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COMPARISON OF PATHOGENICITY OF *Fusarium equiseti* AND *Colletotrichum graminicola* ON *Echinochloa* spp.

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ABSTRACT

Echinochloa spp. are the most important weeds in rice fields. In this research *Fusarium equiseti* and *Colletotrichum graminicola* were isolated from these weeds and then their pathogenicity effects were compared on these weeds and five rice cultivars in a completely random design with three replications in greenhouse conditions. Fungi were inoculated on weeds and rice cultivars using a spore suspension consisting of 10^6 spore· ml^{-1} of distilled water. Results indicated significant effect of *F. equiseti* and *C. graminicola* on *Echinochloa oryzicola* and *E. crus-galli*. Also, these rice cultivars showed significant reaction to *F. equiseti* and *C. graminicola*. The results showed that in comparison between effect of *F. equiseti* and *C. graminicola* on *Echinochloa* spp., disease rating caused by *F. equiseti* on *E. oryzicola* and *E. crus-galli* was more than disease rating caused by *C. graminicola* and these species of weeds were more susceptible to *F. equiseti*, but *C. graminicola* created less damage on rice cultivars and these rice cultivars were more tolerant to this fungus.

Key words: barnyard grass, disease rating, fungi, rice

INTRODUCTION

Barnyard grass, *Echinochloa crus-galli*, and *E. oryzicola* are ranked as important worst weed species and are two of the most serious weeds in rice (*Oryza sativa*) [Zhang et al. 1996, Huang et al. 2001]. These species severely reduce both yield and quality of rice [Huang et al. 2001]. Fungal pathogens can be exploited as biological agents for the managements of agricultural pests and diseases [Rahimian and Banayan 2009]. Generally, different *Fusarium* species play important roles in the biological control of the weeds of different crops. For instance, *Fusarium oxysporum* is considered an important factor in the biological control of weeds [Boari and Vurro 2004]. Studies showed that this fungus caused high pathogenesis levels in *Amaranthus retroflexus* and *Cyperus difformis* [Boari and Vurro 2004]. Its effect was greater during the early stages of growth in the said weeds [Boari and Vurro 2004]. *Colletotrichum gloeosporioides* f. sp. *aeschynomene* is used as a microbial herbicide known as Collego for controlling important weeds in soybean and rice fields, i.e. *Aeschynomene virginica* [Rahimian and Banayan 2009]. Studies showed that of the pathogen fungi isolated from weeds such as *Sesbania exaltata*, *Solanum viarum* and *Striga hermonothica*, being considered as the main problems of soybean and rice cultivation, *Colletotrichum truncata* and *Ralstonia solanacearum* caused the highest disease ratings in the said weeds [Charudattan 2001]. Conducted studies showed that some *Colletotrichum* species are extensively used for

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the biological control of weeds in the US fields [Safari Motlagh and Javadzaheh 2011b]. Also, using *Colletotrichum graminicola* along with applying chemical and mechanical methods controlled weeds [Safari Motlagh and Javadzadeh 2011b]. This fungus was effective in some corn bred cultivars; however, productive cultivars were sensitive to it [Safari Motlagh and Javadzadeh 2011b]. Usually, fungi such as *Fusarium oxysporum* and *Colletotrichum coccodes* being among good mycoherbicides are changed by pectinase, cellulase and expansin coding genes (enzymes which facilitate the influence and growth of fungi inside weed tissues) [Hershkovitz et al. 2007]. Moreover, isolates from *Colletotrichum graminicola* have been used for the biological control of barnyard grass [Yang et al. 2000]. In another study, *Colletotrichum dematium* was introduced for controlling *Epilobium angustifolium* which can replace herbicides such as simazine, atrazine [Watson and Winder 1993]. Also, evaluation of the reaction of *Sagitaria trifolia* and some rice cultivars to *Fusarium equiseti* was done in Iran and its pathogenicity was proved [Safari Motlagh and Sharifi 2011]. In another study, evaluation of *Colletotrichum graminicola* as an eventual bioherbicide for biocontrolling *Alisma plantago-aquatica* in paddy fields of Iran was done [Safari Motlagh and Javadzadeh 2011a]. Also, pathogenicity of *Alternaria pellucida* and *Curvularia lunata* on *Echinochloa* spp. in Guilan province was compared [Safari Motlagh 2015].

In this research, two different indigenous fungal species have been isolated from naturally infected *E. crus-galli* and *E. oryzicola*. To select the best candidate for further development as biological control agent for *Echinochloa* spp. in paddy fields, this study was done to determine the pathogenicity of these fungi and compare the disease raring of these fungi on *Echinochloa* species and rice cultivars in Guilan province of Iran.

MATERIALS AND METHODS

Collection and culture of fungal isolates

Diseased leaves of *Echinochloa crus-galli* and *E. 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

Fungi which had grown were isolated and Koch's postulates were completed for most sample after each collection. Cultures of these fungi were submitted to the Research Plant Pathology Institute of Iran for the confirmation of identification**.**

Pathogenicity tests

***Echinochloa* spp.** This reaction occurred as complete random design (CRD) with 2 treatment and 3 replications. *E. crus-galli* and *E. oryzicola* were planted in plastic pots 2.5 cm in diameter containing farm soil (one sample of each plant). For each treatment, one control was assigned [Zhang et al. 1996]. Pots were placed at 25–30°C, 12 D : 12 L photoperiod and a relative humidity of more than 90%. Inoculation of weeds was performed at its 3–4 leaf stage in greenhouse. To do so, a spore suspension including 10^6 *Fusarium equiseti* and *Colletotrichum graminicola* spore/ml distilled water, separately was used (2 isolates of each fungus). In order to increase adsorption, 1% Tween-20 was used. This suspension (10^6 spore$\cdot$ml^{-1} distilled water) was sprayed on the leaves using a sprayer. It should be mentioned that before inoculation, all pots were sprayed with distilled water. To create a relative humidity higher than 90%, treated plants were immediately covered with

plastic bags for 48 h [Ghorbani et al. 2000]. Evaluation was done 7 days after inoculation based on lesion type and size in reaction to inoculation: 0 = lesions absent, 1 = small, unexpanded lesions, 2 = slightly to moderately expanded lesions and 3 = large lesions [Zhang et al. 1996]. Therefore, standard evaluation system and Horsfall-Barratt system were applied for *Echinochloa* spp. [Zhang et al. 1996, Safari Motlagh 2015].

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

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

Rice. This reaction occurred as complete random design (CRD) with 5 treatment and 3 replications (one sample of each plant). The five rice cultivars including 3 indigenous (Hashemi, Ali Kazemi and Binam) and 2 bred cultivars (Khazar and Sepidroud) were evaluated against inoculation with *F. equiseti* and *C. graminicola*. In order to do so, first, rice seeds germinated and after being transferred to the greenhouse inside pots, 2.5 cm in diameter without any drain, they were planted in the farm soil. When the plants reached their 3–4 leaf stage, thinning was performed. Finally, there were 4 seedlings in each pot. Then, 2 g urea fertilizer was added to the pots for increase vegetative growth. At this stage, inoculation was done by a spore suspension of *F. equiseti* and *C. graminicola* containing 10^6 spore·ml^{-1} of distilled water with 1% Tween-20 (2 isolates of each fungus). Other environmental conditions were similar to those of the weeds. Evaluation was done 7 days after inoculation based on Horsfall-Barrat system. Then, disease ratings were calculated [Safari Motlagh 2015]. It is noteworthy that in both experiments, one control was considered for each replication.

Measuring plant fresh weight, dry weight and height

In order to measure these traits, inoculated weeds and rice cultivars along with controls were transferred from greenhouse to the laboratory. Then, shrubs were cut on the soil surface and weighed by an electric scale. This weight was recorded as their fresh weight. After separately measuring their height, each shrub was placed inside a paper bag and they incubated in an oven at 80–90°C for 48 h. When the bags were taken out of the oven, each shrub was weighed, which was considered as its dry weight [Ghorbani et al. 2000].

Data analysis

Data analysis was done using SAS, SPSS and MSTAT-C softwares. In order to compare average values, Duncan test was used.

RESULTS AND DISCUSSION

Based on analysis of variance for evaluating the disease rating, the effect of the *Fusarium equiseti* on *E. crus-galli*, and *E. oryzicola* spp., was significant at p = 5% (compared to controls). Based on the comparison of disease rating, there was not significant difference in disease rating caused by *F. equiseti* between *E. oryzicola* and *E. crus-galli*, but it was revealed that the first weed was more affected by *F. equiseti* (tab. 1). In the study of the effect of *F. equiseti* on fresh weight, dry weight and height of *Echinochloa* species based on analysis of variance at p = 5%, a significant reaction was observed (compared to controls). Also, the comparison of the traits, mean values, between *Echinochloa* species showed a significant difference in terms of fresh weight, but not in dry weight and height (tab. 1). In this regard, *E. crus-galli* had more reduction (tab. 1). According to the results of this research, *E. crus-galli* was less affected by *F. equiseti* and *E. oryzicola* was more susceptible to this fungus.

Analysis of variance showed significant effect of *Colletotrichum graminicola* on *E. oryzicola* and *E. crus-galli* at p = 5% (compared to controls). Based on the comparison of disease rating, there was not significant difference in disease rating caused by *C. graminicola* between *E. oryzicola* and *E. crus-galli*, but it was revealed that *E. crus-galli* was more affected by *C. graminicola* (tab. 1). In the study of the effect of *C. graminicola* on fresh weight, dry weight and height of *Echinochloa* spe-

Table 1. Comparison of means of the studied traits affected by *F. equiseti* and C. graminicola in Echinochloa species

	Weeds	Disease rating	Height	Fresh weight	Dry weight
Affected by *F. equiseti*	*E. oryzicola*	2.30 ±0.42c	67.713 ±0.334a	7.146 ±0.0082c	1.013 ±0.0643c
	E. crus-galli	1.80 ±0.13c	61.763 ±0.258a	5.32 ±0.136d	0.663 ±0.0425c
Affected by *C. graminicola*	*E. oryzicola*	1.49 ±0.02b	62.330 ±0.037a	7.179 ±0.0257c	0.998 ±0.0569c
	E. crus-galli	2.25 ±0.16b	62.123 ±0.312a	5.475 ±0.141c	0.630 ±0.188c

Treatments with at least one similar letter, did not have a significant difference at p = 5%

Table 2. Comparison of means of the studied traits affected by *F. equiseti* and *C. graminicola* in rice cultivars

	Cultivars	Disease rating	Height	Fresh weight	Dry weight
Affected by *F. equiseti*	Hashemi	2.75 ±0.41bc	66.833 ±1.7934b	5.976 ±0.099b	1.050 ±0.0472b
	Ali Kazemi	3.75 ±0.12b	83.762 ±2.081a	7.673 ±0.128a	1.763 ±0.083a
	Sepidroud	5.50 ±0.02a	70.583 ±2.948b	4.576 ±0.103c	0.74 ±0.0351c
	Khazar	1.25 ±0.04c	62.786 ±2.403a	3.213 ±0.018d	0.4 ±0.0152d
	Binam	1.75 ±0.13c	71.873 ±0.919b	3.316 ±0.223d	0.446 ±0.0375d
Affected by *C. graminicola*	Hashemi	2.21 ±0.02b	68.206 ±0.586b	6.090 ±0.0832b	1.013 ±0.0166b
	Ali Kazemi	1.80 ±0.16b	82.833 ±1.083a	7.956 ±0.0633a	1.673 ±0.155a
	Sepidroud	3.25 ±0.17a	69.206 ±1.149b	4.683 ±0.0392c	0.825 ±0.0173b
	Khazar	2.20 ±0.01b	62.33 ±2.385c	3.157 ±0.0179d	0.407 ±0.0121c
	Binam	1.25 ±0.13b	68.820 ±2.454b	2.760 ±0.261d	0.392 ±0.0416c

Treatments with at least one similar letter, did not have a significant difference at p = 5%

Table 3. Comparison of means of the studied traits affected by *F. equiseti* and *C. graminicola* in *Echinochloa* species compared to control

	Weeds	Reduction of height	Reduction of fresh weight	Reduction of dry weight
Affected by *F. equiseti*	*E. oryzicola*	0.576 ±0.14b	0.216 ±0.103b	0.236 ±0.018a
	E. crus-galli	0.866 ±0.135b	0.186 ±0.079b	0.11 ±0.057ab
Affected by *C. graminicola*	*E. oryzicola*	0.916 ±0.562a	0.231 ±0.093b	0.198 ±0.097b
	E. crus-galli	1.12 ±0.403a	0.351 ±0.163b	0.322 ±0.031b

Treatments with at least one similar letter, did not have a significant difference at p = 5%

Table 4. Comparison of means of the studied traits affected by *F. equiseti* and *C. graminicola* in rice cultivars compared to control

	Cultivars	Reduction of height	Reduction of fresh weight	Reduction of dry weight
Affected by *F. equiseti*	Hashemi	3.08 ±0.798a	0.263 ±0.101a	0.296 ±0.067a
	Ali Kazemi	2.92 ±1.045a	0.185 ±0.204b	0.231 ±0.068a
	Sepidroud	2.04 ±1.119a	0.076 ±0.063a	0.133 ±0.074a
	Khazar	2.25 ±0.943a	0.123 ±0.043b	0.083 ±0.039a
	Binam	0.916 ±0.108a	0.226 ±0.08a	0.189 ±0.027b
Affected by *C. graminicola*	Hashemi	0.823 ±0.151b	0.036 ±0.149a	0.133 ±0.33a
	Ali Kazemi	1.196 ±0.571a	0.046 ±0.129a	0.240 ±0.14a
	Sepidroud	1.816 ±0.183a	0.063 ±0.044a	0.132 ±0.048a
	Khazar	1.19 ±0.052a	0.39 ±0.084b	0.121 ±0.05a
	Binam	0.783 ±0.859b	0.196 ±0.066b	0.118 ±0.046a

Treatments with at least one similar letter, did not have a significant difference at p = 5%

cies study of the effect of *C. graminicola* on fresh weight, dry weight and height of *Echinochloa* species based on analysis of variance at p = 5%, a significant reaction was observed (compared to controls). Also, the comparison of the traits, mean values, between *Echinochloa* species did not show a significant difference in terms of fresh weight, and dry weight and height (tab. 1). In this regard, *E. crus-galli* had more reduction and was more affected by *C. graminicola*.

The results showed that in comparison between effect of *F. equiseti* and *C. graminicola* on *Echinochloa* spp., disease rating caused by *F. equiseti* on *E. oryzicola* was more than disease rating caused by *C. graminicola*, but disease rating caused by *C. graminicola* on *E. crus-galli* was more than disease rating caused by *F. equiseti*. Finally, average disease ratings on *Echinochloa* species caused by *F. equiseti* was more than *C. graminicola*.

The effect of *F. equiseti* in height reduction of *E. crus-galli* was more than other fungus, but in height reduction of *E. oryzicola,* no difference was observed. In the comparison of effect of fungi on fresh weight no difference was observed on *Echinochloa* species. Finally, in terms of dry weight reduction, the effect of *C. graminicola* on *E. oryzicola* was more than other *F. equiseti*, but on *E. crus-galli* no difference was observed.

Results from the variance analysis of the disease rating revealed that the studied rice cultivars showed significant reaction to *F. equiseti* at p = 5%. Based on the mean values of disease rating in the studied rice cultivars revealed that Sepidroud was the most sensitive cultivar. There was no significant difference between Khazar, Hashemi and Binam cultivars and Khazar was more tolerant (tab. 2). In the study of dry weight, fresh weight and height of the said rice cultivars, a significant reaction was observed in all these traits based on analysis of variance at p = 5%. Evaluation of the mean values of the said traits in the studied rice cultivars revealed that regarding height, there was no significant difference between Ali Kazemi and Khazar, also there was no significant difference between Hashemi, Sepidroud and Binam (tab. 2). In terms of fresh weight and dry weight, there was no significant difference between Khazar and Binam, but Ali Kazemi, Hashemi and Sepidroud showed significant difference (tab. 2).

Results from the variance analysis of the disease rating revealed that the studied rice cultivars showed significant reaction to *C. graminicola* at p = 5%. Based on the mean values of disease rating in the

studied rice cultivars revealed that Sepidroud was the most sensitive cultivar. There was no significant difference between Ali Kazemi, Khazar, Hashemi and Binam cultivars and Binam was more tolerant (tab. 2). In the study of dry weight, fresh weight and height of the said rice cultivars, a significant reaction was observed in all these traits based on analysis of variance at p = 5%. Evaluation of the mean values of the said traits in the studied rice cultivars revealed that regarding height, there was significant difference between Ali Kazemi and Khazar, also there was no significant difference between Hashemi, Sepidroud and Binam (tab. 2). In terms of fresh weight, there was no significant difference between Khazar and Binam, but Ali Kazemi, Hashemi and Sepidroud showed significant difference (tab. 2). In terms of dry weight, there was no significant difference between Khazar and Binam, also between Hashemi and Sepidroud but was observed significant difference between Ali Kazemi and other cultivars (tab. 2).

F. equiseti and *C. graminicola* were pathogenic on *Echinochloa* species and on rice cultivars. The disease rating caused by *F. equiseti* on Sepidroud bred cultivar and Ali Kazemi and Hashemi indigenous cultivars was more than disease rating on *Echinochloa* species but on Khazar and Binam cultivars was less. The disease rating caused by *C. graminicola* on Sepidroud bred cultivar was more than disease rating on *Echinochloa* species but on other rice cultivars was less. Based these results in regarding to the disease rating, *C. graminicola* was a better antagonistic fungus compared to *F. equiseti.*

In regarding to the influence of *F. equiseti* and *C. graminicola* on height, fresh weight and dry weight of *Echinochloa* species and rice cultivars compared to control, reduction was observed. *C. graminicola* was more effective in reduction of height, fresh weight and dry weight of *Echinochloa* species and *F. equiseti* was more effective in reduction of height, fresh weight and dry weight of rice cultivars (tabs 3 and 4). In this case, *C. graminicola* was a better antagonistic fungus compared to *F. equiseti.*

In this study, among indigenous cultivars, Ali Kazemi and Hashemi were more affected by the fungi than Binam. Also, responses of the two bred cultivars, Sepidroud and Khazar, in the evaluation of disease rating were totally different and Sepidroud showed the least tolerance. The greater tolerance of Khazar could be attributed to the existence of greater numbers of resistance genes. There is a positive correlation with tolerance under stressful conditions and existence of tolerant genes. For this purpose the multiline varieties can be considered for cultivation [Talebi et al. 2004].

Usually, Khazar and Sepidroud are more tolerant against some major rice diseases such as stem rot and brown spot [Javan Nikkhah et al. 2001, Safari Motlagh et al. 2005]. A resistance response to pathogens is generally developed following the interaction of fungus and plant genes [Rezvani et al. 2002]. Hence, in order to increase plant tolerance, more modification methods should be applied to cultivars [Rezvani et al. 2002].

In this research, traits of fungi-treated cultivars with controls showed that the effect of fungi on the height of the tested cultivars in comparison with fresh and dry weights was greater. Since the genes which control each component of resistance have their own sequence, there would be different responses for each trait because each trait has a specific gene sequence and therefore, there will be different reactions [Xing and Zhang 2010].

Studies conducted by Hurrell et al. [2005] showed that *Fusarium tumidum* affects *Ulex europaeas* and reduces the weed height and the growth of roots by 63% and that the decrease of height has a positive correlation with temperature which can be an indication of environmental effect on different plant responses. It was found that the existence of a dominant gene in the above-mentioned fungus plays a role in the inactivation of the height coding gene [Hurrell et al. 2005]. Zhang et al. [1996] indicated that some species of *Curvularia* such as *C. lunata* and *Exserohilum oryzae* were pathogenic to both rice and *Echinochloa* species while *C. geniculata* and *Exserohilum monoceras* were pathogenic only to *Echinochloa* species. Having more effects of fungi on height of *Echinochloa* species could possibly be related to the plant growth stage. Plants such as weeds are usually more sensitive to pathogens during their early stages of growth. In this study, two fungi could affect the studied traits with high disease rating. Moreover, the type of resistance in plants is very effective in their reaction to pathogens.

Therefore, it can be said that the vertical resistance with the simple Mendelian inheritance is controlled by

one or more genes, but a horizontal resistance is a multifactor inheritance [Xing and Zhang 2010]. Usually, resistance and sensitivity genes in bred cultivars are more than those of the indigenous ones [Xing and Zhang 2010]. As a main factor for introduction of a biological pathogen, it is necessary that the biocontrol agent does not damage the important crops in practice [Rahimian and Banayan 2009].

In this study, Khazar and Binam cultivars showed the highest tolerance to *F. equiseti* and *C. graminicola* than other rice cultivars respectively, and therefore biocontrol of *Echinochloa* species by the two fungi can be done in paddy fields that these cultivars are cultivated.

CONCLUSIONS

F. equiseti caused higher disease ratings in *Echinochloa* spp. compared to *C. graminicola*, but the second fungus created less damage on rice cultivars.

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