaki	Conidiophores single, straight or flexuous, septate, smooth, pale brown to brown, up to 200 um long, 2 - 6 um thick. Conidia straight, cylindrical, 4 - 6 pseudoseptate, 7 - 10 x 11.5 - 38 um.	*Cynodon dactylon* (L.) Pars *Dactyloctenium aegyptium* L.P. Beauv *Digitaria ascenden* (HBK) Henr *Echinochloa colona (L.) Link.* *Zea mays L.*

Table 1. Continued…

Fungal name	Descriptions	Hosts
Bipolaris leerisae (Atk.) Shoem.	Conidiophores single or in groups, straight to flexuous, geniculate, cylindrical, septate, smooth, brown to dark brown, up to 200 um long, 2 - 6 um thick. Conidia fusoid or ellipsoidal, cylindrical, 3 - 12 pseudoseptate, pale brown to brown, 18 - 27 x 37 - 117 um.	*Brachiaria mutica* (Forsk.) Stapf
Bipolaris maydis (Nisikado & Miyake) Shoem.	Conidiophores in groups, septate, smooth, brown to dark brown, up to 500 um long, 5 - 7 um thick. Conidia distintly curved, fusoid, pale brown to brown , usually 6 - 11 pseudoseptate,10 - 12 x 80 - 130 um.	*Zea mays L.*
Bipolaris papendorfii (van der Ae) Alcorn	Conidiophores single, straight to flexuous, geniculate, septate, smooth, pale brown to brown, up to 200 um long, 5 - 7 um thick. Conidia typically curved, smooth, pale brown at the ends, pale brown to brown, 3 pseudoseptate, 20 - 25 x 27 - 45 um.	*Zea mays L.*

Table 1. Continued…

Fungal name	Descriptions	Hosts
Bipolaris sacchari (Butler) Shoem.	Conidiophores single or in groups, straight, flexuous near the apex, septate, smooth, brown to dark brown , up to 250 um long, 4 - 8 um thick. Conidia straight, cylindrical, smooth, pale brown, 5 - 9 pseudoseptate, 8 - 20 x 40 - 100 um.	*Chloris barbala* (SW.)
Bipolaris setaria (Saw.) Shoem.	Conidiophores single, straight, flexuous near the apex, septate, smooth, brown to dark brown, up to 300 um long, 5 - 8 um thick. Conidia straight, cylindrical, slightly curved, pale brown, 8 - 14 pseudoseptate, 11 - 20 x 55 - 117um.	*Cynodon dactylon* (L.) Pars *Dactyloctenium aegyptium* L.P. Beauv *Digitaria ascenden* (HBK) Henr *Echinochloa colona (L.) Link.* *Eleusine indica* (L.) Gaerth *Imperata cylindrical* (L.) Beauv *Penisetum polystachyon* Schult
Bipolaris sorghicola (Lefevre & Sherwin) Alcorn	Conidiophores single, straight or flexuous, septate, smooth, dark brown, up to 300 um long, 6 - 10 um thick. Conidia slightly curved, fusoid, smooth, 4 - 8 pseudoseptate, pale brown to reddish brown, 14 - 16 x 42 - 65 um.	*Eleusine indica* (L.) Gaerth *Imperata cylindrical* (L.) Beauv

Table 1. Continued…

Fungal name	Descriptions	Hosts
Bipolaris sorokiniana (Sacc.) Shoem.	Conidiophores single or in groups, straight to flexuous or geniculate, cylindrical, septate, smooth, dark brown, up to 150 um long, 4 - 9 um thick. Conidia ellipsoidal, cylindrical, mid brown to dark brown, 3 - 12 pseudoseptate, 15 - 30 x 50 - 130 um.	*Dactyloctenium aegyptium* L.P. Beauv
Curvularia andropogonis (Zimm.) Boedijn	Conidiophores in groups, geniculate, cylindrical , septate, smooth, pale brown to dark brown, up to 400 um long, 8 - 15 um thick. Conidia sligthly curved or clavate, third cell from base is larger and darker than others, 3 distoseptate, brown to dark brown, 18 - 30 x 45 - 65 um.	*Cymbopogon citrates Stapf.*
Curvularia brachyspora Boedijn	Conidiophores single or in groups, straight, cylindrical, septate, smooth, brown to dark brown, up to 300 um long, 4 - 5 um thick. Conidia straight or slightly curved, approxximately ellipsoidal, brown to dark brown, usually 3 distoseptate, 9 - 11 x 20 - 23 um.	*Digitaria ascenden* (HBK) Henr

Table 1. Continued…

Fungal name	Descriptions	Hosts
Curvularia borreria (Viégas) M.B. Ellis	Conidiophores sometimes singly but more often in groups, simple, straight or flexuous, septate,smooth, often paler near the apex, up to 200 um long, 5 - 7 um thick. Conidia straight or slightly curved, clavate or pyriform, has a protuberrant hilum, 3 distoseptate, third cell from base is the largest, brown to dark brown, 6 - 13 x 17 - 28 um.	*Borreria latifolia* (Aubl.) k. Sch.
Curvularia clavata Jain	Conidiophores single or in groups, straight or flexuous, septate, smooth, pale brown to brown, up to 150 um long, 3 - 5 um thick. Conidia straight or slightly curved, usually calvate, base cell pale, other cell dark brown, smooth, 3 distoseptate, 7 - 10 x 19 - 21 um.	*Cymbopogon citrates Stapf.* *Eleusine indica (L.) Gaerth* *Heveae brasiliensis Muell. Arg.* *Sorghum vulgare Pers.*

Table 1. Continued…

Fungal name	Descriptions	Hosts
Curvularia eragrostidis (Henn.) J.A. Meyer	Conidiophores in groups, straight or geniculate, cylindrical, septate, smooth, pale brown to brown, up to 350 um long, 3 - 5 um thick. Conidia straight, ellipsoidal or barel shaped, brown to dark brown, uaually 3 disoseptate, 11 - 12 x 27 - 31 um.	*Zea mays L.*
Curvularia fallax Boedijn	Conidiophores single or in groups, branched, septate, smooth, reddish brown to brown, up to 250 um long, 3 - 4 um thick. Conidia straight or curved, ellipsoidal, pale brown, 3 - 4 distoseptate, 8 - 10 x 23 - 27 um.	*Hevea brasiliensis* Muell. Arg.
Curvularia geniculata (Tracy & Earle) Boedijn	Conidiophores in groups, geniculate, flexuous, septate,smooth, brown to dark brown, up to 500 um long, 3 - 5 um thick. Conidia usually curved, geniculate, fusiform, pale brown to brown, 3 - 4 distoseptate, rarely 5 distoseptate, 8 - 10 x 23 - 25 um.	*Brachiaria mutica (Forsk.) Stapf* *Cyperus iria L.* *Digitaria ascenden (HBK) Henr* *Echinochloa crus-galli (L.) Beauv.* *Eleusine indica (L.) Gaerth* *Hevea brasiliensis Muell. Arg.* *Saccharum officinarum Linn.* *Sorghum vulgare Pers.* *Zea mays L.*

Table 1. Continued…

Fungal name	Descriptions	Hosts
Curvularia lunata (Wakker) Boedijn	Conidiophores single or in groups, straight or curved, ylindrical, septate, smooth, brown to dark brown, up to 500 um long, 3 - 4 um thick. Conidia curved, geniculate, third cell from base usually larger and often darker than others, pale brown to brown, 3 distoseptate, 9 - 11 x 23 - 26 um.	*Chloris barbala* (SW.) *Digitaria ascenden* (HBK) Henr *Hymenachne pseudointerrupta* C. Muell. *Sorghum vulgare* Pers.
Curvularia pallescens Boedijn	Conidiophores in groups, geniculate near the apex, cylindrical, septate, smooth, brown to dark brown, up to 200 um long, 2 - 4 um thick. Conidia straight or slightly curved, 3 distoseptate, second septate from base is darker than others, pale brown to brown, 10 - 12.5 x 24.5 - 25 um.	*Hevea brasiliensis* Muell. Arg.
Curvularia peniseti (Mitra) Boedijn	Conidiophores single or in groups, flexuous, cylindrical, septate, smooth, reddish brown to dark brown, up to 150 um long, 3 - 5 um thick. Conidia slightly curved, clavate, third cell from base is larger than others, pale brown to dark brown, 3 distoseptate, 15 - 20 x 34 - 36 um.	*Brachiaria mutica* (Forsk.) Stapf

Table 1. Continued…

Fungal name	Descriptions	Hosts
Curvularia senegalensis (Speg.) Muntanola	Conidiophores single or in groups, straight or flexuous, cylindrical, septate, smooth, brown to dark brown, up to 200 um long, 3 - 5 um thick. Conidia geniculate, mid cell is darker than others, smooth, brown to dark brown, 3 - 4 distoseptate, 10 - 13 x 23 - 26 um.	*Zea mays L.*
Curvularia uncinata Bugnicourt	Conidiophores single or in groups, simple or branched, flexuous, geniculate, septate, smooth, pale brown to brown, up to 400 um long, 2 - 5 um thick. Conidia usually strongly curved or hook shaped, brown to dark brown, usually 4 distoseptate, 7 - 9 x 25 - 27 um.	*Saccharum officinarum* Linn.
Curvularia verusiformis Agarwal & Sahni	Conidiophores arising singly or in groups, simple or branched, straight or flexuous, sometimes geniculate, septate, smooth, pale brown to brown, up to 150 um long, 4 - 7 um thick. Conidia curved, fusiform to ellipsoidal, mid cell often largest and darkest, brown to dark brown, 3 - 5 distoseptate, always 4 distoseptate, 6 - 10 x 18 - 24 um.	*Sorghum vulgare* Pers.