Evaluation of *Colletotrichum graminicola* as an Eventual Bioherbicide for Biocontrolling *Alisma plantago-aquatica* in Paddy Fields

Mohammad Reza Safari Motlagh[1] and Armin Javadzadeh[2]

[1.] Department of Plant Pathology, Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Guilan Province 41335-3516, Iran. Tel:00989111384168

[2.] Department of Plant Breeding and Agronomy, Faculty of Agriculture, Islamic Azad University, Tabriz Branch, Tabriz, Azarbayjan Province Iran

*Corresponding author's email: ssafarimotlagh@yahoo.com

Abstract: *Alisma plantago-aquatica* is one of the most important paddy field weeds in Iran. In this research, *Colletotrichum graminicola* was isolated from the said weed and studied as a biological agent for controlling *Alisma plantago-aquatica*. 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 random completely design with three replications and five treatments was used at a greenhouse. Then, *Colletotrichum graminicola* was inoculated on *Alisma plantago-aquatica*. This 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 indicated that the disease rating caused by the this fungus in the weed was more than that in the studied rice cultivars. Also, the fungus had a significant effect on the height, fresh weight and dry weight of *Alisma plantago-aquatica* and reduced them. Furthermore, *Colletotrichum graminicola* had a significant effect on all the studied rice cultivars and significantly reduced their heights and fresh weights. With consideration of the results of this research, *Colletotrichum graminicola* is not recommended as a probable mycoherbicide for biological controlling *Alisma plantago-aquatica* in paddy fields, unless more tolerant rice cultivars are used when using it.

[Mohammad Reza Safari Motlagh and Armin Javadzadeh. Evaluation of *Colletotrichum graminicola* as an Eventual Bioherbicide for Biocontrolling *Alisma plantago-aquatica* in Paddy Fields. Life Science Journal. 2011;8(1):377-382 (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: *Alisma plantago-aquatica*; biological control; *Colletotrichum graminicola*; rice

1. Introduction

Usually, using chemical herbicides is the most effective method for controlling various weeds (Fryer and Chancellor, 1970). However, there are two obvious difficulties regarding the outcomes of using these herbicides which include extensive increase of tolerance to herbicides in weed populations and complexity of the eradication of weed races or crop families; that is, wherever herbicides are used extensively, interspecies selection is its most dominant result which depending on the type of chemicals that are used, creates many changes in the relative frequency of the species (Fryer and Chancellor, 1970; Harris et al., 2003). On the other hand, it was found that weeds tolerant to an herbicide may be tolerant to other herbicides as well (Kouchaki et al., 2001).

Recent biotechnology developments have increased scientists' interest in microorganisms as potential resources of natural herbicides (Kouchaki et al., 2001). In fact, the management of pathogens and insects is the basis of the biological control of weeds (Hoy and Herzog, 1985). Moreover, using plant pathogens, due to their specificity (specific performances) in terms of hosts and also their lower costing production compared with insects (except for obligate parasites) is very useful and cost-effective (Kouchaki et al., 2001). There are several examples of using different microorganisms such as fungi for the biological control of weeds, but their numbers in terms of controlling paddy field weeds is limited. For example, *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* (Bowers, 1986; TeBeest and Templeton, 1985).

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). In a study conducted in Vietnam, Australia and South Korea for biological controlling *Echinochloa crus-galli* and *Echinochloa* sp. in paddy fields, *Exserohilum fusiforme* and *Colletotrichum graminicola* were used and they prevented their propagation during the growth stages of rice (Johanson et al., 2003). Usually, *Alisma plantago-aquatica* is considered as one of the most important rice weeds in Australia and Asia (Ash et al., 2005). This weed which is among broad-leaf and monocotyledon weeds, due to having too much seeds with long dormancy and its high resistance to many chemical herbicides has greatly propagated in paddy fields (Rezvani et al., 2002).

Investigations revealed that *Plectosporium tabacinum* and *Rhynchosporium alismatic* had considerable effects on controlling *Alisma plantago-aquatica* (Ash et al., 2005). Another study showed that *Alternaria eichhorniae* isolated from Water hyacinth only affected this weed and did not control *Alisma plantago-aquatica* (Martinez and Charudattan, 1998).

Usually, fungi such as *Fusarium oxysporum* and *Colletotrichum coccodes* being among good mycoherbicides are changed by pectinase, cellulase and expancine 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 barnyardgrass (Yang et al., 2000).

In another study, *Colletotrichum dematium* was introduced for controlling *Epilobium angutifolium* which can replace herbicides such as simazine, atrazine (Watson and Winder, 1993).

Also, *Ipomoea lacunosa* is a dangerous and dispersed weed in soybean and cotton fields and many other plants all through the southern regions of the United States and the use of *Colletotrichum capsici* has proved effective in reducing its damages (Cartwright and Tempelton, 1994). Furthermore, a combination of glyphosate and *Colletotrichum truncatum* is effective for controlling *Sesbania exaltata* in a way that by using the said fungus, the level of the used herbicide decreases (Boyette et al., 2008). However, one of the prerequisites which should be observed is that prior to the introduction of each biological factor, ensuring that no probable damages would be made to crops is important. Hence, studying and investigating the reactions of some crop varieties which have been planted in places where a bioherbicide is used is necessary (Bergson and Carter, 2002). One of the most important principles of developing biocontrol is the existence of biological factor-resistant crops (Burdon and Leather, 1990).

The main objective of the present research was to evaluate *Colletotrichum graminicola* as a mycoherbicide for controlling *Alisma plantago-aquatica*. In order to do so, at first, the above-mentioned fungus was isolated from *Alisma plantago-aquatica* and then, its disease rating in the weed and some important rice cultivars of Guilan province in Iran was studied.

2. Materials and Methods

2.1 Collection and culture of fungal isolates

Leaves with symptoms of the disease *Alisma plantago-aquatica* 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 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).

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

This reaction occurred as complete random design (CRD) with one treatment and 3 replications. Inoculation of *Alisma plantago-aquatica* 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. 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%,. 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).Then, five rice cultivars including 3 indigenous (Hashemi, Ali Kazemi and Binam) and 2 bred cultivars (Khazar and Sepidroud) were evaluated in complete random design with three replications 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, 2g 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 for which Horsfall-Barrat system was used. 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 for 48 hours, they were in an oven at 80-90^{0}C. 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, the difference between the average value of each fungus-treated rice cultivars and the controls and for weeds Chi-square test 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 (Figure 1). Among indigenous cultivars, Hashemi showed less tolerance (Figure 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, the said 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 the said 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).

Results from the present research showed that the disease rating caused by *Colletotrichum graminicola* in *Alisma plantago-aquatica* was more than that in the rice cultivars (Figure 1).

On the other hand, based on the Chi-square test, the above-mentioned fungus had a significant effect on all the three studied traits, i.e. fresh weight, dry weight and height (Table 4) and this effect was greater on height than on the other two traits (Table 4).

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. Chi-square values of the studied traits affect by *C. graminicola* in weed.

Weed	Height	Fresh weight	Dry weight
Alisma plantago-aquatica	2.251**	1.21**	1.02**

** : Significance at the probability level of 1%.

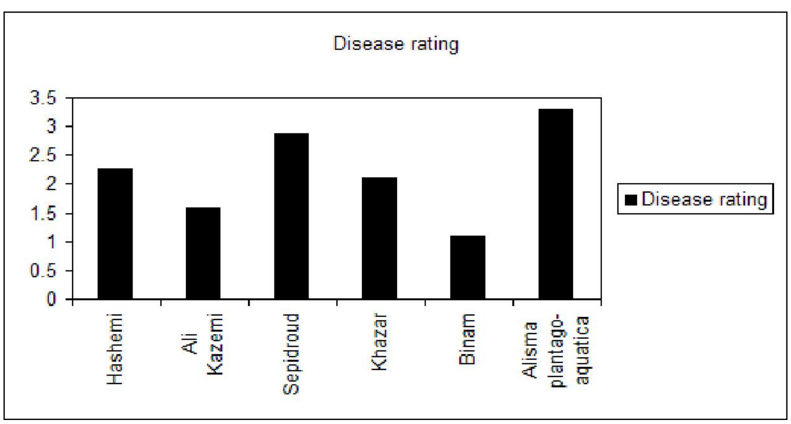

Figure 1. Diagram of the comparison of *Colletotrichum graminicola* mean disease rating in rice cultivars and *Alisma plantago-aquatica.*

4. Discussion

With consideration of the studies conducted in this research, *Colletotrichum graminicola* had a negative effect on both indigenous and bred studied rice cultivars. However, there were some differences between these two groups of cultivars in terms of their tolerance. As has been shown in other studies, different species of *Colletotrichum* including *C. graminicola* and *C. truncata* are considered as effective antagonistic fungi for controlling barnyardgrass and woody nightshade, but in order to extend their uses, the existence of tolerant cultivars is necessary for wheat and rice cultivation (Johanson et al., 2003).

Studies showed that *Colletotrichum graminicola* was effective on *Sorghum halepenes* (one of the weeds in alfalfa fields), but it also caused disease in alfalfa and this prevented the introduction of the said fungus as a biological agent (Templeton and Henry, 1990). Other studies on the reactions of bred and indigenous corn cultivars to *Colletotrichum* sp., isolated from annual mercury in corn fields in the US showed that the bred cultivars were more tolerant of disease rating, but the fungus was more effective on their dry weights yet with the existence of high disease rating in indigenous cultivars, the decrease of dry weight was not noticeable (17). Molecular studies showed that bred cultivars had more resistant genes, but in indigenous ones dry and fresh weight-controlling genes were even more (Norris, 1992). Also, studies showed that *Colletotrichum truncata* and *Ralstonia solanacearum,* which were effective for the biological control of *Sesbania exaltata* and *Striga hermonthica* and caused higher disease ratings, had fewer effects on soybean and rice bred cultivars while causing the same severe symptoms in indigenous ones (Charudattan, 2001).

Therefore, with consideration of the results from this research and similar studies it was concluded that though the disease rating caused by *Colletotrichum graminicola* in *Alisma plantago-aquatica* was more than in the studied rice cultivars, but with the negative effect of the fungus on the experimented traits taken into account, this fungus could not be introduced as a biological agent for controlling *Alisma plantago-aquatica* unless upon using it as a bioherbicide, other rice cultivars are cultivated which are more tolerant to *Colletotrichum graminicola* and this would require conducting more studies and also modifying rice cultivars by doing biotechnological and genetic engineering researches.

Acknowledgments:

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

Corresponding Author:
Dr. Mohammad Reza Safari Motlagh

Department of Plant Pathology, Faculty of Agriculture

Islamic Azad University, Rasht Branch, Rasht, Guilan

Province 41335-3516, Iran. Tel:00989111384168

Email: ssafarimotlagh@yahoo.com

References

1. Ash GJ, Cother EJ, Chung YR, et al. Phylogeny, pathogenicity and diversity of biocontrol agents for alismataceae weeds in Australia and Korea. 2005. Joint Workshop, International Bioherbicide Group and EWRS-Biocontrol Working Group. Bari, Italy.
2. Bergson CH, Carter R. Weed sedage (cyperaceae) of world southern. Weed Sci, 2002; 3:1-18.
3. Bertrand PF, Gottwald TR. Evaluation fungicides for pecan disease control. In: Hickey, KD. Methods for Evaluating Pesticides for Control of Plant Pathogens. 1997; 179-181.
4. Bowers RC. Commercialization of Collego-An industrialist's view. Weed Sci, 1986; 34 (Suppl.1) , 24.
5. Boyette CD, Hoagland RE, Weaver MA. Interaction of a bioherbicide and glyphosate for controlling Sesbania in glyphosate – resistant soybean, Weed Biology and Management, 2008; 8:19-24.
6. Burdon JJ, Leather SR. Pests, pathogens and plant communities, Blackwell Scientific Publication, 1990; 61-72.
7. Cartwright KD, Tempelton GE. Biologically pure phytopathogenic fungus *Colletotrichum capsici*. 1994;United States Patent 5292659.
8. Charudattan R. Biological control of weeds by means of plant pathogens significance for integrated weed management in modern agro-ecolog**y**, Biocontrol, 2001; 8(46):46-229.
9. Fryer JD, Chancellor RJ. Herbicides and our changing weeds in the flora of changing Britain. Perring, F., ED., Bot. Soc. Br. Isles, E.W. Classey, Hampton, Middlesex, 1970; 105 p.
10. Ghorbani R, Seel W, Litterick A, et al. Evaluation of *Alternaria alternata* for biological control of *Amaranthus retroflexus*. Weed Sci, 2000; 474-480.
11. Hershkovitz MS, Larroch C, Al-Ahmad H, et al. Enhancing mycoherbicide activity. Novel and Sustainable Weed Management in Arid and Semi-Arid-Ecosystems, 2007; Rehovot, Israel.

12. Harris P, Dung HP, Schroeder D. Biological control of leafy spurge in North American. Weed Science of American, 2003; 79-92.
13. Hoy MA, Herzog DC. Biological Control in Agricultural IPM Systems. Academic Press, Orlando, Fla. 1985; 589 p.
14. Johanson DR, Wyse DL, Janes KJ. Controlling weeds phytopathogen. Weed Technol, 2003; 10: 621-624.
15. Kouchaki A, Zarif Ketabi H, Nakhforoush, A. Ecological Strategies for Weed Management, Ferdosi University Publications, Mashhad. 2001; 457 p.
16. Martinez M, Charudattan R. Survey and evaluation of Mexican native fungi for potential biocontrol of water hyacinth and Alisma weed. Plant Management, 1998; 5(30): 145-148.
17. Norris RF. Have ecological and biological studies improved weed control strategies? In: Levick KJ, Combellak, JH. (eds.), Victoria University,Weed Science Society, 1992; 1:7-33.
18. Rezvani A, Izadyar M, Faghih A. A guide to pests, diseases and rice weeds. The ministry of agricultural crusade publication. Journal of Organization and Agriculture Research Train, 2002; 5: 1-5.
19. TeBeest DO, Templeton GE. Mycoherbicides: progress in the biological control of weeds. Plant Disease, 1985; 69,6.
20. Templeton GE, Henry DK. Mycoherbicides suppressing agricultural pests and disease, New York: Baker, Liss Inc, 1990; 279-286.
21. Watson AK, Winder RC. Composition for ed biocontrol of firewe. 1993;United States Patent 544659.
22. Yang YK, Kim SO, Chung HS. et al. Use of *Colletotrichum graminicola* kAoo to control barnyardgrass. Plant Dis, 2000; 84: 55-59.
23. Zhang WM, Moody K, Watson AK. Responses of *Echinochloa* species and rice (*Oryza sativa*) to indigenous pathogenic fungi. Plant Dis, 1996; 80, 1053-1058.

10/1/2011