Evaluation of the Reaction of Major Weeds and Some Rice Cultivars to *Colletotrichum graminicola*

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Abstract: *Alisma plantago-aquatica*, *Sagitaria trifolia* and *Echinochloa* spp. are among the most important damaging weeds of rice paddies. In this research, *Colletotrichum graminicola* was isolated from these weeds and studied as a biological agent for controlling weeds. To do so, at first, reactions of five rice cultivars including three indigenous cultivars such as Hashemi, Ali Kazemi and Binam and two bred ones, i.e. Sepidroud and Khazar to *Colletotrichum graminicola* were evaluated. Thus, a complete random design with three replications and five treatments was used at a greenhouse. Then, *Colletotrichum graminicola* was inoculated on these weeds. The experimental design was a randomized completed with three replications. Inoculation was done at the 3-4-leaf stage using a spore suspension consisting of 10^6 conidia/mL distilled water to which Tween-20 1% was added. Results showed that *Colletotrichum graminicola* caused high disease ratings in *Alisma plantago-aquatica, Sagitaria trifolia, E. crus-galli* and *E. oryzicola,* respectively. In addition, the studied rice cultivars showed a significant reaction in terms of the disease rating among which bred cultivars were less tolerant. Moreover, the fungus reduced fresh weight, dry weight and height in the studied weeds and rice cultivars. Therefore, *C. graminicola* can be used as a mycoherbicide for the biological control of these weeds only when other cultivars except the above-mentioned rice cultivars are planted. This issue particularly requires producing new tolerant cultivars with a combination of the desired traits.

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1. Introduction

Weeds are the most important biological barriers in rice production in a way that a noticeable part of the production costs are allocated to them and are among the most important inhibiting factors with regards to increasing rice production (Mudge, 2004). Researchers have reported the reduced crop yield to be 10%, which is due to competition with weeds (Moody, 1991), while this rate in rice is 20%, that is more than other crops (Lindquist and Kropff, 1998). Today, herbicides are considered among the most important factors in cropping systems of the developed countries and a noticeable part of the increase in crop yields in these countries is indebted to the use of these chemicals (Zand et al., 2007). Despite the universal acceptance of herbicides in recent years, willingness to lower their consumption rate has had an increasing trend because more consumption of herbicides increases production costs, damages crops and causes weeds to become resistant (Blackshaw et al., 2006). Furthermore, in the last few years, concerns for unknown side effects of herbicides, their environmental effects and the role they have in threatening mankind's health have increased (Blackshaw et al., 2006).

Accordingly, controlling weeds by living microorganisms is of great significance for maintaining natural ecosystems and preventing environmental hazards, especially pollution of ground waters and the emergence of a new pest related to producing crops throughout the world (Marol and Baroet, 2004).

Fungi are some of the most important factors which might be used for the biological control of weeds in rice paddies (Safari Motlagh, 2010). *Colletotrichum gloeosporioides* f. sp. *aeschynomene* as the anthracnose-causing fungus is used in the state of Arkansas in the US as a microbial herbicide called Collego for controlling the main weed of soybean fields and rice paddies, which is *Aeschynomene virginica* (Kouchaki et al., 2001). Studies conducted by Watson and Winder showed that *Colletotrichum dematium* has strong bioherbicidal characteristics and could control fireweed (*Epilobium* spp.) quite well (Watson and Winder, 1993).. In the state of Florida, it was found that fungi such as *Colletotrichum truncata, Ralstonia solanacearum* and *Fusarium oxysporum* isolated from *Sesbania exaltata, Solanum viarum* and *Striga hermonthica* could be effective against these weeds and eradicated them after five days (Charudattan, 2001). Conducted studies showed that some

Colletotrichum species are extensively used for the biological control of weeds in the US fields (Goodwin, 1995). Poa weed as one of the main weeds in the United States reduces the yield of most crops and chemical control does not have much effect on limiting weeds competition (Goodwin, 1995). Also, using *Colletotrichum graminicola* along with applying chemical and mechanical methods controlled weeds (Goodwin, 1995). This fungus was effective in some corn bred cultivars; however, productive cultivars were sensitive to it (Goodwin, 1995). In another study, for the biological control of *Echinochloa crus-galli* and *Echinochloa* sp. in paddy fields in Vietnam, Australia and South Korea, *Exserohilum fusiforme* and *Colletotrichum graminicola* were used as two pathogens and inhibited the growth of these two weed species at early stages (Johanson et al., 2003).

Ipomoea locunosa is known as a serious and extensively spreading weed in soybean, cotton and many other crop fields of the southern parts of the United States (Cartwright and Tempelton, 1994). *Colletotrichum capsici* was used for its biological control and proved effective in reducing damages (Cartwright and Tempelton, 1994).

Sesbania exaltata is a problematic weed in soybean cultivation (Boyette et al., 2006). In order to biologically control this weed, microsclerotia of *Colletotrichum truncatum* are mixed with some surfactants and turn into granules called 'Pesta' which effectively control this weed (Boyette et al., 2006).

Abutilon theophrasti is a weed with a wide spreading range which damages many crops (Meir et al., 2009). Therefore, a specific pathovar of *Colletotrichum coccodes* has been considered as a specific bioherbicide for selective controlling of this weed (Meir et al., 2009).

While introducing a microorganism as a biological control agent, it is very important to make sure that it won't damage crops (Watson, 1985). In the present study, *Colletotrichum graminicola* was isolated from three main weeds of rice paddies in Guilan province in Iran, i.e. *Echinochloa* spp., *Alisma plantago-aquatica* and *Sagitaria trifolia* and thus, it was investigated as a probable mycoherbicide for the biological control of the said weeds. Also, to examine the effects of the fungus on rice, the reaction of some rice cultivars to *C. graminicola* was studied.

2. Materials and methods

2.1. Collection and culture of fungal isolates

Leaves with symptoms of the disease weeds (*Alisma plantago-aquatica, Sagitaria trifolia, E. crus-galli* and *E. oryzicola*) were collected in Guilan province of Iran, cut to appropriate sizes and transferred to the laboratory. Samples were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on potato dextrose agar (PDA) in Petri dishes. Then, Petri dishes were incubated at 28^0C in darkness or light on a 12 hours light/dark photoperiod for 6-15 days. Conidia were single-sporulated and then, monoconidial isolates of the recovered fungi were maintained on half-strength PDA slants in test tubes as stock cultures (Zhang et al., 1996) 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, 2010).

2. 2. 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.

2.3. Pathogenicity test

Weeds

This reaction occurred as complete random design (CRD) with one treatment and 3 replications. Weeds were planted in plastic pots 2.5 cm in diameter containing farm soil. For each treatment, one control was assigned (Zhang et al., 1996). Pots were placed at 25-30^0C, 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 *Colletotrichum graminicola* spore/mL distilled water was used. In order to increase adsorption, 1% Tween-20 was used. This suspension 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 hours (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, 3= large lesions (Zhang et al., 1996). Therefore, standard evaluation system and Horsfall-Barratt system were applied for *Echinochloa* spp. 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 is number of treatments.

Rice

This reaction occurred as complete random design (CRD) with five treatment and 3 replications. The five rice cultivars including 3 indigenous (Hashemi, Ali Kazemi and Binam) and 2 bred cultivars (Khazar and Sepidroud) were evaluated against inoculation with *Colletotrichum 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 shrubs in each pot. Then, 2 g urea fertilizer was added to the pots. At this stage, inoculation was done by a spore suspension of *Colletotrichum graminicola* containing 10^6 spore/ mL of distilled water with 1% Tween-20. Other environmental conditions were similar to those of the weed. Evaluation was done 7 days after inoculation based on Horsfall-Barrat system. Then, disease ratings were calculated (Bertrand and Gottwald, 1997). It is noteworthy that in both experiments, one control was considered for each replication.

2.4. 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^{0}C for 48 hours. When the bags were taken out of the oven, each shrub was weighed, which was considered as its dry weight (Ghorbani et al., 2000).

2.5. Data Analysis

Data analysis was done using SPSS and MSTAT-C softwares. In order to compare average values, Duncan test was used, while for comparing the reaction of rice cultivars and weeds, the difference between the average value of each fungus-treated rice cultivars and the controls was used.

3. Results

According to the variance analysis table for the evaluation of the disease rating, it was found that the studied rice cultivars showed significant reactions to *Colletotrichum graminicola* (Table 1). Also, based on the comparison of the mean traits in the study of disease rating, the greatest effect of the fungus was seen on Sepidroud, i.e. this cultivar was less tolerant compared with others (Figureure 1). Among indigenous cultivars, Hashemi showed less tolerance (Figureure 1). There was no significant difference between Ali Kazemi, Khazar and Binam with only Binam being more tolerant to this fungus in terms of the number and sizes of the spots created (based on Horsfall-Barratt system) (Figure 1).

On the other hand, based on the variance analysis table for the evaluation of traits including height, fresh weight and dry weight, the studied rice cultivars showed significant reactions (Table 1). According to the comparison of the above-mentioned traits among the cultivars, it was found that for height, there was no significant difference between Hashemi, Sepidroud and Binam cultivars. Also, no significant difference was observed between Ali Kazemi and Khazar. No significant difference was found between the dry and fresh weights of Khazar and Binam as well (Table 2). However, a significant difference was observed in terms of these two traits between Hashemi, Ali Kazemi and Sepidroud (Table 2). Moreover, compared with other rice cultivars, Khazar showed more reductions of the said three traits (Table 2).

In the investigation of the reactions of the studied rice cultivars regarding their heights, fresh weights and dry weights compared with the controls, results showed that for height, Ali Kazemi, Sepidroud and Khazar were more affected by the fungus than the controls (in comparison with the controls, they revealed a decrease in height which compared with that of other cultivars was greater.); however, when compared with each other, they had no significant differences in terms of this trait (Table 3). In the second group, there were Hashemi and Binam for height decrease, yet with no significant difference between each other. But when compared with Ali Kazemi, Sepidroud and Khazar, they were less affected by the fungus (Table 3).

In terms of fresh weight, this fungus was effective on Sepidroud (as a bred cultivar) and indigenous Hashemi and Ali Kazemi compared with the controls, but they showed no significant differences between themselves. *Colletotrichum graminicola* had no effects on the fresh weights of Khazar and Binam. Furthermore, these cultivars did not show any significant differences between themselves (Table 3). In comparison with the effect of the fungus on the fresh weight in bred cultivars, Sepidroud had more fresh weight decrease than Khazar.

Concerning dry weight, it was found that the fungus caused this trait to decrease in the studied cultivars compared with controls and that there was no significant difference between the cultivars.

And in terms of the effect of this fungus on all the three studied traits, it was revealed that the fungus was more effective on height and fresh weight than on the dry weight. Moreover, it was found that bred cultivars were more sensitive to the fungus (Table 3).

Based on the variance analysis table for evaluating the disease rating, the effect of the *Colletotrichum graminicola* on *Alisma plantago-aquatica, Sagitaria* *trifolia* and *Echinochloa* spp., was significant (Table 4). The greatest effect was seen in *Alisma plantago-aquatica* and then in *Sagitaria trifolia, E. crus-galli* and *E. oryzicola*, respectively (Figure. 2).

According to the variance analysis table for evaluating weeds' height, dry weight and fresh weight, it was found that the weeds showed significant reactions in terms of all three studied traits (Table 4). With consideration of the comparison of the traits' mean values in weeds results revealed that in terms of height, there was no significant difference between two *Echinochloa* species; however, *Alisma plantago-aquatica* and *Sagitaria trifolia* showed a significant difference (Table 5). Moreover, conditions for both fresh and dry weights were the same. Two *Echinochloa* species showed no significant difference in terms of these traits, while *Alisma plantago-aquatica* did have a significant difference regarding their fresh and dry weights (Table 5). The examination of the reactions of the studied weeds in terms of the three traits compared with those of the controls revealed that for height, all weeds had reduced heights compared with controls, but the differences were not significant among themselves (Table 6). In terms of fresh weight, *Sagitaria trifolia* and *Alisma plantago-aquatica* had more reductions, but did not show any significant difference compared with each other. The fungus reduced the fresh weight in both *Echinochloa* species as well.

Comparison of the studied weeds' dry weights with those of their controls indicated a reduction in this trait among treatments, but these weeds did not show any significant difference. Therefore, in terms of dry weight, *Sagitaria trifolia* was more affected compared with *Alisma plantago-aquatica* and two *Echinochloa* species and showed more reduction of the trait (Table 6).

4. Discussion

The present study revealed that not only the disease rating caused by *Colletotrichum graminicola* in *A.plantago-aquatica, Sagitaria trifolia, Echinochloa crus-galli* and *E. oryzicola* was high, but also it caused high disease rating in the studied rice cultivars as well. In this research, *C. graminicola* was effective in both bred and indigenous rice cultivars. This finding was consistent with that of Johanson *et al.* According to these studies, *Colletotrichum* species such as *C. graminicola* and *C. truncata* are considered as effective fungi with strong antagonistic characteristics for the biological control of *Echinochloa* and nightshade, but extensive use of this fungus depends on producing tolerant rice and wheat cultivars (Johanson et al., 2003)

The present research showed that bred rice cultivars were more affected compared with indigenous cultivars, which might be related to these cultivars becoming more adapted to indigenous fungi. While in the study of the reaction of bred and indigenous corn cultivars to *Colletotrichum* spp., isolated from annual mercury in corn fields in the US, bred cultivars were more tolerant and the disease rating caused by the fungus in them was lower (Norris, 1992). However, compared with indigenous cultivars, it was more effective in terms of their dry weight (Norris, 1992). Molecular studies revealed that bred cultivars had more resistant genes, but in indigenous cultivars, fresh and dry weight-controlling genes were more in numbers (Norris, 1992).

In another study, it was found that *C. graminicola* was effective in controlling *Sorghum halepense*, but since the fungus caused higher disease rating in alfalfa, it was not introduced as a biological control agent (Tempelton and Henry, 1990).

It has been found that the competence of a plant is directly related to its genetic composition that is, the less the number of resistant genes in a plant, the lower its competence degree and it would be considered a more suitable host for fungi (Blum, 1998).

In conclusion, with the results of this research taken into account, it can be said that *Colletotrichum graminicola* can be introduced as a biological agent for controlling *Alisma plantago-aquatica, Echinochloa* spp. and *Sagitaria trifolia* provided that rice cultivars other than those studied here are planted. This would be especially possible when new bred cultivars have a combination of the useful traits of the existing indigenous and bred rice cultivars all together.

Table 1. Variance analysis of disease rating and the studied traits in rice cultivars affected by *C. graminicola*.

SOV	DF	Squares Mean			
		Disease rating	Height	Fresh weight	Dry weight
Treatment	4	0.778**	171.278**	13.814**	0.834**
Error	10	0.136	8.761	0.049	0.015
C.V.	-	15.62	4.21	4.49	14.19

** Significance at the probability level of 1%.
SOV: sources of variations
DF: degree of freedom

Table 2. Comparison of the reactions of rice cultivars affected by *C. graminicola*.

Cultivars	Height	Fresh weight	Dry weight
Hashemi	68.206±0.586b	6.09±0.0832b	1.013±0.0166b
Ali Kazemi	82.833±1.083a	7.956±0.0633a	1.673±0.155a
Sepidroud	69.206±1.149b	4.683±0.0392c	0.825±0.0173b
Khazar	62.33±2.385c	3.157±0.0179d	0.407±0.0121c
Binam	68.820±2.454b	2.760±0.261d	0.392±0.0416c

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 3. Comparison of the reactions of rice cultivars affected by *C. graminicola* with those of the controls.

Cultivars	Change of Height	Change of Fresh weight	Change of Dry weight
Hashemi	-0.823±0.151b	-0.036±0.149a	-0.133±0.33a
Ali Kazemi	-1.96±0.571a	-0.046±0.129a	-0.24±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 having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 4. Variance analysis of disease rating and the studied traits in weeds affected by *C. graminicola*.

SOV	DF	Squares Mean			
		Disease rating	Height(cm)	Fresh Weight (g)	Dry Weight(g)
Treatment	3	3.652**	432.828**	97.676**	4.710**
Error	8	0.489	1.218	0.989	0.192
C.V.	-	27.74	2.04	8.94	25.16

**:Significance at the probability level of 1%

SOV: sources of variations

DF: degree of freedom

Table 5. Comparison of means of the studied traits affected by *C. graminicola* in weeds.

Weeds	Height(cm)	Fresh weight(g)	Dry weight(g)
E. oryzicola	62.330±0.0378a	7.179±0.0257c	0.998±0.0569c
E. crus-galli	62.123±0.313a	5.475±0.141c	0.630±0.188c
Sagitaria trifolia	54.900±0.907b	17.346±1.128a	3.466±0. 447a
A. plantago-aquatica	36.776±0.837c	14.513±0.891b	1.870±0.227b

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 6. Comparison of the reactions of weeds affected by *C. graminicola* with those of the controls.

Weeds	Change of Height(cm)	Change of Fresh weight(g)	Change of Dry weight(g)
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
Sagitaria trifolia	-0.933±0.466a	-4.926±1.526a	-1.23±0.309a
A. plantago-aquatica	-0.483±0.483a	-3.173±0.164a	-0.036±0.433b

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

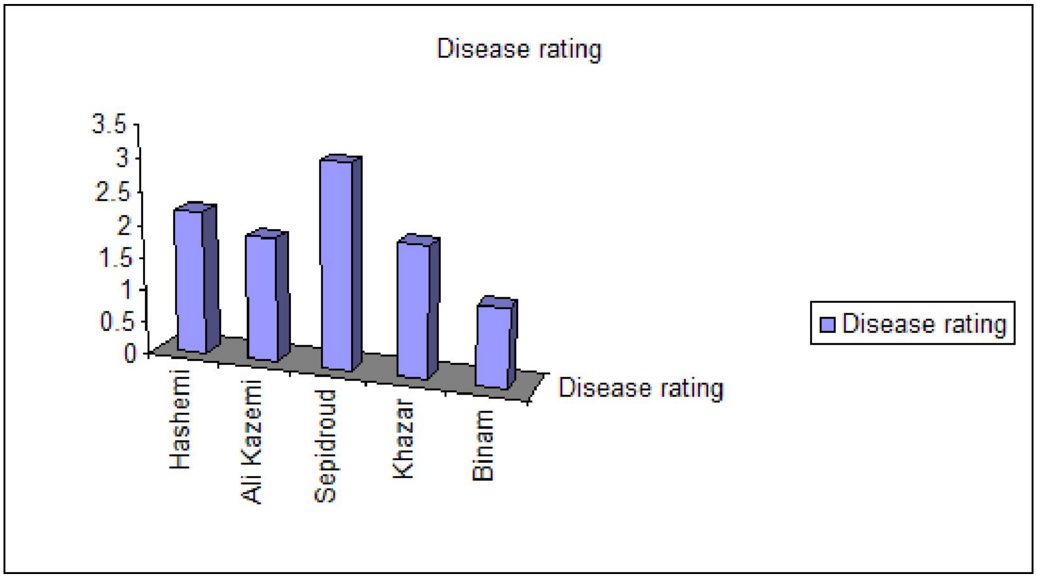

Figure 1. Diagram of the comparison of *Colletotrichum graminicola* mean disease rating in rice cultivars.

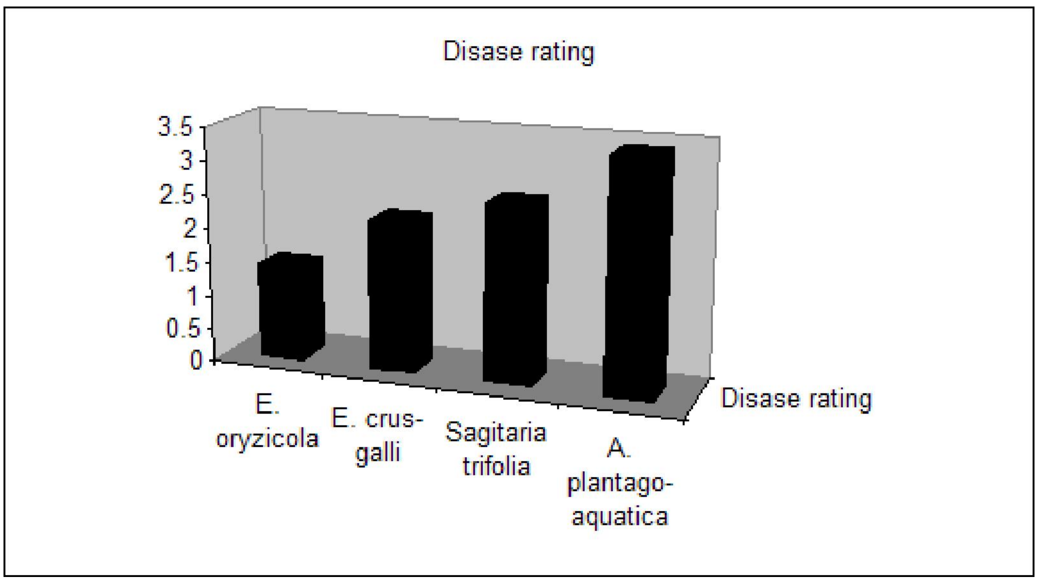

Figure 2. Diagram of the comparison of *Colletotrichum graminicola* mean disease rating in weeds.

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