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Comparison of pathogenicity of *Alternaria pellucida* and *Curvularia lunata* on weed *Echinochloa* species

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Abstract

Echinochloa spp. are the most important weeds in rice fields. In this research *Curvularia lunata* and *Alternaria pellucida* were isolated from these weeds and their pathogenicity effects were compared on these weeds and five rice cultivars in a completely random design with three replications in greenhouse conditions. Fungi were inoculated on weeds and rice cultivars, using spore suspension consisting of 10^6 spore ml^{-1} of distilled water. Results indicated significant effect of *Curvularia lunata* and *Alternaria pellucida* on *Echinochloa oryzicola* and *E. crus-galli*. In the present study, effect of *C. lunata* on fresh weight, dry weight and height of *Echinochloa* species based on variance analysis table, a significant reaction was observed for height and fresh weight, but for dry weight reaction was not significant. The effect of *A. pellucida* on fresh weight, dry weight and height of *Echinochloa* species based on variance analysis table, a significant reaction was observed for all the three traits. Also, rice cultivars did not show any significant reaction to *C. lunata* and *A. pellucida*. The results showed that in comparison between effect of *Curvularia lunata* and *Alternaria pellucida* on *Echinochloa* spp., disease rating caused by *A. pellucida* on *E. oryzicola* and *E. crus-galli* was more than disease rating caused by *C. lunata* and these species of weed were more susceptible to *A. pellucida*. However, *A. alternata* can be considered as a better promising bioherbicide to control *Echinochloa* spp.

Key words

Alternaria pellucida, *Curvularia lunata*, *Echinochlooa* spp., Pathogenicity

Introduction

Echinochloa spp. are annual weed native to Asia and presently can be found throughout the world (Safari Motlagh, 2011). The most widespread and economically important member of genus is annual *E. crus-galli* (Safari Motlagh, 2011). This weed is noxious in rice field and has been found to reduce rice yield by 40% in direct seeded rice in Malaysia and about 21% annually in Indonesia (Tjitrosemito, 1994). It belongs to tribe Paniceae, family Gramineae. *Echinochloa oryzicola* Vasing.(= *Echinochloa phyllopogon* Stapf ex Kessenko) is another obligate weed with an elaborated survival strategy in the rice fields (Yamasue, 2001). The genus may include 20 to 50 species that are widely represented in tropical and warm temperate regions of the world (Martines *et al.,* 1999). With an increase in direct seeding rice practices, *Echinochloa* spp. are becoming a greater problem in rice fields (Azmi and Baki, 2002). In addition, continuous chemical control of barnyard grass in rice plant has caused herbicide resistance to *Echinochloa* spp. (Tasrif *et al.,* 2004).

Hand weeding and hoeing are common practices of controlling this weed in developing countries of the world; but this method is quite expensive and time consuming, thus ineffective as new weed seeds germinate after every hoeing and reinfest the crop, thus using maximum soil nutrients. Moreover, hoeing is not possible during rainy season and labour shortage due to paddy transplanting at that time further accentuates the problem (Aneja *et al*., 2000). It is relevant here to define the two broad approaches to biological control: classical and inundative. Inundative control, in case of fungal pathogens, involves mass production, formulation and application of a product (mycoinsecticide, mycoherbicide) that can be marketed and employed in much the same way as conventional chemical pesticide (Evans *et al.,*

1999). *Curvularia* and *Alternaria* species as facultative parasites are among microorganisms being used for controlling weeds (Safari Motlagh, 2011).

In the present study, two different indigenous fungal species were isolated from naturally infected *E. crus-galli* and *E. oryzicola* in Iran. To select the best candidate for further development as biological control agent for *Echinochloa* spp. in rice, study was carried out to determine the pathogenicity of these fungi on *Echinochloa* spp. and compare the disease raring of these fungi to *Echinochloa* species.

Materials and Methods

Culture and identification of fungal isolates : Diseased leaves of *Echinochloa crus-galli* and *E. oryzicola* were sampled from five locations in each field from Guilan province, Iran. Leaves were transferred to the laboratory and then fungi were isolated from diseased samples. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on potato dextrose agar in Petri dishes at 27º-30 ºC for 2-3 days. PDA medium was used for sporulation. Then Petri dishes containing media were incubated at 27 ºC in dark or artificial light supplied by fluorescent light on a 12 hr light/dark photoperiod for 15-25 days. To avoid bacterial contamination, sulfate streptomycin antibiotic was used. Conidia were single- sporulated. Monoconidial isolates of the recovered fungi were maintained on half- strength potato dextrose agar slants in test tubes as stock cultures.

Fungi, which had grown were isolated and were completed for most sample after each collection. Cultures of these fungi were submitted to the Research Plant Pathology Institute of Iran for identification.

Pathogenicity tests

***Echinochloa* spp. :** This experiment was done as complete random design (CRD) with one treatment and 3 replications. *Echinochloa* spp. were planted in plastic pots 2.5 cm in diameter containing farm soil. For each treatment, one control was assigned. Pots were placed at 25º-30ºC, 12 hr D: 12 L photoperiod and a relative humidity of more than 90%. Inoculation of weeds was performed at 3-4 leaf stage in greenhouse. To do so, a spore suspension including 10^6 *Curvularia lunata* and *Alternaria pellucida* spore/ml distilled water, was used separately. 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%, the treated plants were immediately covered with plastic bags for 48 hrs. Evaluation based on lesion type and size in reaction to inoculation was done 7 days after inoculation: 0= lesions absent, 1= small, unexpanded lesions, 2= slight to moderate expanded lesions, 3= large lesions (Zhang *et al.,* 1996). Therefore, standard evaluation system and Horsfall- Barratt system were applied for *Echinochloa* spp.

Rice : This experiment was done as complete random design (CRD) with five treatment and 3 replications. Five rice cultivars including 3 indigenous (Hashemi, Ali Kazemi and Binam) and 2 bred cultivars (Khazar and Sepidroud) were evaluated against inoculation with *C. lunata* and *A. pellucida*. In order to do so, rice seeds were first germinated and after being transferred to greenhouse inside pots, 2.5 cm in diameter without any drain, they were planted in farm soil. When plants reached 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 *C. lunata* and *A. pellucida* containing 10^6 spore ml^{-1} of distilled water with 1% Tween-20. Other environmental conditions were similar to those of the weed. Evaluation based on Horsfall-Barrat system was done 7 days after inoculation. Then, disease ratings were calculated. It is noteworthy that in both experiments, one control was considered for each replication.

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 laboratory. Then, shrubs were cut on soil surface and weighed by an electric scale. This weight was recorded as fresh weight. After separately measuring their height, each shrub was placed inside a paper bag and incubated in an oven at 80º-90 ºC for 48 hrs. When bags were taken out of the oven, each shrub was weighed, and this weight was considered as its dry weight.

Statistical analysis : Data analysis was done using SAS, SPSS and MSTAT-C softwares. In order to compare average values, Duncan test was applied.

Results and Discussion

Analysis of variance showed significant effect of *Curvularia lunata* on *Echinochloa oryzicola* and *E. crus-galli*. A significant difference of disease ratings between *E. oryzicola* and *E. crus-galli* was observed (Table 1). Comparison of the mean disease rating between *E. oryzicola* and *E. crus-galli* revealed that the first weed was more affected by *C. lunata* (Table 1). In the present study effect of *C. lunata* on fresh weight, dry weight and height of *Echinochloa* species, based on variance analysis table, showed significant reaction for height and fresh weight, but for dry weight the reaction was not significant. Also, based on comparison of the traits, mean values between *Echinochloa* species showed significant difference in terms of fresh weight, but not in dry weight and height (Table 1). In this regard, *E. crus-galli* showed more reduction (Table 1).

According to the results obtained in the present study, *E. crus-galli* was less affected by *C. lunata* and *E. oryzicola* was more susceptible to this fungus. Analysis of variance showed

significant effect of *Alternaria pellucida* on *Echinochloa oryzicola* and *E. crus-galli*. Comparison of mean disease rating between *E. oryzicola* and *E. crus-galli* showed significant difference and revealed that latter weed was more affected by *A. pellucida* (Table 1). The effect of *A. pellucida* on fresh weight, dry weight and height of *Echinochloa* species, based on variance analysis table, showed significant reaction for all the three traits.

Also, based on the comparison of traits, mean values, between *Echinochloa* species showed a significant difference in terms of fresh weight, but not in dry weight and height (Table 1). In this regard, *E. crus-galli* showed more reduction and was more affected by *A. pellucida* (Table 1). The results showed that in comparison between effect of *Curvularia lunata* and *Alternaria pellucida* on *Echinochloa* spp., disease rating caused by *pellucida* on *E. oryzicola* and *E. crus-galli* was more than disease rating caused by *C. lunata* and these species of weed were more susceptible to *A. pellucida*. The effect of *A. pellucida* in height reduction of *E. oryzicola* was more than other fungus. However this fungus had less effect on height of *E. crus-galli*. In comparison to the effect of fungi on fresh weight, *C. lunata* had more effect on *Echinochloa* species. Finally, no significant difference was observed between these fungi in terms of dry weight.

Results from variance analysis of the disease rating revealed that the studied rice cultivars did not show any significant reaction to *Curvularia lunata*. A significant reaction was observed in dry weight, fresh weight and height of the said rice cultivars (Table 2). Evaluation of the mean values of the traits in the studied rice cultivars revealed that regarding height, there was no significant difference between Hashemi, Ali Kazemi, Sepidroud and Binam and it was only Khazar that showed such difference (Table 2). In terms of fresh weight, there was no significant difference between Sepidroud, Khazar and Binam, but Ali Kazemi and Hashemi showed less reduction in their fresh weights (Table 2). For dry weight, the reactions of studied cultivars were similar to those of fresh weight (Table 2). In the indigenous Ali Kazemi cultivar, decrease in fresh weight was less than Hashemi, yet when compared with Sepidroud and Khazar, it was more. In evaluation of this trait, fresh weight decrease was not observed in Binam.

Based on the variance analysis table presented for the evaluation of disease rating, the studied rice cultivars did not show any significant reaction to *A. pellucida*. Variance analysis of the evaluation of height, fresh weight and dry weight indicated that regarding height and fresh weight, rice cultivars showed significant reactions and for dry weight, the reaction was not significant.

No significant difference existed between Hashemi, Ali Kazemi, Sepidroud and Binam, in terms of their heights (Table 2).

Table 1 : Comparison of means of the studied traits affected by *Curvularia lunata* and *Alternaria pellucida* in *Echinochloa* species

Dry weight	Fresh weight	Height	Disease rating	Weeds
		Affected by *Curvularia lunata*		
0.12 ± 0.016a	0.27± 0.062b	2.09 ± 0.85a	2.30 ± 0.42a	*E. oryzicola*
0.10 ± 0.024a	0.94 ± 0.011a	3.37 ± 1.20a	1.37 ± 0.13b	*E. crus-galli*
		Affected by *Alternaria pellucida*		
0.61 ± 0.006a	0.12± 0.054b	3.29 ± 1.37a	2.87 ± 0.02b	*E. oryzicola*
0.08 ± 0.045a	0.29 ± 0.028a	1.89 ± 0.32a	3.32 ± 0.16a	*E. crus-galli*

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

Table 2 : Comparison of means of the studied traits affected by *Curvularia lunata* and *Alternaria pellucida* in rice cultivars.

Cultivars	Height	Fresh weight	Dry weight
	Affected by *Curvularia lunata*		
Hashemi	69.196±1.089a	4.818±0.258b	2.007±0.043ab
AliKazemi	72.416±1.044a	5.817±01.425a	4.182±0.0296a
Sepidroud	72.333±2.103a	3.309±0.11c	0.678±0.201c
Khazar	58.123±1.200b	3.124±0.186c	0.732±0.072c
Binam	68.333±1.622a	2.584±0.163c	0.672±0.059c
	Affected by *Alternaria pellucida*		
Hashemi	71.588±2.105a	4.975±0.21a	0.827±0.097ab
AliKazemi	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%

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 as compared with Hashemi and Ali Kazemi. No significant difference existed between rice cultivars in terms of their dry weights (Table 2). The responses of *Echinochloa* species and rice cultivars to these two fungi were different. *Curvularia lunata* and *Alternaria pellucida* were pathogenic to two *Echinochloa* species but not to rice cultivars.

Differences on the effect of the fungus as a biological control agent depend on the environmental conditions of geographical location, especially humidity and temperature (Huang *et al.,* 2005). For instance, *Exserohilum monoceras* that was isolated from *Echinochloa* in some farms in Philippines, was effective on the weeds of the region.

Furthermore, reactions of weeds to fungi isolated from different hosts might be different. For example, it was found that *Curvularia oryzae* isolated from *Cyperus difformis*, host range of different weeds was effective in their response to the fungus in terms of traits such as fresh weight and stem length. *Curvularia oryzae* reduced the height of *Cyperus difformis*, but it did not affect fresh weight (de Luna *et al.,* 2002). Among all *Alternaria* species, *Alternaria alternata* was modest for biological control of weeds. But, *Alternaria pellucida* was applied for controlling weeds of ornamental and apartment plants (Boland, 2005). Also, *Alternaria* species enjoy high biodiversity, thus the reaction of crops to the effect of fungus might be different (Johanson *et al.,* 2003). Masangkay *et al.* (1999) showed the effect of *Alternaria alternata* on dry weight of *Sphenoclea zeylanica* was significantly correlated with concentrations of spore suspension. A study on biological control of *Amaranthus retroflexus* revealed that *Alternaria alternata* had fewer effects on fresh and dry weight of weeds. Thus, the effect of fungus on height was more conspicuous relative to other traits (Ghorbani *et al.,* 2006).

Results from the other research indicated two isolates of *Curvularia tuberculata* and one isolate of *Curvularia oryzae* had potential to control *Cyperus difformis*, *Cyperus iria* and *Fimbristylis miliacea* in rice. Cross pathogenicity of three *Curvularia* isolates to the other three sedge weeds was demonstrated. However, *Cyperus rotundus* was resistant to infection. Most of the rice seedling varieties included in the present study showed resistance to *C. tuberculata* and *C. oryzae* (de Luna *et al.,* 2002). *Alternaria eichorniae was* used to control water hyacinth (*Eichhornia crassipes*) (Hoagland, 2001). While, *Alternaria macrospora*, was used to control spurred anoda (*Anoda cristata*) (Upadhyaya and Blackshaw, 2007). *Curvularia* sp. used to control Itchgrass (*Rottboellia cochinchinensis*) (Hoagland, 2001).

Alternaria alternata was applied as a bioherbicide for *Amaranthus retrofexus*, to kill this weed at 2–4-leaf stage. 4–5 and 6–7-leaf stages showed significant variability as these plants possess well-developed outer cuticles and are more hard (Juraimi *et al.,* 2006).

Curvularia species, as facultative parasites are among microorganisms used for controlling weeds (de Luna *et al.,* 2002). In South American countries, *Curvularia lunata* and *Phyllachora* sp. have been identified as leaf spot-causing factors in *Hymenachne amplexicaulis* (Rudge) (Monterio *et al.,* 2003). *Bipolaris, Curvularia, Drechslera* and *Exserohilum* species, have been reported to cause lesions on leaves of *Lolium multiflorum* and *Cynodon dactylon* (Pratt, 2006).

In an other study, *Curvularia lunata* was reported as a probable agent for biological control of *Alisma plantago-aquatica*, *Echinochloa* spp. and *Sagitaria trifolia* (Safari Motlagh, 2011).

Considering the fact that *A. pellucida* causes higher disease ratings in *Echinochloa* spp. as compared to *C. lunata*, it can act as a potential agent for biological control of weeds.

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