Australian Journal of Crop Science AJCS
AJCS 5(4):453-457 (2011) ISSN:1835-2707

Evaluation of the reaction of *Sagitaria trifolia* (arrowhead) and some rice cultivars to *Fusarium equiseti*

Mohammad Reza Safari Motlagh*[1] and Peyman Sharifi[2]

[1]Department of Plant Pathology, Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Iran
[2]Department of Agronomy and Plant Breeding, Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Iran

***Corresponding author: ssafarimotlagh@yahoo.com**

Abstract

In this research, *Fusarium equiseti* (Corda) Saccardo was isolated from *Sagitaria trifolia* and studied as a biocontrol agent of this weed in paddy fields. Three Iranian indigenous rice cultivars such as Hashemi, Ali Kazemi and Binam and two bred cultivars, Sepidroud and Khazar were studied in a greenhouse in a random complete design with three replications. Inoculation was done on three or four leaf stage plants. Besides, arrowhead plant was inoculated with *Fusarium equiseti* in three replications. Rice cultivars evaluated for disease rating index, showed a significant reaction to the fungus. However, among the cultivars, Sepidroud and Khazar had the least and most tolerance, respectively. The effect of this fungus on cultivar traits such as height, fresh weight and dry weight was significant, but the greatest effect was observed on plant height. In addition, the disease rating of was signifucanly higher on arrowhead compared to rice cultivars. *Fusarium equiseti* also had a significant effect on the dry weight of the weed as well. In this study we suggest *Fusarium equiseti* as a promising mycoherbicide for controlling *Sagitaria trifolia* under special conditions, when cropping incorporated with tolerant rice cultivars.

Keywords: Biocontrol, *Fusarium equiseti*, *Sagitaria trifolia*.
Abbreviations: PDA- potato dextrose agar.

Introduction

Weeds are the most important biological obstacles for rice production (Lindquist and Kropff, 1988). The average performance decrease for crops due to competition with weeds is about 10% in comparison. This yield loss is even higher in rice and has been reported to be about 20% (Lindquist and Kropff, 1988). Arrowhead (*Sagitaria trifolia* L.) is one of the most important broad leaf weeds in paddy fields (Rezvani et al., 2002). Due to vegetative reproductive capacity, its control is much more difficult than other weeds (Rezvani et al., 2002). Overuse of chemical herbicides to control this group of weeds has resulted the resistance to herbicides (Holt and Lebaron, 1998). Collego is among the most important bioherbicides which widely has been used in rice fields yet (Tempelton et al., 1984). Beside this, Devin (*Phytophthora palmivora*) has also been suitably used to control broad leaf weeds in paddy fields (Yamaguchi et al., 2008). Studies have shown that *Plectosporium tabacinum*, which isolated from arrowhead, is very effective in limiting the development of rhizomes during its early growth stages (Zhang et al., 2002). Also, research results revealed that two fungi, *Plectosporium tabacinum* and *Rhynchosporium alismatic* cause the highest virulence in two broad leaf weeds such as *Alisma plantago-aquatica* (L.) and *Sagitaria trifolia* (Ash et al., 2005). In fact, these two fungi do not cause high levels of virulence in the above-mentioned weeds in all geographical regions (Ash et al., 2005). Generally, different *Fusarium* species can be applied for biological control of the weeds in different crops. For instance, *Fusarium oxysporum* is considered as an important factor in biological control of some weeds (Boari et al., 2003). Studies showed that this fungus causes high pathogenesis levels in *Amaranthus retroflexus* (L.) and *Cyperus difformis* (L.). However, its effect was greater during the early stages of growth in relevant weeds (Boari et al., 2003). On the other hand, *Fusarium tumidum* affects *Ulex europaeas* (L.) and reduces the root growth and the height of weed plants. Moreover, the decrease of height was reported positively correlated with temperature (Hurrell et al., 2005). Molecular studies using SSR markers have shown that the existence of a dominant gene in the fungus influences the height-controlling gene in a plant to become inactive (Hurrell et al., 2005). Prior to introducing any biological agent we need to be ensured that there would not be any damage possibility to environment and crops (Moein and Pourkashani, 1992). Therefore, study of reaction of rice cultivars to biological agents seems to be necessary (Moein and Pourkashani, 1992). In general, assessment of cultivar reactions to pathogens is considered to be the first step of identification of tolerant varieties (Burdon and Leather, 1990). Response of *Echinochloa* (L.) species and rice (*Oryza sativa*) to indigenous pathogenic fungi has been investigated (Zhang et al., 1996). Modification of plants with genetic engineering and sequencing of resistance genes are some key factor in development of resistant and tolerant varieties (Williams et al., 2001). *Sagitaria trifolia* is one of the important weeds in paddy fields. In this study, the reaction of this weed to the pathogenic fungus *Fusarium equiseti* (Corda) Saccardo was evaluated. Moreover, some rice cultivars in Guilan province in the north of Iran were

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

SOV	DF	Squares Mean			
		Disease rating	Height(cm)	Fresh Weight (g)	Dry Weight (g)
Treatment	4	5.249**	183.693**	10.720**	0.935**
Error	10	0.36	13.720	0.053	0.007
C.V.	-	20.68	5.20	4.65	9.51

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

Fig 1. Macroconidia and microconidia of *F. equiseti* (×460)

evaluated in terms of reaction to pathogenesis. Some other rice and weed traits were also studied to assess the potential of this fungus as a mycoherbicide.

Materials and methods

Collection and culture of fungal isolates

Leaves with symptoms of the disease *Sagitaria trifolia* were collected in Guilan province, Iran. They cut to 3-4 cm and transferred to the laboratory. Samples were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on 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).

Identification of fungi

The grown fungi isolated and koch's postulates were completed for most samples after each collection. Cultures of these fungi were submitted to the Research Plant Pathology Institute of Iran for confirmation of identification (Fig. 1).

Pathogenicity test

This reaction was assessed in a complete random design (CRD) with one treatment and three replications. Inoculation of *Sagitaria trifolia* was performed at three-four leaf stage in greenhouse. To do so, a spore suspension including 10^6 *F. equiseti* spores/ml in distilled water was applied. In order to increase adsorption, 1% Tween-20 was also added. Weeds were planted in plastic pots, 2.5 cm in diameter, filled with 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%. Before inoculation, all pots were sprayed with distilled water. The fungus suspension was then sprayed on the leaves. 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 type and size of lesions, scored as: 0= lesions absent, 1= small, unexpanded lesions, 2= slightly to moderately expanded lesions, 3= large lesions (Zhang et al., 1996). Then, five rice cultivars including three indigenous (Hashemi, Ali Kazemi and Binam) and two bred cultivars (Khazar and Sepidroud) were evaluated, in complete random design with three replications, against inoculation with *F. equiseti*. The rice seeds germinated and then planted in the farm soil, inside the 2.5 cm diameter pots, without any drain and transferred to greenhouse. When the plants reached to their three or four leaf stage, thinning was performed. Finally, there were 4 shrubs in each pot. Two grams of urea fertilizer was added to the pots. At this stage, inoculation was done by a spore suspension of *F. equiseti* containing 10^6 spore/ml of distilled water with 1% Tween-20. Other environmental conditions were similar to those for weed. Evaluation was done 7 days after inoculation, for which Horsfall-Barrat system used. Then, disease ratings were calculated (Bertrand and Gottwald, 1997). It is noteworthy that in both experiments, one control was placed for each replication (Safari Motlagh and Javadzadeh, 2010).

Table 2. Comparison of the reactions of rice cultivars affected by *F. equiseti* with those of the controls.

Cultivar	Change of Height(cm)	Change of Fresh weight(g)	Change of Dry weight(g)
Hashemi	-3.08 ± 0.798[a]	-0.263 ± 0.101[a]	-0.296 ± 0.067[a]
Ali Kazemi	-2.92 ± 1.045[a]	0.185 ± 0.204[b]	-0.231 ± 0.068[a]
Sepidroud	-2.04 ± 1.119[a]	-0.076 ± 0.063[a]	-0.133 ± 0.074[a]
Khazar	-2.25 ± 0.943[a]	0.123 ± 0.043[b]	-0.083 ± 0.039[a]
Binam	-0.916 ± 0.108[a]	-0.226 ± 0.08[a]	0.189 ± 0.027[a]

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

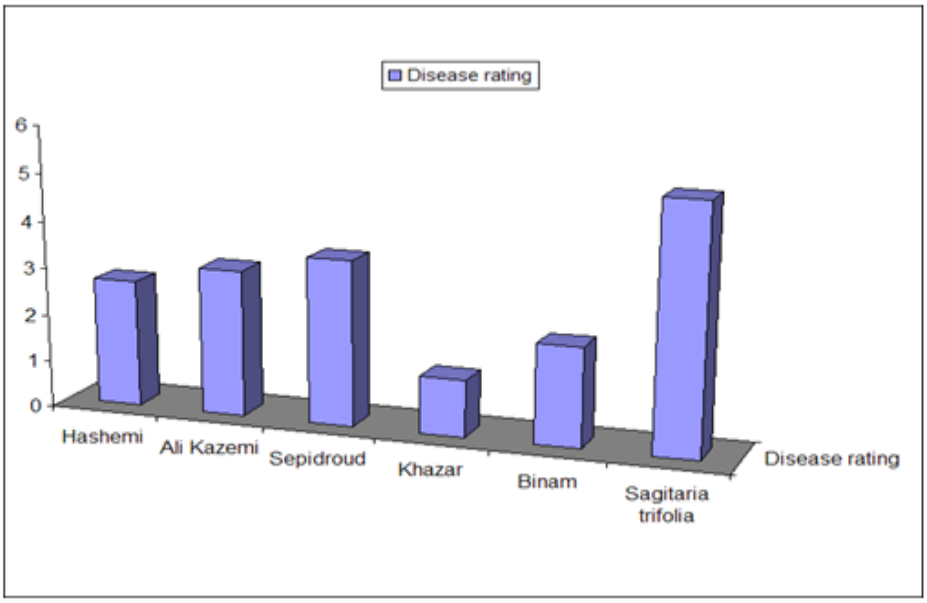

Fig 2. Comparison of *F. equiseti* mean disease rating in rice cultivars and *Sagitaria trifolia*.

Measurement of plant fresh and 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 at the level adjacent to soil surface and weighed by an electric scale. This weight was recorded as their fresh weight. After measurement of their heights, each shrub was placed inside a paper bag and kept for 48 hours in an oven at 80-90^{0}C. When dried, each shrub was weighed, which was considered as its dry weight (Ghorbani et al., 2000).

Data Analysis

Data analysis was done using SPSS and MSTAT-c software. In order to compare average values, Duncan test was used. The reaction of rice cultivars and difference between the average value of each fungus-treated rice, controls and weeds was tested by Chi-square.

Results and discussion

The analysis of variance indicated the significance of reaction in studied cultivars to disease rating (Table 1). Based on the comparison of the averaged traits, the highest level of disease rating was observed in Sepidroud compared to other cultivars (Fig. 2). There was no significant difference between Hashemi, Khazar and Binam and they were more tolerant to fungus. The Khazar cultivar was the most tolerant at all. On the other hand, among indigenous cultivars, Ali Kazemi more affected by the fungus. The responses of the two bred cultivars, Sepidroud and Khazar, were totally different in disease rating as Sepidroud showed the least tolerance. Evaluation of traits such as height, fresh and dry weight revealed that rice cultivars had significant reductions in all three traits compared to controls after treatment with the fungus. Only Binam cultivar kept its dry weight after treatment with agent (Table 2). The comparison of the above-mentioned rice cultivars revealed that in terms of height, they did not have any significant difference. Concerning the fresh weight, Hashemi, Sepidroud and Binam were classified in the same group, while Ali Kazemi and Khazar were placed in another group. The first group showed higher reduction of fresh weight compared to controls (Table 2). Regarding dry weight, Hashemi, Ali Kazemi, Sepidroud and Khazar were grouped together, while Binam was placed in another group. The decrease of dry weight in the first group was much more than those of the controls (Table 2). The fungus-treated cultivars showed the taller height compared to controls and the effect of fungus on plant height was greater in comparison with other studied traits such as fresh and dry weight. Based on the results of this research disease rating of

Table 3. Chi-square values of the studied traits affect by *F. equiseti* in weed

Weed	Height(cm)	Fresh weight(g)	Dry weight(g)
Sagitaria trifolia	0.0198 $^{n.s.}$	0.0282 $^{n.s.}$	2.5356**

n.s: not significant at p=5% ,****** : Significance at the probability level of 1%.

fungus was greater on *Sagitaria trifolia* compared to rice cultivars (Fig. 2). Moreover, the fungus-treated weeds showed a remarkable reduction in the dry weight, but no significant effect observed in weed height and fresh weight (Table 3). In this study, among indigenous cultivars, Ali Kazemi was more affected by the fungus than Hashemi. Also, responses of the two bred cultivars, Sepidroud and Khazar, in the evaluation of disease rating were totally different, while 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 (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, 2002). Hence, in order to increase plant tolerance, more modification methods should be applied to cultivars (Rezvani, 2002). In this research, traits of fungus-treated cultivars with controls showed that the effect of fungus 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 (Chang and Li, 1991). 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 *Exserohilun oryzae* were pathogenic to both rice and *Echinochloa* species while *C. geniculata* and *Exserohilun monoceras* were pathogenic only to *Echinochloa* species. Having more effects on the fresh weight and height of *Sagitaria trifolia Fusarium equiseti* treatment 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, a fungus 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 (Coen and Rotem, 1987). Usually, resistance and sensitivity genes in bred cultivars are more than those of the indigenous ones (Frankel, 1997). 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 (Mousavi, 1993). In this study, Khazar cultivar showed the highest tolerance to *F. equiseti*, than other rice cultivars and therefore biocontrol of arrowhead can be done in its paddies.

Conclusions

Due to the fact that *F. equiseti* could cause a higher disease rating in *Sagitaria trifolia*, it may be regarded as a probable biological agent in controlling this weed. But more tolerant rice cultivars such as Khazar must be planted when this fungus is being used as biocontrol agent.

Acknowledgments

This experiment was supported by the Islamic Azad University, Rasht Branch, Iran.

References

Ash GJ, Cother EJ, Chung YR, Pitt WM, McKenzie C (2005) Phylogeny, pathogenicity and diversity of biocontrol agents for alismataceae weeds in Australia and Korea. Joint Workshop, International Bioherbicide Group and EWRS-Biocontrol Working Group. Bari, Italy

Bertrand PF, Gottwald TR (1997) Evaluation fungicides for pecan disease control. In: Hickey KD(ed) Methods for Evaluating Pesticides for Control of Plant Pathogens. edn. APS.149-164.

Boari A, Abouzeid MA, Zonno MC, Evident A (2003) Microbes and microbial product for biological control of parasitic weed. Bioherbicide Group. Italy.

Burdon JJ, Leather SR (1990) Pests, pathogens and plant communities. Blackwell Scientific Publication, 61-72.

Chang TT, Li CC (1991) Genetics and breeding in rice production**,** New York: Nestrand Reingold Pub., Vol.1, pp. 23-44.

Coen Y, Rotem J (1987) Sporulation of foliage pathogen. Brithish Mycological Society, Cambridge UK., UK. 315-333.

Frankel OH (1997) Genetic resources as the back bone of plant protection. Plant Dis,Vienna, 3-15

Ghorbani R, Seel W, Litterick A, Leifert C (2000) Evaluation of *Alternaria alternata* for biological control of *Amaranthus retroflexus*. Weed Sci 48:474-480

Holt JS, Lebaron HM (1998) Significance and distribution of herbicide resistance. Weed Tech 4: 141-149

Hurrell GA, Bourdot GW, Barton J (2005) Do *Chondrostereum purpureum* and *Fusarium tumidum* have potential as mycoherbicides for gorse? Joint Workshop, International Bioherbicide Group and EWRS- Biocontrol Working Group. Bari, Italy.

Lindquist JL, Kropff MJ (1998) Application of an ecophysiological model for irrigated rice (*Oryza sativa*) and *Echinochloa* competition. Weed Sci 3(44):52-56.

Moein MJ, Pourkashani A (1992) Harmful Factor of Rice. Publication of the organization of agricultural advisory services. 1:1-23.

Mousavi M (1993) Weeds in Rice Paddies and Olive Groves, Journal of Olive, A Specialized Scientific Magazine Published by the Ministry of Agriculture, 46: 1-14.

Nikkhah M, Hedjaroud Gh, Sharifi A, Elahinia A (2001) Study of the tolerance of some rice cultivars and lines of the stem rot disease in Guilan province. J Sci Tech Agri Nat Res 4:189-195.

Rezvani A, Izadyar M, Faghih A (2002) A guide to pests, diseases and rice weeds. The ministry of agricultural crusade publication. J Org Agric Res Train 5: 1-5.

Safari Motlagh M R, Padasht F, Hedjaroud Gh (2005) Reaction of some rice cultivars to brown spot in Guilan province. J Sci Tech Agri Nat Res 2:171-182.

Safari Motlagh MR, Javadzadeh A (2010) Study of *Alternaria pellucida* as a probable mycoherbicide for controlling Arrowhead(*Sagitaria trifolia*) in paddy fields. Plant Omics J 3(6): 172-176.

Talebi R, Rahimiyan H, Nematzadeh Gh, Momeni A (2004) Genetic evaluation of the resistance of different rice cultivars to blast using microsatellite markers. J Agri Sci Na Res Khazar Agr 4:28-43.

Templeton GE, Tebest DO, Smith RJ (1984) Biological weed control in rice with a strain of *Colletotrichum gloeosporiodes*. Crop Protect 74:1171-1175.

Williams E, Backer EM, Shannon F (2001) Development of genetic markers for risk assessment of biological control agent. Plant Pathol 3:13-18.

Yamaguchi K, Ishihara K Fukai S (2008) Wild and weed rice in rice ecosystems in Asia. A review, limited proceeding in IRRI, China, 87-99.

Zhang WM, Moody K, Watson AK (1996) Responses of *Echinochloa* species and rice (*Oryza sativa*) to indigenous pathogenic fungi. Plant Dis 80:1053-1058.

Zhang W, Sulz M, Baily KL (2002) Evaluation of *Plectosporium tabacinum* for control of herbicide- resistant and herbicide- susceptible false cleavers. Weed Sci 50:79-85.