Evaluation of *Curvularia lunata* as an Biological Control Agent in Major Weeds of Rice Paddies

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Abstract: Common water-plantain (*Alisma plantago-aquatica* L.), arrowhead (*Sagitaria trifolia* L.) and *Echinochloa* spp. (L.) are among the most important damaging weeds of rice paddies. In this research, *Curvularia lunata* (Waker) Boedijn was isolated from the said weeds. Then, its effect in different growth stages, i.e. seed, 2-3 leaf stage (seedling) and also in greenhouse conditions was examined in *Alisma plantago-aquatica*, *Sagitaria trifolia*, *Echinochloa* spp., and five rice cultivars including 2 bred (Sepidroud and Khazar) and 3 indigenous (Ali Kazemi, Hashemi and Binam) ones in a totally random design with three replications. To do so, pure fungal colonies and a spore suspension containing 10^6 conidia/ml distilled water were used. The disease rating caused by this fungus in the 2-3 leaf stage (seedlings) of the said weeds was more than that in the rice cultivars. Also, the fungus decreased the germination of the weeds seeds. Results showed that in the evaluation of the disease rating, the studied rice cultivars showed no significant reaction to greenhouse conditions while weeds' reactions were significant. The greatest effect of *C. lunata* was on *Alisma plantago-aquatica*. The evaluation of fresh weight, dry weight and height of the said weeds and rice cultivars indicated that the above-mentioned fungus could affect these traits in weeds and rice cultivars and would reduce them. Hence, *Curvularia lunata* can be considered as a probable agent for the biological control of *Alisma plantago-aquatica*, *Echinochloa* spp., and *Sagitaria trifolia* provided that modification of rice cultivars is done with useful traits.
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Keywords: *Alisma plantago-aquatica*; biological control; *Curvularia lunata*; *Echinochloa* spp. ;*Sagitaria trifolia.*

1. Introduction

Rice (*Oryza sativa*) plays a major role in the nutrition of the people around the world and after wheat is the most important agricultural product (Yamaguchi et al., 2008). There are various factors that reduce rice production, the most important of which are pests, diseases and weeds (Yamaguchi et al., 2008).

In terms of nutrients that exist in rice paddies, these weeds are some strong competitors of rice which prevent adequate light to reach it (Anonymous, 2002). Moreover, they can be suitable hosts for many rice pests and diseases as well and if necessary controlling measures won't be taken, the resulting damages would reach up to 90% (Anonymous, 2002). The importance of weeds in rice plantation is known to be much more than in other cultivations, in a way that in the worst growth conditions of weeds, their damages have been stated to be 25% (Lindquist and Kropff, 1998). About 350 species of 150 genus and 60 families have been reported as rice weeds throughout the world (Hill et al., 1990). Using herbicides is considered as one of the best methods for controlling weeds; however, improper and unduly use of these chemicals along with the resulting pollution in the environment have limited their use and the risk of resistance to pesticides should be added to the previous issues (Holt and Lebaron, 1998). Because of this issue, using other controlling methods such as applying natural weed-controlling microorganisms known as biological controls or bioherbicides has become necessary (Rashed Mohasel et al., 2001). Applying mycoherbicides is the most essential measure in controlling weeds with biological backgrounds, particularly in the sustainable agriculture because they target ecosystem much less than controlling factors such as herbicides (Charudattan, 1993). In addition, they perform quite selectively and cause minimum damages to crops (Charudattan, 1993). *Curvularia* species as facultative parasites are among microorganisms being 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).

Also, *Bipolaris, Curvularia, Drechslera* and *Exserohilum* species, as fungi which cause lesions on the leaves of *Lolium multiflorum* (L.) and *Cynodon dactylon* (L.) were evaluated (Pratt, 2006). Hence, isolates from different *Curvularia* species in *Cyperaceae* were collected and evaluated as probable biocontrol agents of weeds in rice paddies among which *Curvularia tuberculata* was quite effective in

controlling *Cyperus difformis* (L.), *C. iria* (L.) and *Fimbristylis miliacea* (L.) (de Luna et al., 2002).

The first isolation of *Curvularia lunata* from *Lolium perenne* (L.) was reported in 2007 (Goldring, 2007). Also, six pathogenic fungal species were isolated from *Echinochloa* spp. (L.) and were evaluated as controlling agents of this weed in rice that among these fungi, *Curvularia lunata* var. *aeria* and *Exserohilum oryzae* were pathogenic in rice and *Echinochloa* species (Zhang et al., 1996), while *Curvularia geniculata* was only pathogenic in *Echinochloa* spp., but not in rice (Zhang et al., 1996).

In order to modify fungal strains, which could show better efficiency in biological controlling, protoplast fusion was done between *Helminthosporium gramineum* and *Curvularia lunata* and the resulting strains effectively controlled major rice weeds (Zhang et al., 2007).

Curvularia lunata and *Curvularia aeroa* have been reported as biological control agents in *Echinochloa* spp. (Tsukamoto et al., 1998). *Curvularia lunata* isolated from barnyardgrass was evaluated for controlling weeds in bean fields (Bisen, 1983). It was found that this fungus was not effective in rice cultivars, but caused disease in bean varieties (Bisen, 1983). Also, *Curvularia lunata* reduced the growth of *Echornia crassipes* (L.) by 15-20% (Praveena and Nasecma, 2004).

In the Philippines, isolates from *Curvularia tuberculata* and *Curvularia oryzae* were evaluated as probable control agent of *Cyperus difformis* and *Fimbristylis miliacea* (de Luna et al., 2002). Inoculation with spore suspension in these fungi during the foliar stage destroyed the weeds' seedlings (de Luna et al., 2002).

Generally, the substantial condition for introducing a microorganism as a weed biological control factor is ensuring that the microorganism would not damage the main crops (Watson, 1985). Therefore, proving that these fungal isolates would not damage rice is of great significance. In this study, *Curvularia lunata* was isolated from *Echinochloa* spp. (*E. oryzicola, E. crus-galli* L.), *Sagitaria trifolia* (L.) and *Alisma plantago-aquatica* (L.) and as a probable biological control agent for these weeds in rice paddies of Guilan province, it was inoculated to several rice cultivars and the above-said weeds.

2. Materials and Methods

2.1. Collection and culture of fungal isolates

Leaves with symptoms of the disease weeds 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 (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) or colonial of fungal placed onto sterilized filter paper, then cuts of these filters were incubated in sterilized vials at freezer on -20°C (Safari Motlagh, 2010).

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

Pathogenicity tests of weeds in seedling stage were carried out in desiccators. In each of two desiccators (one desiccator as control) two Petri dishes were placed each containing 10 germinated seeds of weeds. At first, seeds of weeds were placed on moistened filter paper in Petri dishes and incubated at 28°C for 24 h in a germinator with 12h light/dark photoperiod. Then, seeds were surface sterilized with 0.2% sodium hypochlorite solution for 2 min. After washing with distilled water, 10 germinated seeds were planted per 10- cm- Petri dishes filled with saturated soil (Zhang et al., 1996), and were incubated at temperature room. Distilled water was added to Petri dishes. Seedlings at the 2-3 leaf stage were inoculated with 10^5 conidia per ml. To increase the surface adsorption, 1% tween-20 was applied. Evaluation of symptoms was performed 7 days after inoculation. Therefore, standard evaluation system and Horsfall-Barratt system were applied for *Echinochloa* spp. (Zhang et al.,1996; Bertrand & Gottwald 1997).

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

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

Pathogenicity tests of rice seedlings were carried out in desiccator. To do so, in each of two desiccator (one desiccator as control) were placed two Petri dishes and in each Petri dish placed 10 seeds of rice, Khazar cultivar. Then, seeds were sterilized in water bath at 52-57°C and cultivated in saturated soil and incubated at 25°C. Distilled water was added to perti dishes. After 16-18 days, seedlings containing 2-3 foliages were inoculated by suspension of spores (Safari Motlagh and Kaviani, 2008). Other conditions including concentration of conidia and evaluation systems were similar.

Pathogenicity tests of weeds in greenhouse conditions occurred as complete random design (CRD) with one treatment and 3 replications. Inoculation of weeds was performed at its 3-4 leaf stage in greenhouse. To do so, a spore suspension including 10^6 *C. lunata* spore/ml distilled water was used. In order to increase adsorption, 1% Tween-20 was used. Weeds were planted in farm soil inside plastic pots, 2.5 cm in diameter. 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%,. 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 disease symptoms 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 *C. lunata*. 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 *C. lunata* 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. Inhibition of Seeds germination test

Seeds of weeds were surface sterilized with 0.2% sodium hypochlorite solution for 2 min. After washing with distilled water, seeds were placed per 10- cm-Petri dishes containing wet filter papers. Then inoculation was done. To do so, 10 seeds were transferred to two Petri dishes containing wet filter papers (one Petri dish as control). Then cuts of fungus colonies were placed on seeds. This test was done with three replications. The Petri dishes were incubated at 28°C on a 12h light/dark photoperiod. Evaluation of symptoms was performed 7 days after inoculation and number and percent of germinated seeds was determined (Zhang and Watson, 1997).

2.6. Data Analysis

Data analysis was done using SPSS, MSTAT-C and NTSYS softwares. In order to compare average values, Duncan test was used, while for comparing the reaction of rice cultivars and weeds, the difference between the average value of each fungus-treated rice cultivars and the controls was used.

3. Results

3.1. Study of the disease rating caused by *Curvularia lunata* in weeds and rice cultivar in desiccators

Evaluation of the disease rating of *Curvularia lunata* isolates in rice, *Alisma plantago-aquatica, Sagitaria trifolia* and *Echinochloa* spp. in accordance with Horsfall-Barratt system using the number and sizes of the spots showed that the disease rating caused by this fungus in weeds was more than in rice and they indicated a significant difference (Figure 1). The first symptoms in weeds appeared 48 hours after inoculation. Initially, chlorotic spots appeared which later on became necrotic and connected and caused necrosis in most parts of the leaf surface. Also, cotton-like colonies of the fungus were observable on the leaves.

On the other hand, the first symptoms in rice appeared 5-6 days after inoculation. They started as small necrotic spots and in some cases, blight occurred on the tip of the leaves.

3.2. Evaluation of the inhibition of seeds germination in weeds

Results showed that *Curvularia lunata* was quite effective on the germination of the studied weeds and even inhibited it. Accordingly, there was a significant difference between controls and treatments (Figure 2).

Based on the dendrogram obtained from the cluster analysis, *Curvularia lunata* isolates were divided into two groups in accordance with the disease rating in weeds. In each group, there were two isolates. Moreover, in the second group, there were two isolates

with a similarity coefficient of more than 95% (Figure 3).

According to the said dendrogram, in terms of disease rating in rice, *C. lunata* isolates were placed in two groups. Group 1 consisted of one isolate, while there were 3 isolates in group 2. The two isolates in group 2 had a similarity coefficient of more than 95% as well (Figure 4).

Also, based on the dendrogram regarding the inhibition of seeds germination in weeds, *C. lunata* isolates were divided into two groups each having two separate isolates. There were two isolates with a similarity coefficient of more than 95% in the second group (Figure 5).

3.3. Greenhouse experiments

Results from the variance analysis of the disease rating revealed that the studied rice cultivars did not show any significant reaction to *Curvularia lunata* (Table 1). Despite the fact that in terms of the disease rating reactions of rice cultivars were not significant, based on direct observations regarding types and sizes of the spots caused by the fungus in the aforesaid rice cultivars, it was found that Sepidroud as a bred cultivar was more tolerant compared with others, while Hashemi was more affected by the fungus among the cultivars. In terms of tolerance, Sepidroud was followed by Ali Kazemi, Khazar and Binam.

In the study of dry weight, fresh weight and height of the said rice cultivars, a significant reaction was observed in all these traits (Table 2). Evaluation of the mean values of the said 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 a 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 the studied cultivars were similar to those of the fresh weight (Table 2).

Evaluation of the fungus' effect on each of the studied traits in rice cultivars compared with the controls revealed that in terms of height, Ali Kazemi, Sepidroud and Binam had no significant difference between them. Moreover, compared with the controls, they showed height decreases as a result of the fungus' effect (Table 3). In terms of height, the highest and lowest height reductions were those of Khazar and Hashemi, respectively (Table 3). Regarding fresh weight, there was no significant difference between Sepidroud and Khazar, while both of them showing reduced fresh weights compared with controls. Furthermore, Hashemi showed more fungus-induced fresh weight decrease in comparison with other cultivars. In the indigenous Ali Kazemi cultivar, fresh weight decrease was less than Hashemi, yet compared with Sepidroud and Khazar it was more. In the evaluation of this trait, fresh weight decrease was not observed in Binam. The dry weight of rice cultivars, compared with that of the controls did not show any significant decrease (Table 3).

For the evaluation of the disease rating caused by *C. lunata* in *Alisma plantago-aquatica*, *Sagitaria trifolia* and *Echinochloa* species, a significant reaction was observed (Table 4). *Alisma plantago-aquatica* was more affected by the fungus while *Sagitaria trifolia* was the most tolerant. Moreover, there was no significant difference between two *Echinochloa* species, i.e. *E. crus-galli* and *E. oryzicola* regarding the effect of this fungus, but based on direct observations relevant to the type and size of the appeared spots by the fungus, the disease in *E. oryzicola* was more severe (Figure 6).

In the study of the effect of the said fungus on fresh weight, dry weight and height of *Sagitaria trifolia*, *Alisma plantago-aquatica* and two *Echinochloa* species based on the variance analysis table, a significant reaction was observed for height and fresh weight. But for dry weight, the reaction was not significant (Table 4).

Also, based on the comparison of the traits' mean values, all the studied weeds showed a significant difference in terms of height (Table 5). In this regard, *Alisma plantago-aquatica* had the highest reduction. It was found that in terms of fresh weight, there was no significant difference between two *Echinochloa* species. However, *Alisma plantago-aquatica* and *Sagitaria trifolia* showed a significant difference. Moreover, in terms of dry weight, no significant difference was found between these weeds (Table 5). It is noteworthy that compared with controls, all studied and treated weeds showed reductions of the said traits (Table 6). Therefore, it could be concluded that the fungus caused reduction in the weeds' height, yet this reduction did not show any significant difference in the four studied weeds. Also, fresh weight conditions were similar to height changes and in terms of the dry weight the fungus caused it to decrease in all the studied weeds compared with controls (Table 6). However, in *Sagitaria trifolia*, the reduction of the dry weight was less in comparison with other weeds and compared with the controls, it was less affected by the fungus (Table 6).

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

SOV	DF	Squares Mean			
		Disease rating	Height(cm)	Fresh Weight (g)	Dry Weight(g)
Treatment	4	0.388n.s	103.008**	5.397**	0.14*
Error	10	0.114	6.464	0.193	0.29
C.V.	-	14.95	3.73	11.17	32.09

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

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

Cultivar	Height(cm)	Fresh weight(g)	Dry weight(g)
Hashemi	69.196 ± 1.089a	4.818 ± 0.258b	2.127 ± 0.043b
Ali Kazemi	72.416 ± 1.044a	5.817 ± 0.425a	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

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. lunata* with those of the controls.

Cultivar	Change of Height(cm)	Change of Fresh weight(g)	Change of Dry weight(g)
Hashemi	-2.21 ± 0.606b	-1.01 ± 0.358a	0.16 ± 0.023a
Ali Kazemi	-3.50 ± 0.5775ab	-0.6 ± 0.286ab	0.063 ± 0.021a
Sepidroud	-2.58 ± 1.21ab	-0.19 ± 0.061b	0.095 ± 0.018a
Khazar	-5.12 ± 0.919a	-0.18 ± 0.053b	0.15 ± 0.091a
Binam	-4.91 ± 0.759ab	0.36 ± 0.066ab	0.16 ± 0.043a

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

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

SOV	DF	Squares Mean			
		Disease rating	Height(cm)	Fresh Weight (g)	Dry Weight(g)
Treatment	3	2.178*	610.230**	122.452**	1.190 n.s.
Error	8	0.328	12.080	1.451	0.329
C.V.	-	18.03	7.19	10.07	24.19

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

Table 5**.** Comparison of means of the studied traits affected by *Curvularia lunata* in weeds.

Weed	Height(cm)	Fresh weight(g)	Dry weight(g)
E. oryzicola	63.500 ± 2.362a	6.175 ± 0.078c	5.018 ± 0.071b
E. crus-galli	53.480 ± 2.992b	7.896 ± 0.406c	0.738 ± 0.056b
Sagitaria trifolia	46.666 ± 1.013c	20.343 ± 0.983a	2.163 ± 0.599b
A. plantago-aquatica	29.600 ± 0.737d	13.403 ± 0.891b	1.563 ± 0.265b

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 *C. lunata* with those of the controls.

Weed	Change of Height(cm)	Change of Fresh weight(g)	Change of Dry weight(g)
E. oryzicola	-3.29 ± 1.37a	-0.12 ± 0.054a	-0.06 ± 0.006a
E. crus-galli	-1.8 ± 0.32a	-0.29 ± 0.28a	-0.09 ± 0.032a
Sagitaria trifolia	-0.6 ± 0.3a	-0.82 ± 1.71a	-0.63 ± 0.245b
A. plantago-aquatica	-1.3 ± 0.83a	-0.43 ± 0.019a	-0.42 ± 0.327a

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

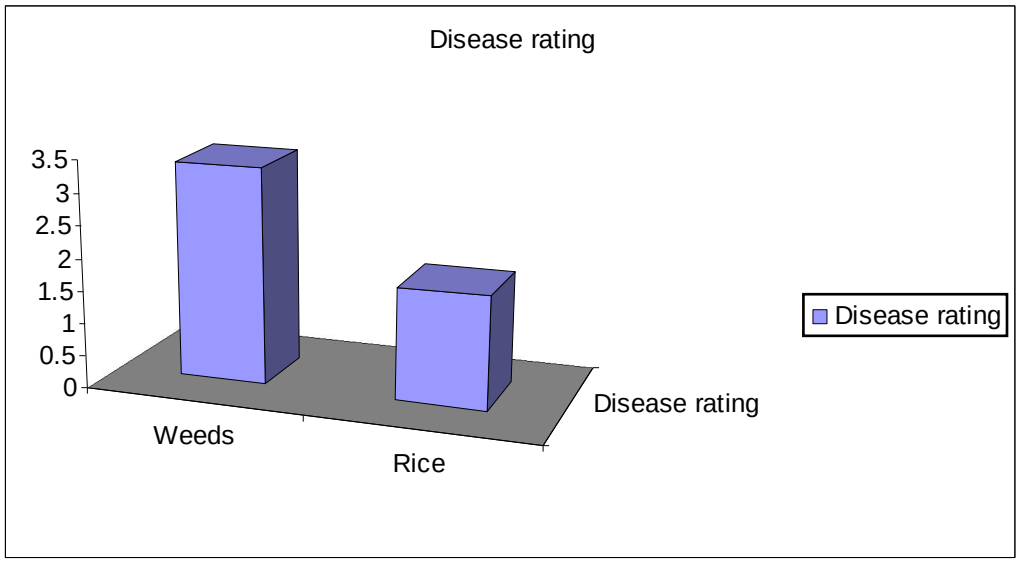

Figure 1. Diagram of the comparison of *C. lunata* mean disease rating in rice and weeds.

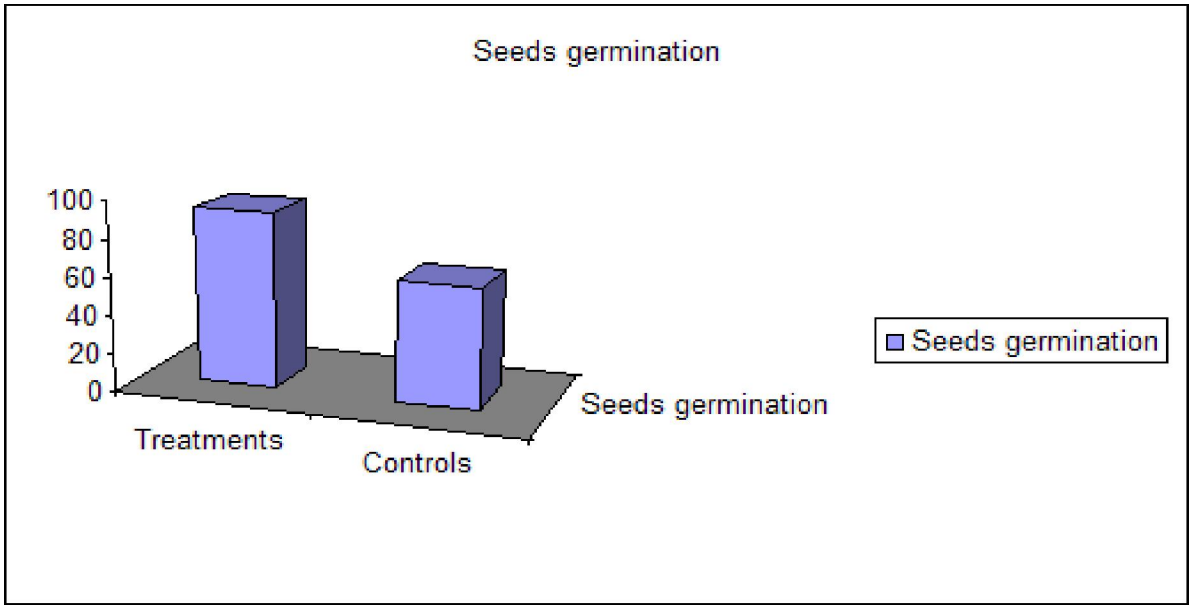

Figure 2. Diagram of the comparison of mean seeds germination percent in treatments and controls of weeds.

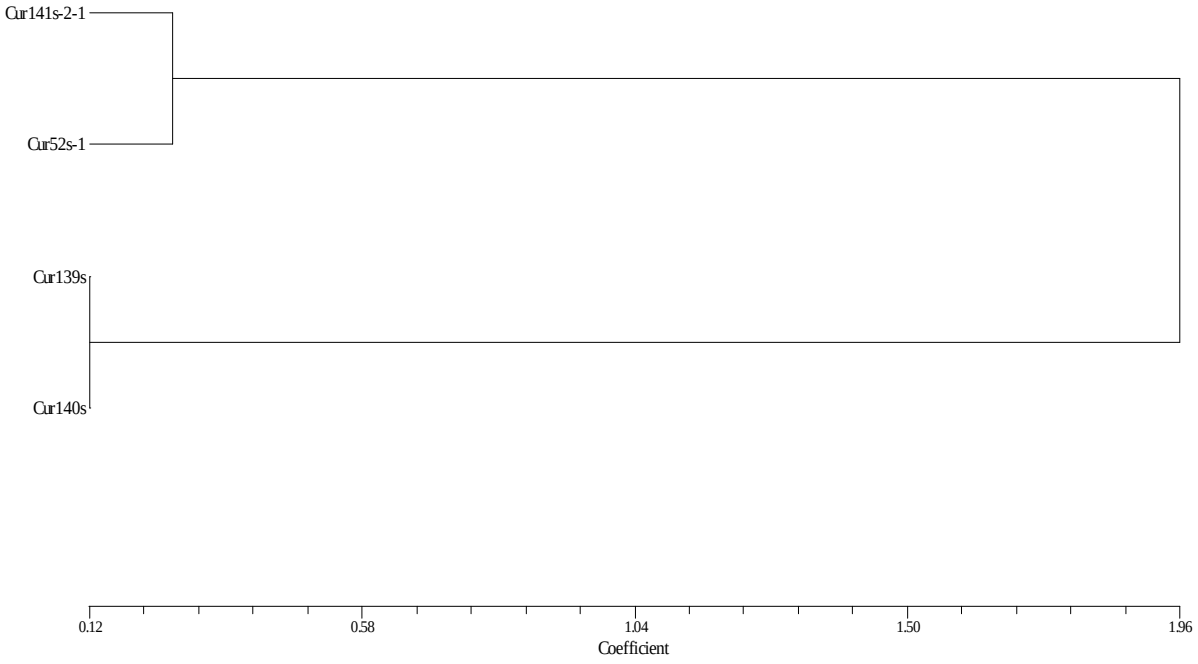

Figure 3. UPGMA-dendrogram for *C. lunata* isolates on weeds.

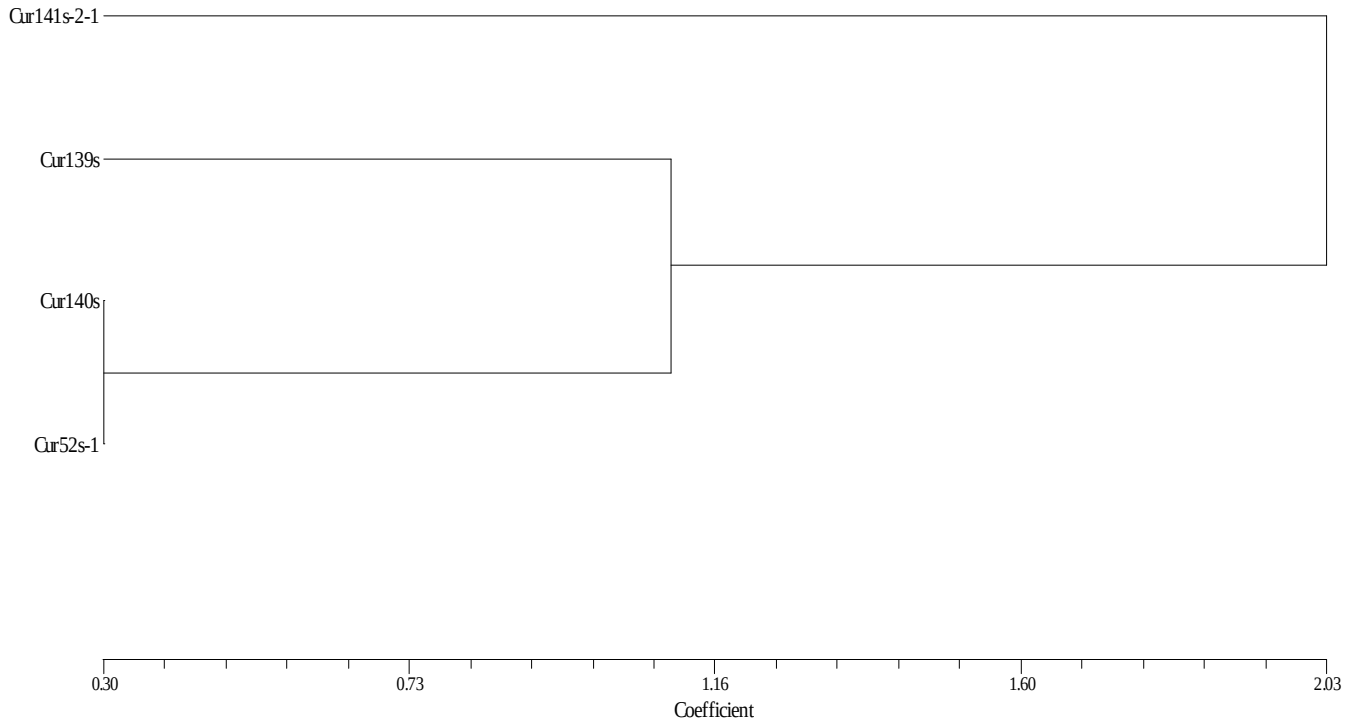

Figure 4. UPGMA-dendrogram for *C. lunata* isolates on rice.

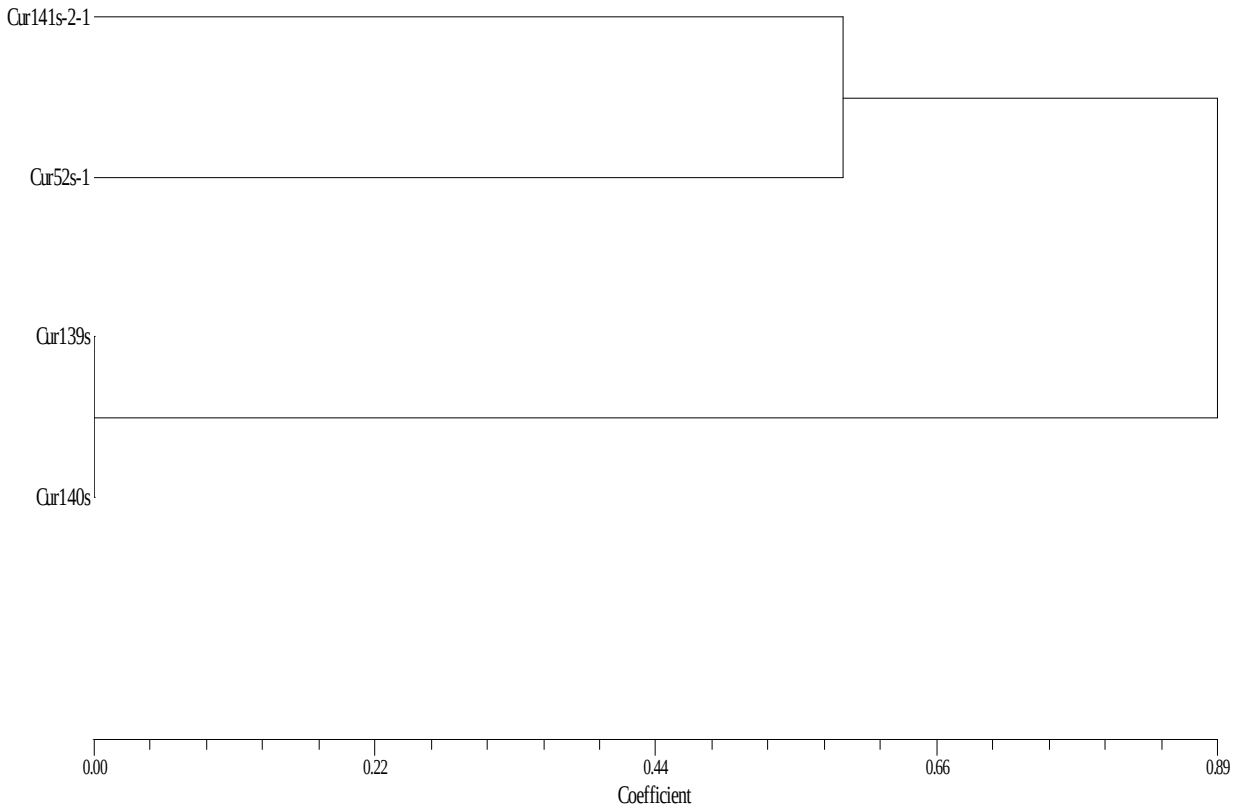

Figure 5. UPGMA-dendrogram for *C. lunata* isolates on weeds (based on inhibition of seeds germination).

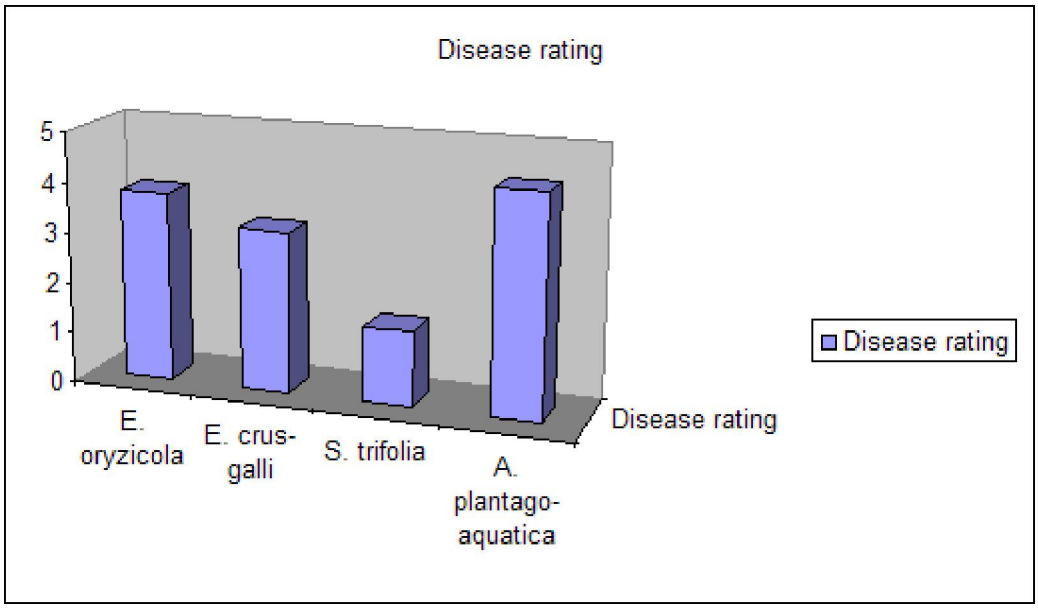

Figure 6. Diagram of the comparison of *C. lunata* mean disease rating in weeds.

4. Discussion

Based on the cluster analysis in the present study, *Curvularia lunata* isolates showed similar reactions in terms of pathogenicity and the inhibition of seeds germination of weeds and thus, were placed in similar groups. Moreover, since two bred cultivars, i.e. Sepidroud and Khazar were used along with some indigenous cultivars, the formers based on the disease rating index were more tolerant compared with Hashemi, Ali Kazemi and Binam.

Also, a study by de Luna *et al*. revealed that indigenous rice cultivars in comparison with bred ones which were exposed to *Curvularia oryzae* were more damaged (de Luna et al., 2002). In the Philippines, the study of rice cultivars' reactions to *Curvularia lunata* isolated from *Echinochloa crus-galli* revealed that the fungus was not pathogenic in six bred and indigenous rice cultivars (Zhang et al. ,1996).

Based on the results of this research regarding the evaluation of fresh weight, dry weight and height in rice cultivars treated with *C. lunata*, it was observed that *Hashemi* and *Alikazemi* cultivars were less affected by the said fungus. This reaction could be related to less genetic diversity and more adaptability of indigenous cultivars to environmental conditions and the inoculated fungus (Kimber, 1983).

According to the results from the present study, although the disease rating was higher in Hashemi, in terms of height, fresh weight and dry weight, the cultivar was less affected by the said fungus. To elucidate this finding, one can say that the interaction between the genes in every plant's genome and the genotype of each fungal races leads to developing different responses of each trait to a given fungus. As researches have shown, one of the main problems regarding the modification of plant resistance to a parasite while studying the biological control of weeds to develop resistant cultivars is encountering a wide range of diverse creatures with different genetic structures (Kimber, 1983). This had led to obtaining inconsistent results in some researches. For example, according to the study conducted by Zhang *et al*. *Curvularia lunata* did not cause a high disease rating in *Echinochloa* spp. (Zhang et al., 1996) while in the present research, the fungus was the cause of a high disease rating in this weed, particularly in *Echinochloa oryzicola*.

Differences of the effect of a fungus as a biological control agent could depend on the environmental conditions of a geographical location, especially humidity and temperature (Huang et al. ,2005). For instance, *Exserhilum monoceras* that was isolated from *Echinochloa* in some farms the Philippines was effective on the weed in the region. However, when it was used for controlling this weed in rice paddies in South Korea, the response was quite different and in fact, it was not effective at all (Chung *et al*. 2005). Studies showed that different climatic conditions between the two geographical regions had led to different responses of the fungus (Chung et al., 2005).

Furthermore, reactions of weeds to fungi isolated from different hosts might be different. For example, in the study of *Curvularia oryzae* isolated from *Cyperus difformis*, it was found that the host range of different weeds was effective in their responses to the fungus in

terms of traits such as fresh weight and stem length. In the said study, *Curvularia oryzae* reduced the height of *Cyperus difformis*, but it did not affect the fresh weight (de Luna et al., 2002).

Different species of one fungal genus may also have different effects on a weed. Studies conducted by Zhang et al. showed that *Curvularia lunata* and *C. geniculata* (isolated from *Echinochloa*) had different effects on *Echinochloa* and rice that is the former had no effect on *Echinochloa* yet was pathogenic in rice cultivars while the latter affected *Echinochloa* but not rice cultivars (Zhang et al., 1996). Thus for different responses of rice cultivars and weeds, different factors such as genetic diversity, geographical and climatic conditions and interactions between different weed and fungal species are effective.

Furthermore, in the study of the effect of *C. lunata* on different growth stages such as seed, 2-3 leaf stage (seedlings) and also in the greenhouse, it was observed that the said fungus was effective in weeds at all stages. This meant that the disease rating caused by the fungus in different growth stages of weeds was more than in rice. Also, it reduced germination in weeds and did not have a significant effect on the said rice cultivars.

With consideration of the effect of the fungus on some performance traits of the studied rice cultivars, using it as a biological control agent is only recommended when the modification of rice cultivars is done using desirable traits of the regional indigenous cultivars so that its probable damages to rice production are minimized.

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