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# Reaction of major weeds and some rice cultivars to *Alternaria pellucida*- a potential biocontrol agent

**Mohammad Reza Safari Motlagh**

**Department of Plant Pathology, Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Iran,**

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

**Abstract**

*Sagitaria trifolia*, *Alisma plantago-aquatica* and *Echinochloa* spp. are among the most important damaging weeds in paddy fields. In this research, *Alternaria pellucida* was isolated from the weeds and then its pathogenicity effects was examined on these weeds and five other rice cultivars, including two bred (Sepidroud and Khazar) and three landraces (Ali Kazemi, Hashemi and Binam), in a completely random design with three replications in greenhouse conditions. *A. pellucida* caused a high disease rating on the weeds compared to rice cultivars. Results revealed that *A. pellucida* caused high disease ratings in *S. trifolia, E. crus-galli, A. plantago-aquatica,* and *E. oryzicola,* respectively. The reaction of weeds to this fungus, in terms of disease rating, was significant while rice cultivars showed no significant reaction. In addition, the fungus caused a significant reduction of height, fresh weight and dry weight of the evaluated weeds, whereas no significant reduction was observed in the dry weight of rice cultivars. Nevertheless, the fresh weight and height of cultivars showed significant reactions. Based on the results of this research, *A. pellucida* could be introduced as a potential mycoherbicide for controlling these weeds.

**Keywords:** weeds, *Alternaria pellucida,* rice, biological control, mycoherbicide.
**Abbreviations**: PDA- potato dextrose agar.

## Introduction

Rice (*Oryza sativa*) plays a very important role in the nutrition of people around the world. There are various factors that reduce rice production, the most important of which are pests, pathogens and weeds. In terms of consuming the existing nutrients in rice paddies, weeds are strong competitors of rice in many cases. They prevent sunlight to reach rice and in the mean time, they can be suitable hosts for numerous rice pests and pathogens. Unless necessary measures are taken for controlling the weeds, the resulting damage might include up to 90% of the crop production. Various practices such as applying farming methods, planting resistant cultivars and application of herbicides have been suggested for controlling weeds in rice paddies. The adverse effects of constant use of these chemical compounds have led to the emergence of different resistant weed varieties (Rezvani et al., 2002). Therefore, application of other methods such as natural microorganisms, known as bioherbicides, became necessary for the biological control of weeds (Rezvani et al., 2002). Fungi are among the important microorganisms that are being used as mycoherbicides for controlling weeds in rice paddies (Rezvani et al., 2002). In one of the studies, *Puccinia xanthii* and *Alternaria helianthi* were used for controlling *Xanthium* spp. which is the third most important crop weed throughout the world (Kouchaki et al., 2001). The results revealed that *Alternaria helianthi* efficiently controlled *Xanthium* spp. under greenhouse conditions (Bassi and Quimby, 1985). Ghorbani et al. (2000) studied *Alternaria alternata* for the biological control of *Amaranthus retroflexus* and found that as the spore concentration and the moisture duration increased, the pathogenicity of fungus increased accordingly. *Alisma plantago-aquatica* is the other main weed in rice paddies (Rezvani et al., 2002). Another study showed that *Alternaria eichhorniae* isolated from *Eichhornia crassipes* did not affect on *A. plantago-aquatica* and only was effective in controlling water hyacinth (*Eichhornia crassipes*) (Martinez and Charudattan, 1998). Comparison of the efficiency of some fungi such as *Fusarium* sp., *Alternaria* sp. and *Plectosporium alismatis* for controlling *Alisma plantago-aquatica* revealed that *Plectosporium alismatis* was more capable of being applied as a pathogen (Jahromi, 2007). The biological control of the most important and damaging weed of sorghum and corn, *Striga hermonthica,* in the semi-tropical south African region studied and the results revealed that *Alternaria alternata*, *Aspergillus* sp., *Bipolaris* sp. and *Curvularia* sp. are among the effective pathogenic agents to control this weed (Abbasher, 2000). However, most of the damage on weed was caused by *Fusarium* sp. and *Bipolaris* sp. (Abbasher, 2000). *Alternaria eichhorniae, Curvularia lunata* and *Fusarium* sp. reduced the growth of *Echornia crassipes* by 15-20% (Praveena and Nasecma, 2004). *Echinochloa* spp., *Alisma plantago-aquatica* and *Sagitaria trifolia* are considered as the most important weeds of rice paddies of Guilan province in Iran (Safari Motlagh, 2010). Hence, for the biological control of these weeds, some researches were conducted and fungi such as *Curvularia lunata* and *Fusarium equiseti* were introduced as mycoherbicides (Safari Motlagh, 2010). In the present study, *Alternaria pellucida* was isolated from the current weeds and evaluated as potential biological agent to control the mother weeds. To ensure that this biological controlling factor does not damage the main crop,

**Table 1.** Variance analysis of disease rating , height, fresh and dry weight, in rice cultivars affected by *A. pellucida*.

| SOV | DF | Squares Mean | | | |
|---|---|---|---|---|---|
| | | Disease rating | Height | Fresh weight | Dry weight |
| Treatment | 4 | 0.049 n.s. | 134.349 ** | 4.857 ** | 0.036 n.s. |
| Error | 10 | 0.16 | 7.598 | 0.309 | 0.02 |
| C.V. | - | 21.95 | 3.99 | 14.82 | 18.36 |

** Significance at the probability level of 1%, n.s.: not significant at p=5%, SOV: sources of variations, DF: degree of freedom

**Table 2.** Comparison of means of height, fresh and dry weight affected by *A. pellucida* in rice cultivars

| Cultivars | Height | Fresh weight | Dry weight |
|---|---|---|---|
| Hashemi | 71.588±2.105a | 4.975±0.21a | 0.827±0.097ab |
| Ali Kazemi | 70.536±0.74a | 5.196±0.651a | 0.736±0.111ab |
| Sepidroud | 75.273±2.171a | 3.361±0.19b | 0.881±0.0131ab |
| Khazar | 57.606±1.486b | 2.927±0.059b | 0.828±0.042ab |
| Binam | 69.953±0.87a | 2.317±0.078b | 0.601±0.094ab |

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

**Table 3.** Comparison of the reactions of rice cultivars affected by *A. pellucida* with those of the controls.

| Cultivar | Change of height | Change of fresh weight | Change of dry weight |
|---|---|---|---|
| Hashemi | -1.075 ± 0.287a | -0.859 ± 0.179a | -0.077 ± 0.022a |
| Ali Kazemi | -1.57 ± 0.238a | -0.525 ± 0.395ab | -0.138 ± 0.438a |
| Sepidroud | -0716 ± 0.236a | 0.023 ± 0.048b | -0.041 ± 0.027a |
| Khazar | -0.906 ± 0.243a | -0.22 ± 0.034ab | -0.107 ± 0.04a |
| Binam | -1.87 ± 0.573a | -0.439 ± 0185ab | -0.068 ± 0.039a |

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

the reaction of some important local rice cultivars to *Alternaria pellucida* also evaluated.

## Materials and methods

### *Collection and culture of fungal isolates*

Leaves with symptoms of disease were collected from weeds (*Alisma plantago-aquatica, Sagitaria trifolia, E. crus-galli* and *E. oryzicola*) in Guilan province of Iran then were cut into the 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. Petri dishes then incubated at $28^0$C in darkness or light on a 12 hours light/dark photoperiod for 6-15 days. Conidia were single-sporulated and 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 -$20^{\circ}$C (Safari Motlagh, 2010).

### *Identification of fungi*

The grown fungi were isolated and koch's postulates were completed for sample after each collection. Cultures of these fungi were submitted to the Research Plant Pathology Institute of Iran for identification.

### *Pathogenicity test*

#### *Weeds*

This test was laid out 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-$30^0$C, 12 D: 12 L photoperiod and a relative humidity of more than 90%. Before inoculation, all pots were sprayed with distilled water. Inoculation of weeds was performed at 3-4 leaf stage in greenhouse. After all, a spore suspension including $10^6$ *Alternaria pellucida* spore/ml distilled water was sprayed on weeds. In order to increase adsorption, 1% Tween-20 was added to the suspension prior to inoculation. 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 lesion, where as: 0= lesions absent, 1= small, unexpanded lesions, 2= slightly to moderately expanded lesions, 3= large lesions (Zhang et al., 1996). The standard evaluation and Horsfall- Barratt system were applied for *Echinochloa* spp. (Zhang et al., 1996; Bertrand and Gottwald 1997).

Disease rating also calculated as follows:

$$\text{Diseaserating} = \frac{(N_1 \times 1) + (N_2 \times 2) + .... + (Nt \times t)}{(N_1 + N_2 + .... + N_t)}$$

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

#### *Rice*

This experiment was conducted in a complete random design (CRD) with five treatments and three replications. Five rice cultivars including three indigenous (Hashemi, Ali Kazemi and Binam) and two bred cultivars (Khazar and Sepidroud) were evaluated against *A. pellucida*. First, rice seeds germinated and then transferred to the greenhouse inside the 2.5 cm diameter pots filled with farm soil, without any drain. The thinning was performed when the plants reached the 3-4

**Fig 1.** The symptoms of *A. pellucida* on rice (Binam cv.)

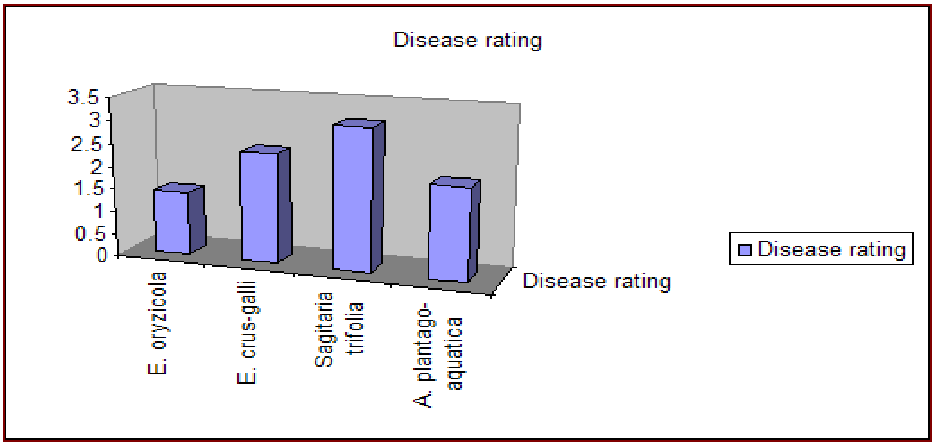


**Fig 2.** Diagram of the comparison of *A. pellucida* mean disease rating in weeds.

**Fig 3.** The symptoms of *A. pellucida* on leaf of *Sagitaria trifolia*

leaf stage. Finally, four shrubs kept in each pot for experiment. Two gram of urea fertilizer was added to the pots. Inoculation was done by a spore suspension of *A. pellucida* containing $10^6$ spore/ ml of distilled water containing 1% Tween-20 as surfactant. Other environmental conditions were similar to those applied for weed. Evaluation was done 7 days after inoculation based on Horsfall-Barrat system. Then, disease ratings were scored (Bertrand and Gottwald 1997). One control was considered for each replication.

***Measuring plant fresh weight, dry weight and height***

Inoculated weeds and rice cultivars along with their controls were transferred from greenhouse to the laboratory. Shrubs were cut from the soil surface and weighed by an electric scale. This was recorded as 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. Each shrub was weighed after drying as dry weight (Ghorbani et al., 2000).

***Data Analysis***

Data analysis was done using SPSS and MSTAT-C packages. In order to compare average values, Duncan test was used. The reaction of rice cultivars and weeds to fungi evaluated by the average value of each fungus-treated rice cultivar and the controls.

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

| SOV | DF | Squares Mean | | | |
|---|---|---|---|---|---|
| | | Disease rating | Height(cm) | Fresh Weight (g) | Dry Weight(g) |
| Treatment | 4 | 1.758* | 780.106** | 102.075** | 4.778** |
| Error | 10 | 0.336 | 0.336 | 0.599 | 0.147 |
| C.V. | - | 24.87 | 1.25 | 6.83 | 20.55 |

**:Significance at the probability level of 1%, *: Significance at the probability level of 5%, SOV: sources of variations

**Table 5.** Comparison of means of the studied traits affected by *A. pellucida* in weeds.

| Weed | Height(cm) | Fresh weight(g) | Dry weight(g) |
|---|---|---|---|
| *E. oryzicola* | 64.306 ± 2.645a | 6.208 ± 0.074c | 0.952 ± 0.037b |
| *E. crus-galli* | 54.610 ± 3.276b | 7.402 ± 0.225c | 1.452 ± 0.139b |
| *Sagitaria trifolia* | 36.466 ± 3.183c | 18.966 ± 0.189a | 3.806 ± 0.301a |
| *A. plantago-aquatica* | 29.266 ± 0.433c | 12.780 ± 0.84b | 1.253 ± 0.29b |

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 *A. pellucida* with those of the controls.

| Weed | Change of Height (cm) | Change of Fresh weight (g) | Change of Dry weight (g) |
|---|---|---|---|
| *E. oryzicola* | -2.09 ± 0.85ab | -0.27 ± 0.057b | -0.429 ± 0.016a |
| *E. crus-galli* | -3.37 ± 1.20ab | -0.094 ± 0.11b | -0.106 ± 0.024a |
| *Sagitaria trifolia* | -6.03 ± 1.58a | -0.15 ± 0.075b | -0.296 ± 0.176a |
| *A. plantago-aquatica* | -1.7 ± 1.12ab | -0.7 ± 0.151a | -0.416 ± 0.165b |

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

**Results and discussion**

Analysis of variance for disease rating showed no significant reaction to *Alternaria pellucida* in rice cultivars (Table 1). However, the fungus was effective on some rice cultivars. Comparisons were done based on visual observations with the size and type of the emerged spots on the leaves on cultivars. Results showed that Binam cv. was more susceptible than others as most of the leaves showed disease symptoms (Fig 1). Conversely, Sepidroud cv. was less affected and revealed a lower disease rating. Taking this finding into account, this fungus was less effective on Sepidroud, so the cultivar was more tolerant having healthier leaves without any disease symptoms. In total, bred cultivars such as Khazar and Sepidroud less affected by the fungus than the indigenous ones. Mousavi (1993) revealed that the effect of fungi isolated from host weeds, in terms of causing diseases in indigenous cultivars, would be specific and important for controlling weeds. Some *Alternaria* species have more antagonistic characteristics and a number of them severely damage crops (Hammed et al., 2002). As a result, crops with higher tolerance thresholds are more important in developing these biological agents. Thus, wild species of crops which show more tolerance could be considered as important sources of tolerance for *Alternaria* species (Hammed et al., 2002). The reaction of rice cultivars to these species, isolated from weeds, has not been studied widely. In wheat and corn, this fungus was more capable of controlling annual mercury and nutsedge weeds, as yet not effective on wheat bred cultivars (Mintez et al., 1992). Analysis of variance for the evaluation of height, fresh and dry weight revealed that, in terms of height and fresh weight, the studied rice cultivars showed a significant reaction, while for dry weight, the reaction was insignificant (Table 1). No significant difference existed between Hashemi, Ali Kazemi, Sepidroud and Binam in terms of their heights (Table 2). For fresh weight, no significant difference was found between Hashemi and Ali Kazemi. Also, no significant difference in fresh weight was observed between Sepidroud, Khazar and Binam. However, these cultivars showed significant differences compared with Hashemi and Ali Kazemi. No significant difference existed between rice cultivars in terms of their dry weights (Table 2). For height, rice cultivars were more affected by *A. pellucida* compared to controls, but they showed no significant difference when compared with one another. With regard to fresh weight, the Hashemi cv. was more affected by the fungus compared to other cultivars and there was no significant difference between Ali Kazemi, Khazar and Binam. In terms of fresh weight, Sepidroud was not affected by the fungus at all. Moreover, there was no significant difference between rice cultivars in terms of dry weight (Table 3). Reduced dry weight could be more influenced by environmental factors. Based on the comparison of the mean values of studied traits in rice cultivars, a reliable performance was achieved. The reduction of fresh and dry weights was not significant when compared to controls. Therefore, these studied cultivars could possibly be used to manage the weed using *Alternaria* species as bio-control agent. Studies conducted to examine the reaction of rice cultivars to stem rot showed that cultivars with less affected traits have more resistant genes (Figoni et al., 1985). Analysis of variance showed significant effect of the fungus on *Alisma plantago-aquatica, Sagitaria trifolia* and *Echinochloa* spp. (Table 4). It was observed that there is no significant difference of disease ratings between *Sagitaria trifolia* and *Echinochloa crus-galli*. Te fungus only caused some more symptoms on *Sagitaria trifolia* than *Echinochloa crus-galli* indicating more susceptibility of *Sagitaria trifolia*. Comparison of the disease rating between *E. oryzicola* and *Alisma plantago-aquatica* revealed that the latter weed, in some extent, was more affected by *Alternaria pellucida* (Fig 2 and 3). Among all *Alternaria* species, *Alternaria alternata* is one of the modest for the biological control of weeds. But, *Alternaria pellucida* has been applied for controlling weeds of ornamental and apartment plants yet (Boland, 2005).

*Alternaria alternata* is also usually used as a biological agent in most of crops. Its disease rating depends on the type of crop and the source weeds which is isolated from (Johanson et al., 2003). Also, *Alternaria* species enjoy a high biodiversity, thus the reaction of crops to effect of fungus might be different (Johanson et al., 2003). Based on table of variance analysis, effect of this fungus on all the three traits such as height, fresh and dry weight of *Alisma plantago-aquatica, Sagitaria trifolia* and *Echinochloa* spp. was significant (Table 4). Only two *Echinochloa* species showed a significant difference in terms of their heights. Regarding fresh weight, there was no significant difference between these two species (Table 4). Comparison of weeds with controls showed that effect of fungus on height of *S. trifolia* was remarkably significant (Table 6). *Echinochloa* spp. and *A. plantago-aquatica* did not show any significant difference in height and were ranked after *Sagitaria trifolia*, respectively. In *A. plantago-aquatica,* the fresh weight was more affected than other weeds. Also, *Echinochloa* spp. and *S. trifolia* were not significantly different from one another (Table 6). In total, the fresh weight in *A. plantago aquatica* was more affected by the fungus than the other two traits. This indicates that biodiversity, existing host range, effect of fungus and environmental conditions among the weeds lead to different reactions to each of the studied traits. For dry weight of weeds, *Echinochloa* spp. and *S. trifolia* were not significantly different. Moreover, all of them showed a reduction in dry weight, affected by the fungus. However, it did not have any effect on *A. plantago aquatica'*s dry weight (Table 6). Studies conducted by Mintez et al., (1992) revealed that the disease rating caused by *Alternaria alternata* on *Amaranthus retroflexus* during the 3-4 leaf stage was more than in the 5-6 leaf stage. Also, the reduction of fresh and dry weights was more considerable during the 3-4 leaf stage. As a result, the growth stage of the weed affected its response to the fungus (Mintez et al., 1992). Masangkay et al. (1999) showed that the effect of *Alternaria alternata* on the dry weight of *Sphenoclea zeylanica* is significantly correlated with concentrations of spore suspension (Masangkay et al., 1999). A study of biological control of *Amaranthus retroflexus* revealed that *Alternaria alternata* had fewer effects on the weed fresh and dry weights. Thus, the effect of the fungus on height was more conspicuous relative to the other traits (Ghorbani et al., 2006). Results obtained by Ghorbani et al. (2006) were consistent with the present research, in terms of the effect of *A. pellucida* on height. Similar conditions were observed with regards to dry and fresh weights of *S. trifolia* and *A. plantago-aquatica* and showed a significant difference, in terms of the two traits when compared. However, in the comparison of fresh and dry weights of *E. oryzicola* and *E. crus-galli*, the performance was completely different. This might be an indication that fungus is more effective on the fresh weight of *Echinochloa* species in a particular environmental condition (Table 5). The weeds had suitable conditions in terms of the dry weight as no considerable reduction was observed. Other studies showed that *Fusarium* sp. was more effective on the weeds than *Alternaria* sp. (Turkington et al., 1980). The effect of *Fusarium* sp. on the dry weight and root growth of these weeds was more than *Alternaria* sp. It was suggested that the mutation in fungi populations can increase the biodiversity and activity of fungus and lead to a better bio-control agent (Turkington et al., 1980).

**Conclusions**

Consideration of the fact that *A. pellucida* causes higher disease ratings in *Sagitaria trifolia*, *Echinochloa* spp. and *Alisma plantago-aquatica* compared to rice cultivars, it can be a potential agent for the biological control of the weeds.

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