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Inhibition of Germination and Seedling Growth of Wild Oat by Rice Hull Extracts

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Abstract: In this study, phytotoxicity of rice hull extracts (*Oryza sativa* L.) on wild oat (*Avena ludoviciana* Durieu) was investigated. Hull extracts from 13 cultivated rice cultivars (*Oryza sativa* L.) were used to determine their allelopathic potential on seed germination and seedling growth of wild oat (*Avena ludoviciana* Durieu). The allelopathic effects of water hull extracts from selected cultivars were investigated. In the screening the Red anbarbo extract inhibited germination 24% very closely fallowed Daniyal is 27%. Seedling growth bioassays demonstrated that the wild-oat (*Avena ludoviciana* Durieu) responded differently to the allelopathic potential of rice. For wild-oat (*Avena ludoviciana* Durieu) shoot length and germination were more depressed than root length. Some hulls extracts including Champa, Sahel almost didn't affect root length. The greatest total seedling length inhibition was from the Daniyal extracts. Extract of rice hulls significantly reduced roots length of wild oat. Extracts of Daniyal reduced root length of wild oat by 2.19 cm. This cultivar have the highest inhibitory on root length of wild oat. These results suggest that rice hull extracts may be a source of natural herbicide. There may be genetic differences among rice cultivars for allelopathic potential on Wild-oat. The breeding of rice cultivars with greater allelopathic potential may be possible.

Key words: Allelopathy, Phytotoxicity, *Oryza sativa* L., *Avena ludoviciana* Durieu

INTRODUCTION

Since, the 1950s, agriculture depended on the use of herbicides to suppress weeds and ensure high yields. The application of weed controlling chemical agents has therefore steadily increased, although a number of herbicides have had well-documented negative consequences on the environment and on human health. Biological control offers a number of alternative approaches for weed control in agriculture (Bond and Grundy, 2001; Mason and Spaner, 2006), but the application of biological weed control has often proved difficult in practice (Müller-Schärer *et al*., 2000). Allelopathy is defined as any direct or indirect effect of one plant (or microorganism) on another mediated through the production of chemical compounds that escape into the environment (Macas *et al*., 2007).

In general, the role of allelopathy in plant-plant interactions and especially its potential for weed control in agriculture are controversial, because evidence for direct allelopathic effects and ecological relevance is often difficult to prove (Blum *et al*., 1999; Inderjit and Weston, 2000; Inderjit and Weiner, 2001; Inderjit, 2006). Nevertheless, crop plants with superior weed suppressive ability under field conditions would be highly desirable in agriculture (Olofsdotter, 2001).

Much attention has focused on the allelopathic effects of rice (*Oryza sativa* L.) as a potential tool for ecologically sound weed control (Kuk *et al*., 2001; Kim and Shin, 2008). These researchers evaluated the allelopathic potential of rice cultivars by analyzing leaf and straw extracts, decomposing straw and the soil where rice was grown. In field experiments, Khanh *et al*. (2007) evaluated about 10000 accessions of different origin from a worldwide rice germplasm collection (including the USA) for allelopathic effects on ducksalad (*Heteranthera limosa* (Sw.) Willd.) and other annual broadleaf weeds. This is study evaluated allelopathic potential by measuring the weed-free radius surrounding the base of rice plants within a hill. Kim and Shin (2008) compared the phytotoxicity of leaf, stem and hull extracts and their mixture for 47 rice cultivars on barnyardgrass (*Echinochloa crus-galli* P. Beauv. var. *oryzicola* Ohwi). These results showed significant variability for allelopathic effects among cultivars that rice hull extracts contained more water-soluble substance toxic to barnyardgrass than leaf or stem extract. These studies suggested potential for developing allelopathic weed suppression through plant breeding. Heavy use of fertilizers, herbicides and other pesticides may pollute water and soil in the paddy ecosystems (Lin *et al*., 2004). The ineffectiveness of herbicides on resistant weed

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species and environmental imperatives, have prompted the search for non-herbicidal innovations to manage weed populations (Wu *et al*., 1999). As cultural practices for rice change from transplanted to direct-seeded to reduce production costs, weed problems like wild - oat will be more serious because rice and weeds can emerge together. The main purpose of this research was to study the effect of aqueous hull extracts from 13 cultivated rice cultivars on wild-oat germination and seedling growth.

MATERIALS AND METHODS

Thirteen Rice (*Oryza sativa* L.) varieties namely Champa, Chaparsar5, Hoveyzeh, Kadus, Shafagh, Chaparsar3, Line6, Jahesh, Tabesh, Gachsaran, Daniyal, Red anbarbo and Sahel were grown at the Shahoor farm of Research Institute of Forests and Rangelands, Ahwaz, Iran, in 2005. From soil preparation to crop harvest, standard cultural practices of the semiarid zone were applied. Plants under experiment were irrigated whenever severe wilting of plants was observed.

Since, aqueous hull extracts had shown more activity than leaf extracts in a previous study (Lin *et al*., 2004), hulls from these cultivars were dried (24°C) and ground in a Wiley mill through a 40-mesh screen. Ground hulls (5 g) were soaked in 100 mL distilled water for 24 h at 24°C in a lighted room. The solutions were filtered through four layers of cheese cloth to remove debris and then centrifuged at 3000 rpm for 4 h. The supernatant was filtered through one layer of Whatman No. 42 filter paper. To prevent microorganism growth, the solutions were filtered again through a 0.2 mm Nalgene filter.

To determine the allelopathic effect of Rice hull extracts, Wild-oat (*Avena ludoviciana* Durieu) seeds were collected in October 2005, cleaned and stored at -35°C. Before the start of experiments for the determination of allelopathic effect, the *Avena ludoviciana* seeds were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min and rinsed several times with distilled water. These sterilized seeds were placed on a paper towel for about 2 h. Then *Avena ludoviciana* seeds were placed on a filter paper in sterilized 9 cm diameter petri dishes. Ten mL of the extract solution was added to each petri dish and distilled water was used as a control. All petri dishes were placed in a lighted growth chamber at 24°C.

Percent germination, seedling root, shoot lengths and wed weight were determined and measured after 4 days. The experiment was designed under Completely Randomized Design (CRD) with four replications. A non-amended treatment was included as a control. Analysis of variance was conducted using Dunkan program of MSTATC (Steel and Torrie, 1980). Data from two experiments were pooled and mean values were separated on the basis of Least Significant Difference (LSD) at the 0.05 probability level.

RESULTS

Maximum seed germination percentage was recorded in hull extracts of rice variety champa, very closely followed by kadus variety, Indicating very less allelopathic effect on seed germination of wild oat (Table 1, 2). Minimum seed germination percentage recorded was noted in hull extracts of rice variety Red anbarbo showing a considerable allelopathic effects on seed germination.

Extracts of all rice varieties significantly affected root length of wild oat plants. As compared to control, maximum was recorded in extracts of variety Champa, very closely followed by Tabesh and Sahel varieties, indicating very less allelopathic effect on root length of wild oat (Table 1). Minimum root length was recorded was noted in extracts of rice variety Daniyal showing a considerable allelopathic effect on root length. With regard to shoot length of plant maximum inhibitory effect was showed by the extract of Daniyal variety, closely followed by extracts of Line 6 and Jahesh varieties, respectively. Maximum shoot length of wild oat plants was retarded by the hull extracts of rice variety Tabesh. In comparison, inhibition of shoot length was greater than that of root length.

Table 1: Analysis of variance table of different parameters

SOV	d.f	Germination (%)	Root length	Shoot length	Wet weight
Treatment	13	804.533*	1.623*	1.346*	0.032*
Error	42	243.452	0.672	0.397	0.012

*Significantly different from control $p<0.05$ assessed by Duncan' s Multiple Range Test

Table 2: Inhibitory effect of rice extracts hulls on wild-oat germination, root length, shoot length and wet weight

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Wet weight (g)
Control	100.0^{a}	4.447^{a}	4.410^{a}	0.7560^{a}
Champa	74.00^{b}	3.987^{ab}	3.160^{bc}	0.6790^{ab}
Chapar sar 5	62.00^{c}	3.368^{abcd}	2.885^{c}	0.6670^{abc}
Hoveyzeh	62.00^{c}	2.788^{bcd}	2.852^{c}	0.5130^{bcd}
Kadus	71.00^{b}	3.607^{abc}	2.902^{c}	0.7090^{a}
Shafagh	60.00^{c}	2.933^{bcd}	3.072^{bc}	0.5720^{bcd}
Chapar sar 3	57.00^{c}	3.388^{abcd}	2.835^{c}	0.5130^{bcd}
Line 6	55.00^{c}	2.855^{bcd}	2.445^{c}	0.5740^{abcd}
Jahesh	55.00^{c}	2.640^{bcd}	2.462^{c}	0.5050^{bcd}
Tabesh	52.00^{c}	3.685^{abc}	3.920^{ab}	0.6470^{abc}
Gachsaran	52.00^{c}	2.383^{cd}	2.570^{c}	0.4340^{d}
Daniyal	27.00^{d}	2.194^{d}	2.312^{c}	0.4840^{cd}
Red anbarbo	24.00^{d}	2.985^{bcd}	2.660^{c}	0.4300^{d}
Sahel	40.00^{b}	3.685^{abc}	3.268^{bc}	0.6050^{abcd}

Values are the means of four replications. Variants possessing the same letter(s) are not statistically significant at $p<0.05$ level, according to Duncan's Multiple Range Test

Maximum wet weight inhibitory effect was noted in the extract of Red anbarbo variety, very closely followed by the extracts of Gachsaran and Daniyal, respectively, showing a considerable allelopathic effect on wet weight. Minimum seedling wet weight inhibitory effect was recorded was noted in extracts of rice variety Kadus that showed no inhibition wet weight accumulation.

DISCUSSION

Results of present study suggested that the response by wild-oat (*Avena ludoviciana*) varied depending on the source of allelochemicals and kind of the variety. Some results of researches showed that hulls extract of rice have more allelopathic effect than the other part of rice (Kim and Shin, 2008).

The inhibition of wild-oat germination and seedling growth by rice hull extracts may reflect the allelopathic potential of individual rice cultivars. The magnitude of allelopathic effects varied among the rice cultivars studied. Results of this study are in agreement with those of Wu *et al*. (2001), Kim and Shine (2008) and Olofsdotter *et al*. (1995), who concluded that variation in allelopathic activity existed among cultivars. Although it has not been determined if this difference in allelopathic activity is a result of higher concentrations of the same chemical or a result of different chemicals between cultivars, response differences in the current study were attributed to genetic differences between cultivars, since comparisons were made among extracts with the same hull rates. It is possible that cultivars may produce different amounts of one or more allelopathic substances at a given extract concentration or hull rate.

Generally, the allelopathic effect was known to involve many secondary metabolites, which reacted with one another and allelochemicals were synthesized by either the shikimic acid or acetate pathways. Uphoff *et al*. (2006), Olofsdotter *et al*. (1995) and Geally *et al*. (2000) reported that allelopathic chemicals, including ferulic acid, are abundant in rice straw. Lin *et al*. (2004) also isolated phenolic acids, including o-hydroxy phenyl acetic acid, from rice straw and nine phenolic acids from rice hulls. These chemicals inhibited seed germination and seedling growth of barnyardgrass at concentrations of 1, 10, 3 M and sometimes even lower (Kim and Shin, 2008). Among the 13 rice cultivars used in this study, Daniyal yielded extracts that reduced wild-oat root and shoot length. This cultivar may provide one of the most important gene resources for breeding rice cultivars with highly allelopathic hulls. Allelopathic potential would be a valuable trait to incorporate in rice cultivars for improved weed control. It's one of the best rice cultivars that adapted on condition in our province (Khozestan-Iran). Hull extracts inhibited wild-oat growth. These results are not in agreement with those of Khanh *et al*. (2007) and Kim and Shin (2008) that they reported stimulatory effects at low concentrations of allelopathic substances.

When using similar bioassay to screen cultivars for allelopathic potential without authentic allelochemical separation and identification, it is advisable to ensure that extract concentrations are similar to those found under natural conditions, since the concentration of inhibitory substances is probably greater in aqueous extracts than in the field. Based on the bioassay hull rates (50 gl L^{-1}) used in this study, 13750 kg hulls ha^{-1} would be theoretically required for allelopathy to occur in the field. However, since factors other than extract concentration are involved in allelopathic activity, it is more appropriate to generalize that the more rice hulls remaining in the paddy soil, the greater the concentration of allelopathic compounds released during the decomposition. Inhibitory chemicals of hull extracts are actually greater in the field than in this study and it is possible to understand why farmers in Korea generally leave a large amount of rice hulls in the field (Chung *et al*., 2001). This study suggests that the allelopathic compounds present in rice hulls may serve as a potential natural herbicide by inhibiting seed germination and growth of wild oat, which has become problem because of increased use of direct seeding of rice to reduce production costs. If these varieties are Moddy (ed.) Proc. Int. Workshop on Constraints, Opportunities used to contribute to the control of wild-oat, they may also be used as genetic markers to identify allelopathic varieties. The same results reported by researchers about allelopathic potential of other plants such as sunflower (Bogatek *et al*., 2004), alstonia (Javaid *et al*., 2006) and barley (Moncef *et al*., 2001) supported, utilization of plant allelopathy for biological control of weeds and plant pathogens. One limitation of this study is that the concentration of allelopathic substances in residue mixture may be greater than in fresh material and subject to positive or negative effects from soil components. The duration of allelopathic substances contained in the residue or released by decomposition, which were not evaluated may be shorter in the field. Further investigations are needed to investigate potential allelopathic cultivars under field conditions.

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