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Study of *Fusarium equiseti*, As Bicontrol Agent of *Echinochloa oryzicola* in Paddy Fields

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Echinochloa oryzicola **is an obligate weed with an elaborated survival strategy in the flooded rice. Fungal pathogens can be exploited as biological agents for the management of agricultural weeds. In this research, *Fusarium equiseti* (Corda) Saccardo was isolated from *Echinochloa oryzicola* and studied as a biocontrol agent against this weed in paddy fields. Pathogenicity test of isolates revealed the pathogenicity of this fungus and its ability to cause leaf spot and leaf blight on *Echinochloa oryzicola*. Inoculation was done using a spore suspension consisting of 10^5 spore/ml distilled water at the 2-3 leaf stage. Also, inhibition of seeds germination test was done. The results showed that *Fusarium equiseti* able to infect *Echinochloa oryzicola* in a higher rate comparing with rice cultivar. Moreover, the results indicated that seeds germination of *Echinochloa oryzicola* was significantly affected by *Fusarium equiseti* in comparison with controls. Hence *Fusarium equiseti* can be considered as a probable bioherbicide for controlling of *Echinochloa oryzicola* at the 2-3 leaf stage of growth of weed.**

Key words: *Echinochloa oryzicola*, *Fusarium equiseti,* biological control.

Echinochloa oryzicola Vasing.(= *Echinochloa phyllopogon* Stapf ex Kessenko) is an obligate weed with an elaborated survival strategy in the rice fields(Yamasue, 2001). *Echinochloa* spp. commonly occur throughout tropical Asia and Africa in fields and along roadsides, ditches, along railway lines, and in disturbed areas such as gravel pits and dumps (Barrett, 1983). The increased use of herbicides will accelerate development of herbicide resistance in weed populations and will increase environmental and societal concerns related to pesticide use (Zhang *et al*., 1996). The use biological control agents such as fungi is one alternative tactic to reduce herbicide inputs (Watson, 1994). This species is considered an invasive species (Barrett, 1983). The weed is distributed in flooded rice (Yamasue, 2001). 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 rice production (de Luna *et al*., 2002). Fungal pathogens can be exploited as biological agents for the management of agricultural pests, diseases and weeds (Evans, 1999). Generally, different *Fusarium* species play important roles in the biological control of the weeds of different crops (Boari *et al*., 2003). For instance, *Fusarium oxysporum* (Schlechtendahl) is considered an important factor in the biological control of weeds (Boari *et al*., 2003). Studies showed that this fungus caused high pathogenesis levels in *Amaranthus retroflexus* (L.) and *Cyperus difformis* (L.) (Boari *et al*., 2003). Its effect was greater during the early stages of growth in the said weeds (Boari *et al*., 2003). On the other hand, *Fusarium tumidum* (Sherb.) affects *Ulex europaeas* (L.) and reduces the root's growth and the height of the plant (Hurrell

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et al., 2005). An isolate of *Fusarium solani* (Martius) obtained from infected *Striga* plants in Sudan and six other isolates from Japan were evaluated for their effects on *Striga* germination (Ahmed *et al*., 2001). *Alternaria alternata* and *Fusarium equiseti* were reported as eventual biological agents for the management of *Echinochloa* spp. (Safari Motlagh, 2010). The organic extract from liquid cultures of one strain of *Fusarium compactum* (Wollenweber), caused total inhibition of germination of *Orobanche ramosa* (Andolfi *et al*., 2005). The microbial proteins of *Fusarium oxysporum*, induced ethylene biosynthesis and caused necrosis in leaves of *Papaver somniferum* and *Acropilon repens* (Jennings *et al*., 2000).

The major goal of this research was to evaluate of *Fusarium equiseti* (Corda) Saccardo isolated from *Echinochloa oryzicola*, as eventual mycoherbicide against this weed in Guilan province of Iran.

MATERIALS AND METHODS

Collection and culture of fungal isolates

Diseased leaves of *Echinochola oryzicola* were sampled from five locations in each field from Guilan province in Iran. Leaves were transferred to the laboratory and then isolated the fungi 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 27-30°C for 2-3 days. PDA medium 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 15-25 days (Zhang *et al*., 1996). For avoid of bacterial contamination, sulfate streptomycin antibiotic was used (Safari Motlagh, 2010). 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 (Safari Motlagh, 2010).

Study and identification of fungi

Morphological studies were carried out on potato dextrose agar and water agar media. Cuts of colonies were placed onto potato dextrose agar medium for 2-3 days. Then, section of colonies was transferred to water agar medium for 7-10 days in incubator at 27°C and 12 h photoperiod. Afterward, morphological observations were taken based on colony, conidium and conidiophore morphology and other characters morphological (Leslie and Summerell, 2006).

Pathogenicity tests

Pathogenicity tests of *E. oryzicola* 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 *E. oryzicola*. At first, seeds of *E. oryzicola* 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, 10 germinated seeds were planted per 10-cm-Petri dishes filled with saturated soil (Safari Motlagh, 2010), and were incubated at temperature room. Distilled water was added to Petri dishes. Seedlings at the 2-3 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. Therefore, standard evaluation system and Horsfall- Barratt system were applied for *E. oryzicola* (Bertrand and Gottwald, 1997; Zhang and Watson, 1997).

Pathogenicity tests of rice cultivar were carried out in desiccator. To do so, in each of two desiccator (one desiccator as control) were placed two Petri dishes and in each Petri dish placed 10 seeds of rice, Khazar cultivar. Then, seeds were sterilized in water bath at 52-57°C and cultivated in saturated soil and incubated at 25°C. Distilled water was added to Perti dishes. After 16-18 days, seedlings containing 2-3 foliages were inoculated by suspension of spores (Safari Motlagh, 2010). Other conditions including concentration of conidia and evaluation systems were similar.

Seeds germination inhibition tests

Seeds of *E. oryzicola* were surface sterilized with 0.2% sodium hypochlorite solution for 2 min. After washing with distilled water, seeds were placed per 10- cm-Petri dishes containing wet filter papers. Then inoculation was done. To do so, 10 seeds were transferred to two Petri dishes containing wet filter papers (one Petri dish as control). Then cuts of fungus colonies were placed

on seeds. This test was done with three replications. The Petri dishes were incubated at 28°C on a 12 h light/ dark photoperiod. Evaluation of symptoms was performed 7 days after inoculation and number and percent of germinated seeds was determined (Safari Motlagh, 2010).

Data Analysis

Data analysis was done using NTSYS software.

RESULTS AND DISCUSSION

The results showed that the fungi isolates belonged to *Fusarium equiseti* (Corda) Saccardo (Leslie and Summerell, 2006).

The first symptoms of *F. equiseti* appeared 48 h after inoculation on *E. oryzicola*, but symptoms did not appear on rice (Fig 1). Symptoms on *E. oryzicola* were white and chlorotic spots that were transformed to necrotic spots. The spots were expanded and leaves with blight symptoms resulted in death (Fig 2 and 3).

Based on the sizes and types of the spots appeared on the rice and Horsfall-Barratt system, rice cultivar was less affected by the *F. equiseti* compared with *E. oryzicola*, and its disease rating was lower and showed much tolerance (Fig 4).

The results showed that seeds germination of *E. oryzicola* was affected by *F. equiseti* and significant difference was observed between treatments and controls. Also, speed of seed germination in controls was more (Fig 5, 6 and 7).

According to dendrogram from cluster analysis, *F. equiseti* isolates in disease rating index on *E. oryzicola* 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 (Fig. 8).

Based on dendrogram from cluster analysis, *F. equiseti* isolates in disease rating index on rice divided into 2 groups. The first group consisted of 2 isolates. The second group consisted of 1 isolate. Also, in the first group, 2 isolates had similarity coefficient of 0.95 (Fig. 9).

According to dendrogram obtained from cluster analysis, *F. equiseti* isolates in seeds germination inhibition of *E. oryzicola* divided into 2 groups. The first group consisted of 2 isolates. The second group consisted of 1 isolate. Also, in the first group, 2 isolates had similarity coefficient of 0.95 (Fig. 10).

Based on the results of this research the disease rating caused by the fungus in *E. oryzicola* in compared with rice cultivar, was greater. Based on cluster analysis, was observed significant difference between isolates of *F. equiseti*. The different isolates showed different reactions in seeds germination test and pathogenicity test. This

Fig. 1. Pathogeniticity test on rice cultivar.

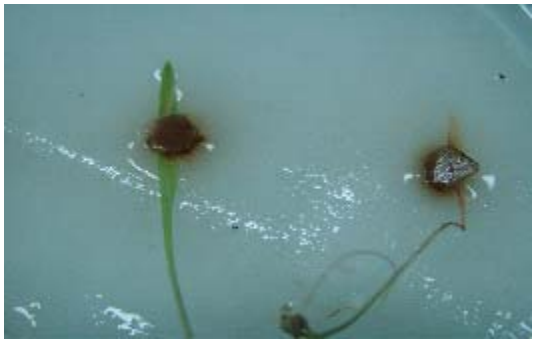

Fig. 2. The symptoms of *F. equiseti* on *E. oryzicola.*

Fig. 3. The symptoms of *F. equiseti* on *E. oryzicola.*

subject can be related to more genetic diversity in different isolates of *Fusarium* spp. (Oliveira *et al*., 2002).

In the study of rice cultivar, the existence of more resistant genes had a positive correlation with a plant's tolerance under stress conditions. Usually, identifying resistance-related genes of a plant is important for producing multi-line varieties (Talebi *et al*., 2004).

Fusarium oxysporum is considered among important factors of the biological control. Its disease rating in various weeds (*Amaranthus*

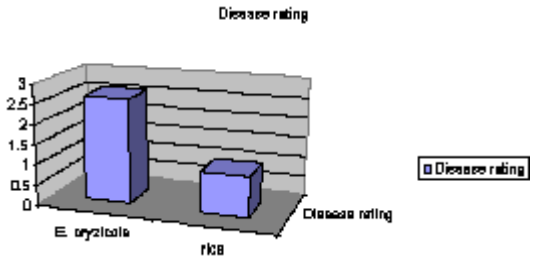

Fig. 4. Diagram of the comparison of *F. equiseti* mean disease rating in rice and *E. oryzicola.*

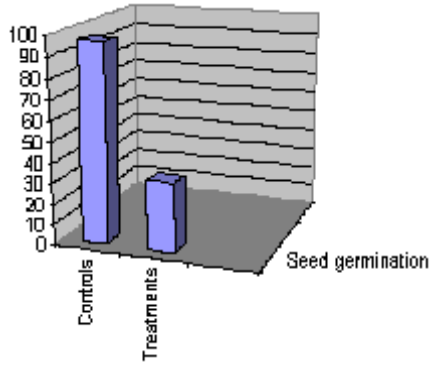

Fig. 5. Diagram of the comparison of mean seeds germination percent in treatments and controls of *E. oryzicola.*

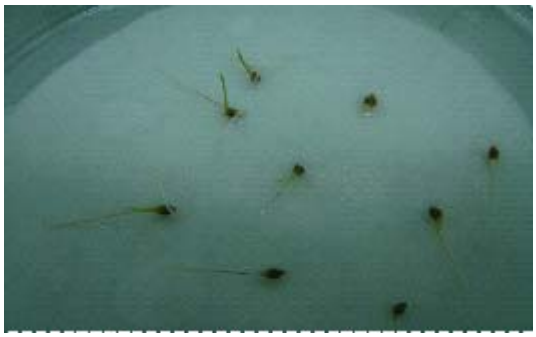

Fig. 6. The seeds germination in controls of *E. oryzicola*

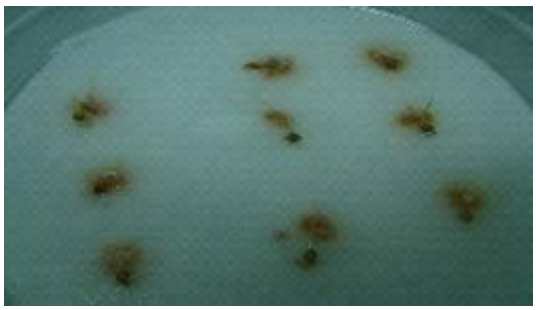

Fig. 7. The seeds germination in treatments of *E. oryzicola.*

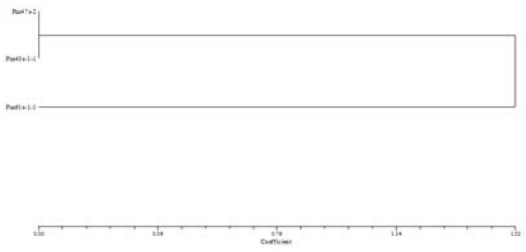

Fig. 8. UPGMA-dendrogram for *F. equiseti* isolates on *E. oryzicola.*

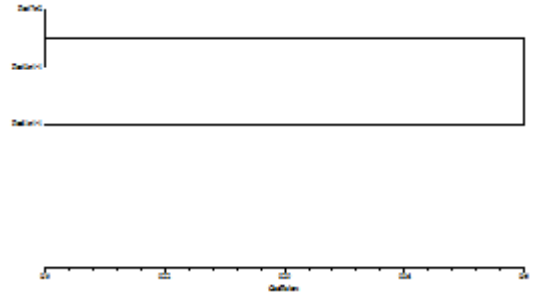

Fig. 9. UPGMA-dendrogram for *F. equiseti* isolates on rice.

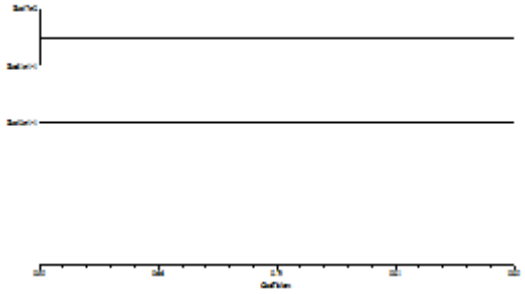

Fig. 10. UPGMA-dendrogram for *F. equiseti* isolates (based on inhibition of seeds germination).

retroflexus and *Cyperus difformis*) was different and it was found that disease rating was related to a plant's growth stage. The effect of this fungus was more obvious during early growth stages (Boari *et al*., 2003).

The ability of 53 strains of fungi to produce bioactive metabolites in solid and liquid cultures was ascertained that among them, the organic extracts from liquid cultures of one strain of *Fusarium compactum* caused total inhibition of germination in *Orobanch ramosa* (Andolfi *et al*., 2005). Metabolites of *Fusarium solani* inhibited *Striga hermonthica* germination induced by the germination stimulant GR24 (Sugimoto *et al*., 2002). An isolate of *Fusarium anthophilum* was obtained from barnyardgrass and after inoculation on this weed caused severe disease expression and death of seedlings (Montazeri *et al*., 2006).

The study indicated that *Fusarium equiseti* able to infect *Echinochloa crus-galli* in a higher rate comparing with rice cultivar, but, seeds germination of *Echinochloa crus-galli* was not significantly affected by *Fusarium equiseti* in comparison with controls (Safari Motlagh, 2011).

As a main factor for introduction of a biological agent, it is necessary that the biological agent does not damage the important crops in practice (Safari Motlagh, 2011).

CONCLUSION

Due to the fact that *Fusarium equiseti* could cause a higher disease rating in *E. oryzicola* and inhibited germination of seeds, it may be used as eventual mycoherbicide for control of *Echinochloa oryzicola*, in 2-3 leaf stage in paddy fields.

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