



**Identification of new fungi isolated from *Echinochloa* spp.,
as potential biological control agents
in soybean fields in India**

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Abstract

Echinochloa spp. is the most important weeds of soybeans fungal pathogens can be exploited as biological agents for the management of agricultural weeds. Two pathogenic fungal species were isolated from naturally infected *Echinochloa* species and identified. In order to isolate the fungus from disease tissues, the obtained samples were cultured on potato dextrose agar medium. Isolates were cultured due to sporulation on water agar medium. Morphological characters of isolates were studied in order to identify the taxonomy. According to the results, isolates belonged to *Bipolaris maydis* (Nisikado and Miyake) Shoem, and *Bipolaris australiensis* (Tsuda and Ueyama) Alcorn. Pathogenicity test of isolates in species was done in desiccators, and revealed the pathogenicity of the species and their ability to cause leaf blight on *Echinochloa* spp. Inoculation was done using a spore suspension consisting of 10⁵ spore/ml distilled water and 1% Tween-20 at the 2 to 3 leaf stage. Results indicated that the disease rating caused by *B. maydis* and *B. australiensis* in *Echinochloa* spp. was higher than that observed in the studied Soybean cultivar. Hence *B. maydis* and *B. australiensis* can be considered as a probable mycoherbicide for controlling *Echinochloa* species.

Key-Words: *Echinochloa* spp., fungi, biological control, *Bipolaris* spp.

Introduction

Soybeans (*Glycin max*) are one of the most important oil-seed crops in the world, being the staple food for millions of people in Asia (Deluna et al., 2002). Weeds are considered a major constraint to world soybeans production (Moody, 1995). The *Echinochloa* spp. is major weeds wherever soybeans are grown (Holm et al., 1977). Their distribution is truly global from temperate to tropics in a wide variety of crops (Gressel, 2002). *Echinochloa* spp., severely reduce both yield and quality of soybeans (Holm et al., 1977). Current weed management practices of mechanical, cultural, and chemical methods need to be reassessed in the wake of increasing concerns about economical and environmental sustainability of intensive soybeans production (Deluna et al., 2002). Fungal pathogens can be exploited as biological agents for the management of agricultural pests and diseases (Evans, 1999). *Bipolaris hawaiiensis* had been reported on bermudagrass (*Cynodon dactylon*) and other *Cynodon* spp. from subtropical areas around the world (Pratt, 2001).

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Virulence of *Bipolaris hawaiiensis* on bermudagrass, compared with *B. cynodontis* and *B. spicifera*. All three pathogens induced symptoms of chlorosis and necrotic lesions (Pratt, 2001). Six pathogenic fungal species were isolated from naturally infected *Echinochloa* species in soybeans (Zhang et al., 1996). *Bipolaris sacchari*, *Curvularia geniculata*, *Dactylaria dimorphospora*, and *Exserohilum monoceras* were pathogenic only to *Echinochloa* species (Zhang et al., 1996). Two fungal pathogens, *B. sacchari* and *Drechslera gigantea* were identified as promising biological control agents for cogongrass (*Imperata cylindrica*) (Yandoc et al., 2005). *B. sacchari* was capable of causing foliar blight on cogongrass (Yandoc et al., 2005). In Japan, a fungal pathogen, identified as *Drechslera monoceras* is being evaluated as a bioherbicide for control of *Echinochloa* species in fields (Gohbara et al., 1996; Goto, 1992). In Malaysia and Indonesia, ten barnyardgrass (*Echinochloa crus-galli* var. *crus-galli*) ecotypes were tested for variation in their susceptibility to the leaf blight pathogen (*Exserohilum longirostratum*) (Jurami et al., 2006). *Alternaria alternata* and *Fusarium equiseti* were reported as eventual biological agents for the management of *Echinochloa* spp. (Safari Motlagh, 2010). The principle goal of this research was to

identify new genus and species of fungi isolated from *Echinochloa* spp., as eventual agents biological, control of this weed in India.

Material and Methods

Collection and culture of fungal isolates

Diseased leaves of *Echinochloa* spp. were sampled from five locations in each field of Madhya Pradesh each sampled location was approximately 5 x 8 m and locations were approximately 35 m apart together (Xia et al., 1993). Leaves were transferred to the laboratory and the fungi was then isolated from disease samples. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on potato dextrose agar in Petri dishes at 27 to 30°C for 2 to 3 days. Related to fungus, potato dextrose agar or water agar was used for sporulation. Then Petri dishes containing media were incubated at 27°C in the dark or artificial light supplied by fluorescent light on a 12 h light/dark photoperiod for 5 to 30 days (Montazeri et al., 2006).

To avoid bacterial contamination, sulfate streptomycin antibiotic was used. Conidia were single- sporulated. Monoconidial isolates of the recovered fungi were maintained on half- strength potato dextrose agar slants in test tubes as stock cultures or colonial of fungal placed onto sterilized filter paper, then cuts of these filters were incubated in sterilized vials at freezer on -20°C

Study and identification of fungi

Morphological studies were carried out on potato dextrose agar and water agar media. Cuts of colonies or each of filter papers were placed onto potato dextrose agar medium for 2 to 3 days. Then, section of colonies was transferred to water agar medium for 7 to 30 days in incubator at 27°C and 12 h photoperiod. Afterward, morphological observations were taken based on colony, conidium and conidiophore morphology and other morphological characters (Ellis, 1971; Sivanesan, 1987).

Pathogenicity tests

Pathogenicity tests of weed were carried out in desiccators. In each of two desiccators (one desiccator as control) two Petri dishes were placed each containing 10 germinated seeds of *Echinochloa* spp. At first, seeds of *Echinochloa* spp. were placed on moistened filter paper in Petri dishes and incubated at 28°C for 24 h in a germinator with 12 h light/ dark photoperiod. Then, seeds were surface sterilized with 0.2% sodium hypochlorite solution for 2 min. After washing with distilled water, ten germinated seeds (coleoptiles and radical just emerged) were planted per 10- cm- Petri dishes filled with saturated soil (Zhang et al., 1996), and were incubated at room temperature.

Distilled wafer added to Petri dishes. Seedlings at were applied for *Echinochloa* spp. (Zhang et al, 1996; Bertrand and Gottwald, 1997).

$$\text{Disease rating} = \frac{(N_1 \times 1) + (N_2 \times 2) + \dots + (N_t \times t)}{(N_1 + N_2 + \dots + N_t)}$$

Where N is number of leaves in each of rate and t is number of treatments.

Pathogenicity tests were earned out in desiccators. To do so, in each of two desiccators (one desiccators as control) were placed two Petri dishes and in each Petri dish was placed 10 seeds of rice, 'Glazer cultivar. Then, seeds were sterilized in water bath at 52 to 57°C and cultivated in saturated soil and incubated at 25°C. Distilled water was added to Petri dishes. After 16 to 18 days, 2 to 3 foliages seedlings were inoculated by suspension of spores (Safari Motlagh and Kaviani, 2008). Other conditions including concentration of conidia and evaluation systems were similar.

Data analysis

Data analysis was done using NTSYS software (Sneath and SAM, 1973).

Results and Discussion

The fungi isolates belonged to *Bipolaris* spp. These isolates were divided into 2 groups based on morphological characters, as follows:

Characteristics of first group

Colonies fast-growing, fluffy, with concentric rings is given in Figure 1. Conidiophores single or often in groups from flat, dark brown to black stromata, straight to flexuous, septate, smooth, geniculate, mid to dark brown, paler towards the apex, up to 700 µm long, 5 to 10 µm thick (Figure 2). Conidiogenous nodes verruculose. Conidia distinctly curve, fusoid, pale to mid dark golden brown, smooth, 5-11-distoseptate, 70 to 160 x 15 to 20 µm, hilum 3 to 4.5 µm wide (Figure 3). The characteristics of this group corresponded with *B. maydis* (Nisikado and Wake) Shoem (Sivanesan, 1987; Ellis, 1971).

Characteristics of second group

Conidial colonies effuse, grey to blackish brown, velvety (Figure 4). Hyphae pale to dark brown, smooth, septate. Stromata erect, straight, cylindrical, black, formed in culture on rice grains. Conidiophores single, flexuous, geniculate, septate, smooth, cylindrical, reddish brown, up to 150 µm long, 3 to 7 µm thick (Figure 5). Conidiogenous nodes verruculose. Conidia straight, ellipsoidal or oblong rounded at the ends, pale brown to mid reddish brown, usually 3, rarely 4-5-distoseptate, and 14 to 40 x 6 to 11 µm (Figure 6). The characteristics of this group corresponded with *Bipolaris australiensis* (Tsuda fluffy colonies. On rice

appeared pinhead specks and on top of leaves was created blight.

Based on the sizes and types of the spots that appeared on the rice and Horsfall-Barratt system, this cultivar was less affected by the *B. maydis* compared with *Echinochloa* spp., and its disease rating was lower and showed much tolerance (Figure 7).

Based on dendrogram from cluster analysis, *B. maydis* isolates in disease rating index on barnyard grass divided into 2 groups. The first group consisted of 2 isolates. The second group consisted of 1 isolate. Also, in first group, 2 isolates had similarity coefficient of 0.95 (Figure 9).

Based on dendrogram from cluster analysis, *B. maydis* isolates in disease rating index on soybeans is divided into 2 groups. The first group consisted of 1 isolate. The second group consisted of 2 isolates. Also, in second group, 2 isolates had similarity coefficient of 0.95 (Figure 10).

Moreover, the rice cultivar was less affected by the *B. australiensis* compared with *Echinochloa* spp. and its disease rating was lower and showed much tolerance (Figure 8).

Disease rating index of *B. maydis* compared with it in *B. australiensis* on soybeans and *Echinochloa* spp. did not show any significant difference (Figures 7 and 8). Because the number of *B. australiensis* was limited (one isolate), cluster analysis was done. But, on the base of dendrogram, cluster analysis of *B. maydis* and *B. australiensis* together in disease rating index on barnyardgrass, isolates divided into 2 groups. In first group, 2 isolates of *B. maydis* and 1 isolates of *B. australiensis* were placed. In second group, 1 isolate of *B. maydis* was placed (Figure 11).

On the base of dendrogram cluster analysis of *B. maydis* and *B. australiensis* together in disease rating index on rice, isolates were divided into 2 groups. In the first group, there was 1 isolate of *B. maydis* and in the second group; there were 1 isolate of *B. maydis* and 1 isolate of *B. australiensis* (Figure 12).

In the study of the reaction of *Echinochloa* spp. to *B. maydis* and *B. australiensis*, it was found that the disease rating caused by these fungi on the said weed was higher than what was observed in the studied rice cultivar.

In this research based on cluster analysis, isolates of *Bipolaris* spp. indicated similar reactions. But this similarity in isolates of same species was more than other species of this genus. This subject can be related to more genetic diversity in different species (Oliveira et al., 2002).

The virulence of *B. hawaiiensis* on bermudagrass is compared with *B. cynodontis* and *B. spicifera*. All three

pathogens induced symptoms of chlorosis and necrotic lesions (Pratt 2001).

In other study, sporulation by nine species of *Bipolaris*, *Curvularia*, *Drechslera* and *Exserohilum* was observed on symptomatic leaves of ryegrass. The most isolates of *B. cynodontis* were virulent and caused necrosis (Pratt;2006).

Studies indicated that *Bipolaris sacchari* was pathogenic only to *Echinochloa* species and was not pathogenic to rice. When provided a 24-h dew period, *B. sacchari* resulted in 100% mortality of seedlings of *Echinochloa* spp. (Zhang et al., 1996). Therefore, *B. sacchari* have potential to control *Echinochloa* species (Zhang et al., 1996). Also was indicated that *B. sacchari* was capable of causing foliar blight on cogongrass (Yandoc et al., 2005).

Phytotoxins have been reported to be produced by *B. sacchari* (Steiner and Stroble, 1971) that are biologically active on *Echinochloa* spp. (Zhang et al., 1996). It is often assumed that a virulent, highly aggressive pathogen (that is, one that causes a high level of mortality) is a preferred bioherbicide candidate (Zhang et al., 1996). Therefore, identification of fungi associated with weeds, helps us in finding biological control agents of weeds.

In conclusion, *B. maydis* and *B. australiensis* can be exploited as eventual biological agents for the management of *Echinochloa* spp. in paddy fields.

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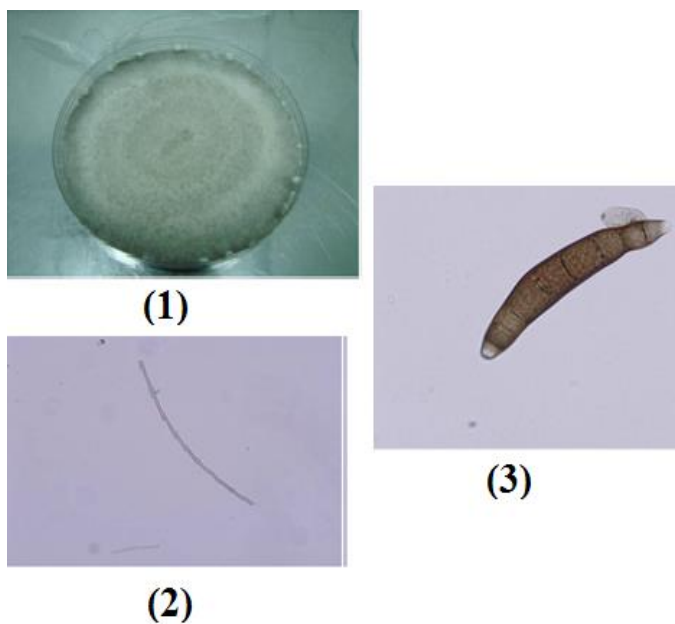


Fig. 1: Colony of *B. maydis* on PDA. 2. Conidiophore of *B. maydis* ($\times 460$). 3. Conidium of *B. maydis* ($\times 1200$)

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