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Isolation and characterization of some important fungi from *Echinochloa* spp. the potential agents to control rice weeds

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Abstract

Barnyard grass (*Echinochloa* spp.) is the most important weeds in rice fields. Some fungal pathogens can potentially be used as biological agents for the control and management of these weeds. For this reason, two pathogenic fungal species were isolated and characterized from naturally infected *Echinochloa* species. Morphological characters of isolates were studied in order to identify the taxonomy. According to the results, isolates were belonged to *Alternaria alternata* (Fries) Keissler, and *Fusarium equiseti* (Corda) Saccardo. Pathogenicity test of isolates was done in desiccators, and revealed the pathogenicity level of the species and their ability to cause leaf blight on *Echinochloa* spp. The results indicated that not only the symptoms but also the virulence of these two fungi are significantly different.

Keywords: *Echinochloa* spp.; fungi; biological control; *Alternaria*; *Fusarium*

Abbreviations: PDA- potato dextrose agar; WA- water agar

Introduction

Barnyard grass, *Echinochloa crus-galli*, jungle rice, *Echinochloa colona* and *E. oryzicola* are ranked as three most important and serious weed species in rice (*Oryza sativa*) (Holm *et al*., 1997; Zhang *et al*., 1996). These species severely reduce both yield and quality of rice (Holm *et al*., 1977; Smith, 1983). Fungal pathogens can be exploited as biological agents for the management of agricultural pests and diseases (Evans, 1999). In Korea, a fungal pathogen *Exserohilum monoceras* was found to cause leaf blight on *E. crus-galli*, but this isolate was also pathogenic to several important crops including rice (Chung *et al*., 1990). In Japan, a fungal pathogen identified as *Drechslera monoceras* is being evaluated as a bioherbicide for control of *Echinochloa* species in paddy fields rice (Gohbara *et al*., 1996; Goto, 1992). In Malaysia and Indonesia, ten Barnyard grass (*E. crus-galli* var. *crus-galli*) ecotypes were tested for variation in their susceptibility to the leaf blight pathogen (*Exserohilum longirostratum*) (Jurami *et al*., 2006). Factors affecting on the herbicidal activity of *Exserohilum monoceras* against *Echinochloa oryzicola* young seedlings were examined using a drop inoculation method under greenhouse conditions (Tsukamoto *et al*., 1998). Bioassays have shown that *E. monoceras* causes leaf blight in *Echinochloa* species and produces phytotoxins which are biologically active against *Echinochloa* species (Zhang and Watson, 2000). Six pathogenic fungal species were isolated from naturally infected *Echinochloa* species and evaluated as biological control agents of *Echinochloa* species in rice (Zhang *et al*., 1996). The principle goal of this research was to identify genus and/or species of fungi which are causal agents of leaf spot in *Echinochloa* spp., in Guilan province on the north of Iran. In fact, this study was carried out due to lack of information regarding the fungi species associated with *Echinochloa* spp.

Materials and methods

Collection and medium culture of fungal isolates

Diseased leaves of *Echinochola* spp. were sampled from five different locations of rice producing province, Guilan, in Iran. Each sampling location was approximately 5 × 8 m and locations were approximately 35 m apart from each other (Xia *et al*., 1993). Leaves were transferred into the laboratory and then the fungi isolated from diseased leaves. Leaf pieces containing lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on the PDA medium in petri dishes at 27-30°C for 2-3 days. The PDA or WA media was used for sporulation depends on type of fungi. The petri dishes containing media were incubated at 27°C in dark or artificial light supplied by fluorescent light on a 12h light/dark photoperiod for 5-30 days (Montazeri *et al*., 2006). Sulfate streptomycin antibiotic was used to avoid the bacterial contamination (Safari Motlagh, and Kaviani, 2008). Conidia were single- sporulated. Monoconidial isolates of the recovered fungi were maintained on half-strength PDA slants in the 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 (Safari Motlagh and Kaviani, 2008).

Study and identification of fungi

Morphological studies were carried out on water agar medium. Cuts of colonies or each of filter papers were placed onto PDA medium for 2-3 days. Then, section of colonies was transferred on to WA medium for 7-30 days in incubator at 27°C and 12h photoperiod. Afterward, morphological observations were taken based on colony, conidium and conidiophore morphology and other morphological characters (Simmons, 2007; Leslie and Summerell, 2006).

Pathogenicity tests

Pathogenicity tests 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. In first step, seeds of *Echinochloa* spp. were placed onto moistened filter paper in petri dishes and incubated at 28°C for 24 h in a germinator with 12h light/ dark photo-period. Then, seeds were surface sterilized with 0.2% sodium hypochlorite solution for 2 min. After washing with distilled water, ten germinated seeds (coleoptile and radical just emerged) were planted per 10- cm- petri dishes filled with saturated soil (Zhang *et al*., 1996), and then incubated at temperature room. Distilled water was added to petri dishes. Seedlings at the one or two- leaf stage were inoculated with 10^5 conidia per ml. To increase the surface adsorption, 1% tween-20 was applied. Evaluation of symptoms was performed 7 days after inoculation. Finally, the standard evaluation system and Horsfall- Barratt system were applied for *Echinochloa* spp. (Zhang *et al*., 1996; Bertrand and Gottwald, 1997).

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

Where N is number of leaves in each of rate, t=Number of treatments

Results and discussion

Totally 148 collected isolates belonged to *Alternaria* spp., and *Fusarium* spp..These isolates were divided into 2 groups based on morphological characters, as follows: Characteristics of first group: The 4 cm diameter colony with four pairs of concentric rings of growth and sporulation on agar surface (Fig 1). Surface sporulation in light- exposed rings was a dense turf of multiple-branched chains of conidia (Fig 2). The aerial portion of the colony (light- deprived rings) consisted of a less dense growth of abundant subarborescent hyphae that produce open, entangled heads of branched chains of conidia. The typical sporulation pattern comprised a single suberect conidiophores and an apical cluster of branching chains of small conidia separated by short secondary conidiophores (Fig 3). In light-exposed areas of a colony, the primary conidiophore was comparatively short, 40-70 × 3-4 µm; it remained simple or may become 1-3 branched or geniculate, with corresponding numbers of primary conidia chains. Branching within the primary chains was initiated when a few secondary conidioph-ores developed a series of conidiogenous loci or when secondary conidiophores arised from conidium body cells. In

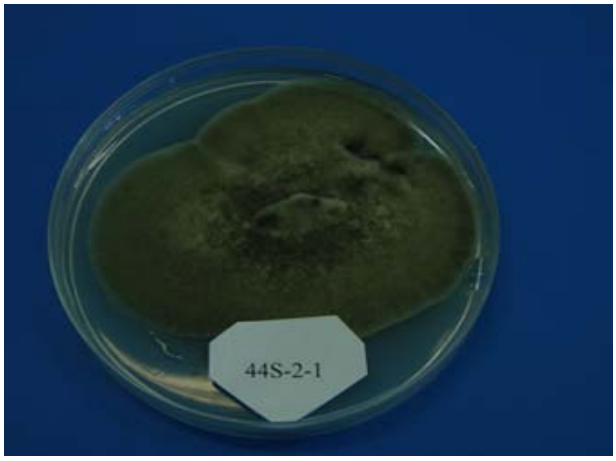

Fig 1. Colony of *Alternaria alternata* on PDA

Fig 2. Conidia of *A. alternata* on WA (×460)

Fig 3. Conidiophore of *A. alternata* (×460)

Table 1. Disease rating caused by *A. alternata* and *F. equiseti* (based on Horsfall- Barratt system)

Fungus	Disease rating
A. alternata	3.11
F. equiseti	2.90

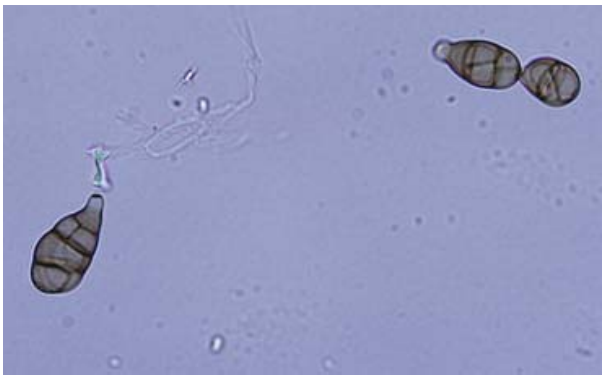

Fig 4. Conidia of *A. alternata*(×1200)

Fig 5. Colony of *Fusarium equiseti* on PDA

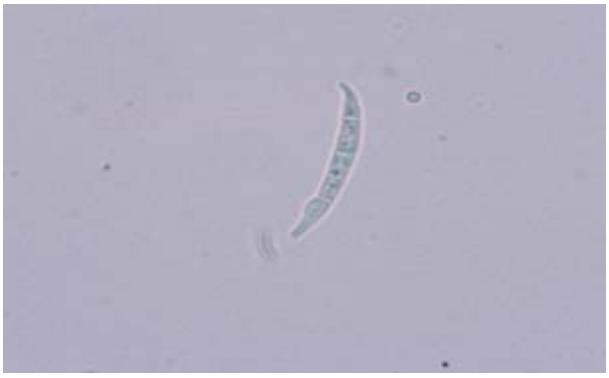

Fig 6. Macroconidia of *F. equiseti* (×1200)

older portions of a colony, the erect conidiophores and their sporulation heads presented a picture of a solid turf of overlapping, interwoven conidial chains. Single chains of conidia in the branching head may have up to 15- 20 conidia. The first 1-2 conidia in a chain usually remain long- elliptical as they mature; conidia produced later in the chain became ovoid, ellipsoid, or subsphaeroid (Fig 4). Initial elliptical conidia were 25-30 (-40) × 5-9 µm, with 4-7 transverse septa and a few or no longisepta; subsequent spores were 7-25 (-40) × 5-12 µm, with 1-7 (very commonly 3) transepta and very few or no longisepta. The secondary conidiophores in chains may be obsolescent, but almost always was a single cell 2-3 × 3-5 µm; occasionally it became 2-celled and, in the case of lateral conidiophores produced from conidium cells, up to 30 × 3-4 µm. Conidia appeared olivaceous a dull grey- green- brown, when first mounted in lactic acid for microexamination; they lose the green tone after a few days and exhibited pale to moderate shades of yellowish to golden brown (Fig 4). The wall ornamentation was densely punctulate in juvenile conidia, became granular to variously verrucose as conidia mature. The characteristics of this group corresponded with *Alternaria alternata* (Fries) Keissler, Beih (Simmons, 2007).

Characteristics of the second group**:** The **a**bundant mycelium that initially was white, but became brown with age (Fig 5). The spore mass may be pale orange to dark brown and annular zonations may develop in response to a light – dark cycle. This species formed a pale brown to dark brown pigment where the colony contacts the agar. Dark brown spots or flecks of pigment were formed in the agar. The macroconidia were long to very long and slender (Fig 6). The macroconidia have pronounced dorsiventral curvature typical. Apiacl cell was tapered and elongated or even whip-like. Basal cell of macroconidia was prominent foot shape that elongated in appearance. Number of septa was usually 5 – to -7- septate. Microconidia were absent. The characteristics of this group corresponded with *Fusarium equiseti* (Corda) Saccardo (Leslie and Summerell, 2006).

Alternaria alternata and *Fusarium equiseti* caused necrotic lesions on all tested *Echinochloa* species. Nectrotic symptoms appeared within 48 h and leaf blight was observed 4-7 days after inoculation with this fungi. The results indicated that not only the symptoms but also the virulence in these two fungi was different (Table1). The fungal pathogens of *A. alternata* and *F. equiseti* can potentially be applied as biological agents for reduction of *Echinochloa* spp. It is usually assumed that a virulent, aggressive pathogen is a preferred bioherbicide candidate (Watson and Wymore, 1990). Some examples for bioherbicide agents are as fllows: *Dactylaria dimorphospora* caused limited disease on *Echinochloa* species but *Curvularia geniculata* caused 100% mortality on *E. colona.* Application of *Bipolaris sacchari* resulted in 100% mortality on *E. colona* and *Exserohilum monoceras* caused 100% mortality on *E. crus-galli*, *E. colona* and *E. glabrescens* (Zhang *et al*., 1996). *Alternaria alternata* was used for biological control of *Amaranthus retrofflexus* (Ghorbani *et al*., 2000). *A. alternata* also caused leaf blight on *Sphenoclea zeylanica* (Masangkay *et al*., 1999). Utilising some metabolites such as tenuaznoic acid, iso- tenuazonic acid and their salts from the cultures of *A. alternata* could lead to control weeds like *Echinochloa* spp. (Qiang *et al*., 2008). The influence of adjuvants on spore germination, desiccation tolerance and virulence of *Fusarium anthophilum* on *Echinochloa crus- galli* has also been evaluated (Montazeri *et al*., 2006).

In total, identification of fungi associated with weeds, greatly helps us to find biological control agents. In conclusion, *A. alternata* and *F. equiseti* can be exploited as eventual biological agents for the management of *Echinochloa* spp. in rice fields of Guilan in Iran but further studies on reaction of rice cultivars against the controlling fungi agents are required.

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