Plant Omics Journal POJ

POJ 3(6):172-176 (2010) ISSN:1836-3644

Study of *Alternaria pellucida* as a promising mycoherbicide for controlling Arrowhead (*Sagitaria trifolia*) in paddy fields

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Abstract

Arrowhead (*Sagitaria trifolia*) is one of the most important weeds in paddy fields of Iran. In this research, *Alternaria pellucida* was isolated from *Sagitaria trifolia* and evaluated as a possible biological agent for controlling arrowhead. In this investigation, reactions of three indigenous (Hashemi, Ali Kazemi and Binam), two bred cultivars (Sepidroud and Khazar) and arrowhead to *Alternaria pellucida* were studied in a completely randomized design with three replications in a greenhouse. *Alternaria pellucida* was inoculated on arrowhead using a spore suspension consisting of 10^6spore/ml of distilled water and 1% Tween-20 at 3-4 leaf stage of cultivars. Results indicated that the rate of infection caused by *A. pellucida* was higher in arrowhead compared to rice cultivars. Among them, bred cultivars (Khazar and Sepidroud) showed more tolerance than indigenous cultivars and less affected by the fungus. *A. pellucida* reduced the height, fresh weight and dry weight of some rice cultivars. Effect of *A. pellucida* on the height and fresh weight were significantly higher than dry weight of rice cultivars. However, it did not affect fresh and dry weight, but altered the height of arrowhead weed. We suggest that *A. pellucida* can be considered as a promising mycoherbicide to control *Sagitaria trifolia*.

Keywords: *Sagitaria trifolia, Alternaria pellucida,* Rice, Biological Control.
Abbreviations: PDA_potato dextrose agar; WA_water agar

Introduction

Weeds reduce product yield due to absorbing nutrients, occupying space and competition with a main crop (Zoschke, 1990). Weeds have been evaluated as important limiting factor for rice plantation. Their damage with considering different planting conditions can be reached up to 46-67% (Lindquist and Kropff, 1988). One of the ways to control population of weed is direct control using chemical herbicides (Mohammadsharifi, 2005). However, the increase of using these chemicals, known as herbicide, has caused public concerns regarding to environmental pollution and most importantly, resistance to herbicides (Holt and Lebaron, 1998). The major cause of these problems is the improper use of herbicides (Williams, 1992). Extra usage of herbicides, in terms of types and amounts, can lead to damage on rice seedlings (Mohammadsharifi, 2005). Contamination of soil and water of rice fields and its adverse effects on the next cultivation results a severe damages to rice ecosystems, mainly due to the penetration of chemical herbicides in water soil and water of paddy fields. Hence, biological control by biomicro-organisms such as fungi, which are referred to as mycoherbicides, is a great chance in organic agriculture (Moein and Pourkashani, 1992). There is very limited information about application of biological controlling factors in paddy fields. Inoculation of barnyard grass by *Fusarium anthophilum* in rice paddy field has shown a high disease rating in the 2 leaf stage of weed (Montazeri et al., 2006). Collego (*Colletotrichum gloeosporioides*) can be mentioned as one of the most important fungal herbicides which is currently being used in rice plantation of Arkansas in the United States (Fisher, 1996). Studies showed that *Plectosporium tabacinum* is effective in the early growth stages of arrowhead by preventing the expansion of its rhizomes (Zhang et al., 2002). It was reported that *Alternaria eichhonia* does not have much effect on contolling *Alisma plantago-aquatica* (Martinez and Charudathan, 1998). Other studies revealed that *Plectosporium tabacinum* and *Rhynchosporium alismatic* had the highest disease ratings in *Alisma plantago-aquatica* and *Sagitaria trifolia* (Ash et al., 2005). Among the various known species of *Alternaria, Alternaria alternata* has proved to be effective in the biological control of different weeds in rice paddies and wheat fields (Abbasher, 2000). *Alternaria helianthi* is capable of controlling *Xanthium* spp. (the third most important crop weed in the world) in greenhouse conditions (Kouchaki et al., 2001; Bassi and Quimby, 1985). Another fungus, *Alternaria alternata* was examined for the biological control of *Amaranthus retroflexus*. The disease rating of this fungus increased with the increase of spore concentration and humidity (Ghorbani et al., 2000). *Alternaria cassiae* is among species which could cause typical lesions and even death in weeds such as *Cassia obtusifolia*, *Cassia occidentalis* and *Crotalaria spectabilis* (Walker, 1983). It has been confirmed that *Alternaria alternata* f. sp. *sphenocleae* could result in leaf blight and finally, the death of *Sphenocleae zeylanica* (Masangkay et al., 1999).

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

Arrowhead (*Sagitaria trifolia)* is one of the most important paddy fields weeds in Iran whose population in rice paddies of Guilan province is ever-increasing. The main reason for this population growth could be the difficulty of its control due to its rhizomes and also, its resistance to chemical herbicides which are typically used in this area (Rezvani et al., 2002). Generally, one of the main elements of developing biological control is the existence of cultivars which are resistant to biological factors (Burdon and Leather, 1990). Producing crops with genetic resistance to pathogens has been one of the greatest achievements of breeders in recent years (Blum, 1998) ensuring that no damage is made to a crop by the applied biological factor, which is the important prerequisites of the biological control (Moein and Pourkashani, 1992). Breeders usually expose their plants to pathogens, by natural or artificial induction, with the purpose of the distinction between sensitive and resistant genotypes, for later selection (Johanson and Jellis, 1993).

In this study, reaction of *Sagitaria trifolia* to application of *Alternaria pellucida* as bio-controlling agent was investigated. Also, the pathogenicity reactions of some rice cultivars were evaluated with the purpose of investigating the capability of *Alternaria pellucida* as a probable mycoherbicide.

Materials and methods

Collection and culture of fungal isolates

Leaves with symptoms of the disease *Sagitaria trifolia* were collected from paddy fields in Guilan province of Iran. Leaves cut to appropriate sizes and transferred to the laboratory. Leaf samples were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and grew on PDA culture medium in petri dishes at 28^0C for 3-4 days. WA medium was used for sporulation. The petri dishes containing leaf pieces were incubated at 28 ^{0}C in darkness or light on a 12 hours light/dark photoperiod for 6-10 days (Safari Motlagh and Kaviani, 2008). Conidia were single-sporulated and then, pure colonies were placed onto sterilized filter paper. Then, paper filter pieces were incubated in sterilized vials in freezer at -20^0C (Safari Motlagh and Kaviani, 2008).

Study and identification of fungi

Grown fungi 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 identifications (Fig 1).

Pathogenicity test

This test carried out in a completely randomized design (CRD) with one treatment and 3 replications. Weeds were planted in field soil inside plastic pots with 2.5 cm diameter.

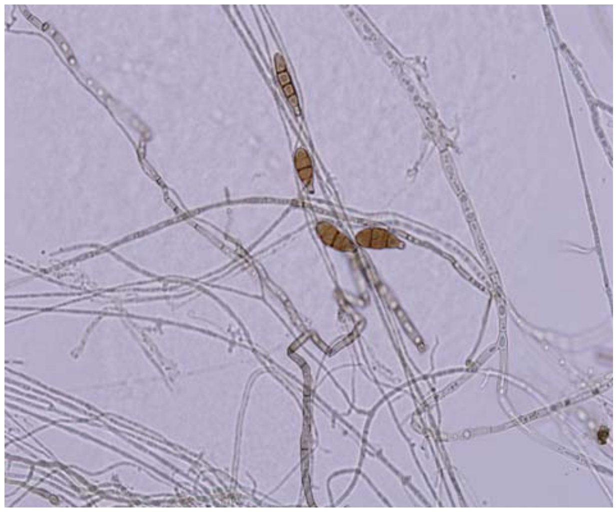

Fig 1. Conidia of *A. pellucida* (×460)

For each treatment, one control was allocated (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%. Inoculation of *Sagitaria trifolia* was performed at its 3-4 leaf stage in greenhouse. To do so, a spore suspension including 10^6 *A. pellucida* spore/ml of distilled water was used. In order to increase adsorption, 1% of 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 of symptoms was done 7 days after inoculation based on lesion type and size in reaction to inoculation: 0= lesions absent, 1= unexpanded small 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 completely randomized design with three replications against inoculation with *A. pellucida*. First, rice seeds germinated and then transferred into 2.5 cm diameter pots in greenhouse without drainage. They were planted in the farm soil. When the plants reached their 3-4 leaf stage, thinning was performed. 2g of urea fertilizer was added to the pots containing 4 plants each. At this stage, inoculation was done by a spore suspension of *A. pellucida* containing 10^6 spore/ml of distilled water including 1% Tween-20. Other environmental conditions were similar to those applied to weed. Evaluation of symptoms 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.

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%.

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 from the soil surface and weighed by an electronic scale. This weight was recorded as fresh weight. After separately measuring their height, each shrub was placed inside a paper bag and then placed in an oven at 80-90^{0}C for 48 hours. When the bags were taken out of the oven, each shrub was weighed as dry weight (Ghorbani et al., 2000).

Data Analysis

Data analysis was done using SPSS and MSTAT-S package. 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.

Results and Discussion

Variance analysis of disease rating did not show any significant reaction to *A. pellucida* in rice cultivars (Table 1). However, based on the sizes and types of the spots appeared on cultivars, Sepidroud a bred cultivar, was less affected by the fungus compared with others and its disease rating was lower (Fig 2). On the other hand, in comparison with other cultivars, Binam showed the least tolerance and the disease rating caused by the fungus was significantly higher (Fig 2). Following these two cultivars as most and least tolerant, Khazar, Hashemi and Ali Kazemi, ranked as more tolerants, respectively (Fig 2). Interestingly, bred cultivars were less affected by *A. pellucida* compared to indigenous ones and showed much tolerance. Studies conducted by Mintez et al. (1992) revealed that indigenous rice cultivars are considered as more suitable hosts for antagonist fungi. Different species of *Alternaria* have shown high potential for controlling *Cyperus difformis* in wheat and corn fields; however, this fungus did not have any effect on wheat bred cultivars (Mintez et al., 1992). Moreover, bred rice cultivars are more resistant to major rice diseases such as sheath blight and blast. Studies have shown that resistant genes in bred cultivars are more than those of the indigenous ones (Frankel, 1997). In order to have a suitable resistance, competition between fungus races and plant tolerance should be taken into account (Eskes and Thoma-Braghini, 1982).

One of the major problems of modifying resistance to pathogen is the complexity of tolerance mechanism caused by wide range of organisms available in practice. Each of these organisms has various species which may be genetically diverse like plant species (Kimber, 1983).

Variance analysis of height, fresh weight and dry weight indicated that height and fresh weight of rice cultivars showed significant reactions but for dry weight, the reaction was not significant (Table 1). Each studied trait, in term of the symptoms, caused by the fungus in a treated environment as follows: for height, there was no significant difference between Hashemi, Ali Kazemi, Sepidroud and Binam and it was only Khazar which showed a significant difference. For fresh weight, no significant difference was observed between Hashemi and Ali Kazemi cultivars. Also, Sepidroud, Khazar and Binam did not show any significant difference relative to this trait. However, compared with Hashemi and Ali Kazemi, these cultivars exhibited a significant difference in term of fresh weight. For dry weight, there was no significant difference between the rice cultivars (Table 2). The evaluation of dry weight, fresh weight and height of each treated rice cultivar compared to controls revealed that, in term of height, the studied cultivars were more affected by *A. pellucida*. However, when compared with each other, they did not have any significant difference (Table 3). With regard to fresh weight, Hashemi more affected by the fungus compared to other cultivars and its fresh weight was decreased, but no significant difference was found between Ali Kazemi, Khazar and Binam (Table 3). The fungus did not have any significant effect on the fresh weight of Sepidroud (Table 3). For dry weight, there was no significant difference between the studied rice cultivars (Table 3). Results showed that the effect of this fungus on fresh and dry weights of the studied cultivars compared with their controls was little and this small effect was even less in the bred rice cultivars.

The reaction of arrowhead to *A. pellucida* and disease rating caused by this fungus was significantly higher than studied rice cultivars (Fig 2). The Chi-square test revealed that weed had a significant reaction in term of height, although it was not significant for dry and fresh weights (Table 4).

Recent studies have suggested that *A. pellucida* could be potentially used for controlling the weeds of ornamental and apartment plants (Boland, 2005). Furthermore, it was found

Table 4. Chi-square values of height, fresh and dry weight affect by *A. pellucida* in weed

Weed	Height	Fresh weight	Dry weight
Sagitaria trifolia	2. 91*	0.005 n.s.	0.097 n.s.

n.s.: not significant at p=5%

*****: Significance at the probability level of 5%.

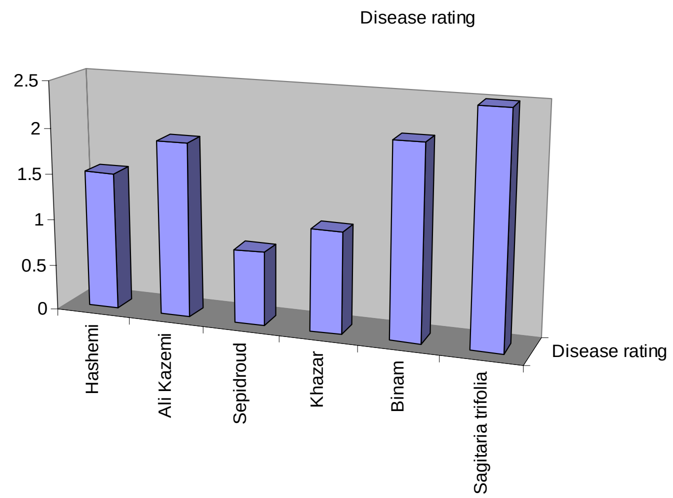

Fig 2. Diagram of the comparison of *A. pellucida* mean disease rating in rice cultivars and *Sagitaria trifolia*

that the effect of *Alternaria alternata* on the dry weight of *Zeylanica sphenoclea* is related to different concentrations of spore suspensions (Masangkay et al., 1999). Studies also revealed that *Alternaria alternata* has little effects on the fresh and dry weights of *Amaranthus retroflexus*, but its effect on weed height was higher compared to other two traits (Ghorbani et al., 2006). These results are consistent with our results regarding with the effect of *A. pellucida* on arrowhead.

The production of resistant cultivars for controlling plant diseases and reducing costs of chemicals, with consideration of their long-term effect on nature, would be of more importance in the future. This goal could be achieved by collaboration of botanists, plant breeders and phytopathologists (Frankel, 1997). In order to introduce the effective biological agent existing of resistant crops is necessary. Study of reactions of cultivars must also be taken into more consideration when new cultivars are developed. These cultivars must be resistant to antagonist agents so finally, weeds could be controlled (Fisher, 1996).

Conclusions

A high disease rating was caused by *A. pellucida* in arrowhead, based on typical scales of disease rating index and high differences among scales. On the other hand, reactions of the studied rice cultivars all taken into account. The Iranian bred cultivar Sepidroud were more tolerant to fungus infection. *A. pellucida* could be regarded as a promising mycoherbicide for controlling *Sagitaria trifolia* in rice paddies.

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