Potential Allelopathic Effects of Rice Hull Extracts on Barnyardgrass (*Echinochloa crus-galli*) Seedling Growth

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ABSTRACT

Some rice cultivars have demonstrated allelopathic potential against most of the troublesome weed species in paddy fields. Hull extracts from 46 rice cultivars at various concentrations were used in bioassay tests to evaluate their effects on seedling growth of barnyardgrass. Hull extracts of cultivars Dashtisard and Dinorado showed higher inhibitory effects on root growth than the others while Alikazemi and Sepidrood had low inhibitory effects. Dinorado and Neda had high inhibitory effects on shoot growth, whereas hull extracts of Line-229 and USEN showed high stimulatory effects on shoot growth of barnyardgrass seedlings. The barnyardgrass roots were more sensitive to hull extracts than the shoot extracts. Hull extracts of five rice cultivars were identified with the highest inhibitory effect on barnyardgrass seedling growth, from which Dinorado, Neda, Dashtisard and IR-60 had inhibitory activities and Line-229 had stimulatory potential. Inhibitory activity of rice hulls can be used to screen allelopathic rice cultivars. The cultivars with more hull allelopathic effects can lead us to cultivars with a natural source of herbicides. The genetic differences among rice cultivars exhibit variation in allelopathic effects on barnyardgrass growth. Breeding of

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rice cultivars with greater allelopathic potential may provide natural herbicides for controlling weeds.

Key words: Allelopathic potential, Barnyardgrass, Rice hulls, Seedling growth**.**

چکیده

بعضی از ارقام برنج توان اللوپاتیک خوبی بر روی بسیاری از علف‌های هرز مزاحم شالیزارهای برنج از خود نشان داده اند. تاثیر عصاره پوسته 46 رقم برنج با غلظت‌های مختلف در آزمایش‌های زیست سنجی بر رشد گیاهچه‌های سوروف مورد بررسی قرار گرفتند. عصاره پوسته رقم‌های دشتی سرد و دیناردو بیشترین تاثیر بازدارندگی و ارقام علی کاظمی و سپیدرود کمترین اثر بازدارندگی را بر روی رشد ریشه سوروف نشان دادند. ارقام دینورادو و ندا بیشترین اثر بازدارندگی را روی رشد اندام‌های هوایی نشان دادند در حالی که عصارهای لاین 229 و رقم یواس ای ان بیشترین اثر تحریک کنندگی رشد را بر اندام‌های هوایی گیاهچه های سوروف نشان دادند. ریشه های سوروف نسبت به عصاره های پوسته برنج حساس‌تر از اندام‌های هوایی بودند. در مجموع، عصاره های پوسته 5 رقم برنج بیشترین خاصیت اللوپاتیک را بر سورف نشان دادند که از میان آنها ارقام دینورادو، ندا، دشتی سرد و آی آر-60 دارای پتانسیل بازدارندگی و لاین 229 پتانسیل تحریک کنندگی رشد داشتند. ویژگی بازدارندگی پوسته برنج می تواند برای غربال سازی ارقام اللوپاتیک برنج مورد استفاده قرار گیرد. ارقام دارای پوسته با توان بازدارندگی بیشتر می تواند ما را به یافتن ارقام با ویژگی علف‌کشی طبیعی رهنمون سازد. تفاوت های ژنتیکی بین ارقام برنج، تنوع اثرات اللوپاتیک بر روی سوروف را نشان می دهد. به نژادی ارقام برنج با توان اللوپاتیک بالا ممکن است علف‌کش‌های طبیعی را برای کنترل علف‌های هرز فراهم سازد.

واژه‌های کلیدی: توان اللوپاتیک، سوروف، پوسته برنج، رشد گیاهچه.

INTRODUCTION

Any direct or indirect harmful or beneficial effect of plants and microorganisms on other plants through production of chemical compounds released to the environment is defined as allelopathy (Rice, 1984). Such phenomenon occurs widely among natural

plant communities and is postulated to be one mechanism by which weeds interfere with crop growth (Rice, 1984, Smith & Martin, 1994).

Allelopathic potential of rice has received a great deal of attention since Dilday *et al*. (1998) identified rice cultivars exhibiting allelopathic potential against ducksalad *Heteranthera limosa* (Sw.) willd. About four percent of the rice cultivars have demonstrated allelopathic potential against some of the most troublesome weed species in paddy fields such as barnyardgrass *Echinochlea crus-galli* (L.) Beauv, redstem (*Ammannia* spp.), sedge (*Cyperus* spp.) and ducksalad. A number of secondary metabolites, phenolic acids, phenylalkanoic acids, hydroxamic acids, fatty acids, terpenes and indoles, were identified in rice extracts (Rimando & Duke, 2003). Chung *et al*. (2005) suggested that rice hulls produce phytotoxic substances that could serve as natural herbicides. They isolated and identified seven compounds from rice hulls and reported high inhibitory effects of the momilactones A and B on germination of three weed species. Kato *et al.* (1977) isolated and determined the chemical structure of growth and germination inhibitors in rice husk cultivar Koshihikari including ineketone, *S* (+)-dehydrovomifoliol, momilactone-C , *p*-coumaric acid, momilactones A, and B. Direct inhibition of PSII components, interruption of dark respiration and ATP synthesis and disruption of amino acids metabolism were reported as some of the biochemical and physiological mechanisms that may be mediated by allelochemicals (Weir *et al.*, 2004). Chung *et al*. (2003) compared the phytotoxicity of leaf, stem, and hull extracts and their mixtures obtained from 47 rice cultivars on barnyardgrass. They reported significant variability for allelopathic effects among cultivars and plant organs, and found that rice hull extracts contained more water-soluble toxic substances to barnyardgrass than leaf or stem extracts. Kawaguchi *et al*. (1997) reported a promotive allelopathic potential of rice extracts on germination of *Monochoria vaginalis* Burm.F. seed. In general, the promotive effects of shoot extracts were slightly higher than that of root extracts. To assess the weed suppressing potential of rice cultivars on barnyardgrass, 111 cultivars and the weed seeds were sown in the

field by Olofsdotter *et al*., 1999. Significant differences were observed among cultivars in their potential to suppress the growth of barnyardgrass. Tests were carried out on the same rice cultivars for allelopathic potential. Correlation between the laboratory screening and the field experiments showed that field performance could be described to some extent by barnyardgrass root length reduction in the laboratory (Olofsdotter *et al*., 1999). Asghari and Musavi (2002) studied the allelopathic potential of 10 Iranian rice varieties on barnyardgrass and Umbrella sedge (*Cyperus difformis* L.) in a mixed-cropping system in pots. They found significant reduction in seedling growth of the weeds compared to the control, and three rice varieties with high allelopathic potential.

The purpose of this study was to determine the effects of aqueous hull extracts of 46 rice cultivars on barnyardgrass seedling growth as an allelopathic screening method, In addition, the allelopathic potential of Iranian rice cultivars compared with IRRI approved allelopathic cultivars in the collection.

MATERIALS AND METHODS

Preparation of Rice Hull Extracts

The mature paddy of 30 Iranian and 16 imported rice cultivars, four of which were obtained from International Rice Research Institute (IRRI), approved allelopathic cultivars grown in Rice Research Institute of Iran (RRII), (Rasht, Guilan: 37°, 12´N, and 49°, 38´E), were threshed and the hulls of each cultivar were separated in September 2005. The hulls were dried at room temperature, powdered to less then 2 mm mesh using a milling machine (TEFAL, Prep'line. 850. France) and stored at -70°C as needed. Aqueous extract (w/v) of each sample was prepared using 100 g ground hull samples with 1000 ml of distilled water (stock solution), and stirred for 24 h at 25°C with a slight modification of the method developed by Ahn and Chung (2000). The solutions were filtered through four layers of cheesecloth to remove fiber

debris. The resultant solution was filtered through a one layer filter paper (Whatman no. 42). Fungal contamination was not observed during the processes.

Bioassay in laboratory

Barnyardgrass seeds were collected from paddy farms of Guilan University and RRII's farms in fall 2005. The seeds were collected after removing the trash and were defected by soaking in distilled water. Before the bioassay, seeds were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 minutes and rinsed several times with distilled water. The seeds were pre-germinated in a germinator, and 10 uniform germinated seeds were placed on each filter paper (Whatman no. 42) in a sterilized 9-cm Petri dish. Three concentrations of each cultivar extract were prepared using 0, 50, or 100 percent of each cultivar from the extract stock solution. Each Petri dish was treated with 10 ml of each solution concentrations and stored in laboratory (25/20 °C day/night temperature and 10 hours daily light). The treatments were replicated four times. After seven days incubation, seedling root and shoot lengths and their fresh weights determined. Roots and shoots were separated, oven dried at 65°C for 4 h and then weighed.

The percentage of growth inhibition traits and average root and shoot growth inhibition of seedlings were determined using the following equations:

Percentage Inhibition = [(Control – Aqueous extracts)/Control] 100.

Average root inhibition = (Root length+ Root dry weight + Root fresh weight)/ 3

Average shoot inhibition = (Shoot length + Shoot dry weight + Shoot fresh weight)/3

Statistical Analysis

All data were subjected to analysis of variance using statistical Analysis system (SAS) software. The means of treatments were compared based on high significant difference (HSD) at 0.05 probability levels.

Results

The differences among rice hull extract concentrations and rice cultivars and their interactions were highly significant in all parameters. Rice cultivars hull extracts exhibited different allelopathic potential on barnyardgrass seedling growth. The original stock solution showed higher inhibitory activities as compared with 50% concentration and the control. The barnyardgrass root growth was more sensitive to rice hull extracts than shoot growth (Figure 1).

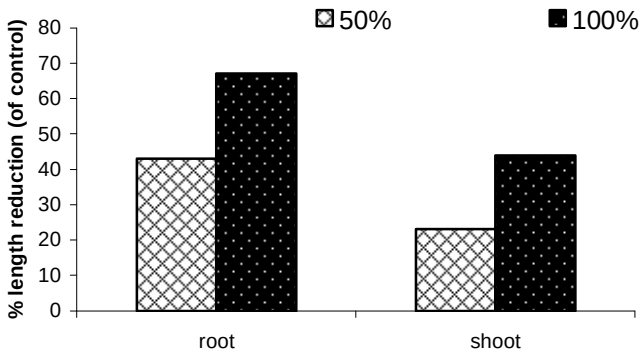

Figure 1. Percent reduction of barnyardgrass seedlings root and shoot length with rice hull extracts.

Seedlings Root Fresh Weight

Hull extracts from Dinorado and Musatarom had the most and least inhibitory effects on barnyardgrass root fresh weight, respectively (Table 1). Rice cultivars were grouped two categories according to their allelopathic activity on root fresh weight. The first group (55-80% inhibitory effect) consisted of 28 cultivars and the second group (40-55% inhibitory effect) consisted of 18 cultivars.

Seedlings Shoot Fresh Weight.

Both stimulatory and inhibitory effects on shoot fresh weight were found in 46 tested cultivars. Line-229 was the most stimulating cultivar, while the hull extract of Dinorado showed the most inhibitory activity on barnyardgrass seedling shoot fresh weight (Table 1). Rice cultivars were grouped into five categories. The first group consisted of six cultivars with stimulating range between15-33%. Second group consisted of eight cultivars (stimulating range: 1-14%). The third group consisted of 10 cultivars with inhibitory activity between 1-18%. The fourth group consisted of 19 cultivars (18-35% inhibitory) and the last group with inhibitory activity between 35-51% consisted of 3 cultivars: Dinorado, Neda and IR-60 (Table 1).

Seedlings Shoot Dry Weight

USEN cultivar's hull extract was the most stimulating (16.5%) and Dinorado was the most inhibitory one (48%). Rice cultivars were grouped into 4 categories. First group consisted of 6 cultivars with stimulating effect (0-16.5%). Second group consisted of 18 cultivars with inhibitory activity between 1-20%. The third group consisted of 20 cultivars (20-38%) and the last group (more than 40% inhibitory) consisted of two cultivars: Dinorado and Neda (Table 1).

Seedlings Root Dry Weight

Rice cultivars showed inhibitory activity between 12-53% on the root dry weight of barnyardgrass seedlings. Soluble hull extracts of Mehr and Neda showed the least and the most inhibitory activity on root dry weight of barnyardgrass seedling, respectively (Table 1).

Seedlings Root Length

Hull extracts of Alikazemi and Sepidrood showed a stimulatory effect on seedling root length of barnyardgrass seedlings (Table 1). Other rice cultivars showed inhibitory

activity between 10-80% on barnyardgrass seedling root length. Hull extracts from Gharib and Dinorado had the least and the most inhibitory effect on barnyardgrass seedling root length, respectively. Comparing the data based on the statistical differences, rice cultivars were grouped into four categories, including group I (1-20%) with seven cultivars, group II (20-40%) with 20 cultivars, group III (40-60%) with 16 cultivars and group IV (60-80%) with 3 cultivars (Table 1).

Seedlings Shoot Length

Hull extracts from rice cultivars were grouped into four categories, including group I with 12 cultivars having stimulating activities (50-80%), in which Line-229 had the highest stimulating activity, group II with 15 cultivars (30-50% stimulating), group III (0-30% stimulating) with 15 cultivars and group IV (1-34% inhibitory) with four cultivars. Dinorado showed the highest inhibitory activity (34%) on shoot length of barnyardgrass seedling (Table 1).

Effect on Root and Shoot Growth

By calculating the average inhibition of root growth, hull extracts of Dashtisard and Dinorado, showed the highest inhibitory effect on root growth. Alikazemi and Sepidrood had the cultivars with the least inhibitory effect. The cultivars with more than 60% inhibition effect were: Neda, Kadus, Dinorado and Dashtisard (Figure 2).

Similar evaluation was done for barnyardgrass seedling shoot growth. Dinorado and Neda were the cultivars with the highest inhibitory effect. Hull extracts of Line-229 and USEN showed the highest stimulating effect on shoot growth of barnyardgrass seedlings (Figure 3).

Table 1- Percent inhibitory or stimulatory effects of rice hull extracts on growth parametery of barnyardgrass seedlings

Treatment	RFW	SFW	SDW	RDW	RL	SL
DASHTISARD	-72	-25	-26	-52	-73	28
DASHTISARD	-72	-25	-26	-52	-73	28
DINORADO	-72	-51	-48	-40	-81	-33
KADUS	-69	-23	-34	-51	-62	45
NEDA	-69	-45	-47	-53	-58	-12
IR-28	-68	1	-11	-42	-60	64
IR-60	-66	-37	-37	-52	-44	-6
BEJAR	-63	-14	-24	-46	-46	55
IR-29	-61	-33	-35	-42	-52	11
IR-36	-58	-29	-29	-42	-52	27
DASHT	-62	-19	-13	-38	-49	51
HASANSARAE1	-64	-17	-26	-39	-43	40
SANGTAROM	-65	-20	-22	-30	-44	45
FAON 110	-65	5	-17	-37	-37	66
ANBARBOO	-55	-32	-36	-42	-42	41
DOLLAR	-56	-32	-27	-46	-37	1
TAICHUNG	-62	-21	-31	-44	-32	48
AMOL1	-64	9	-2	-35	-38	50
NEMAT	-62	13	-1	-28	-46	58
HASANSARAE2	-61	-13	-21	-40	-35	63
USEN	-59	23	17	-26	-49	57
AMOL3	-57	-19	-18	-25	-52	11
LINE-213	-60	-31	-30	-36	-36	24
IR-30	-60	19	7	-25	-46	65
DORFAK	-57	-24	-26	-33	-40	8
BINAM	-56	-4	-11	-34	-37	56
IR-3441	-58	-30	-22	-27	-40	44
GILL1	-52	1	-2	-32	-40	41
IR-67	-54	-6	-7	-22	-46	30
SHAHPASAND	-58	14	-10	-29	-35	58
MEHR	-59	-27	-27	-13	-49	-2

Continue table1

Treatment	RFW	SFW	SDW	RDW	RL	SL
CHAMPA	-57	16	-4	-28	-35	59
NP-125	-50	-13	4	-29	-37	38
KHAZAR	-57	-9	-17	-26	-32	43
AMOL2	-53	-9	-19	-33	-24	50
DOM SORKH	-54	14	-2	-28	-23	63
DOMZARD	-54	-19	-21	-19	-30	23
HASHEMI	-57	-9	-20	-32	-11	48
SANGJU	-44	20	10	-24	-25	33
LINE-229	-50	34	6	-27	-16	78
ZENIT	-46	-20	-32	-29	-18	9
M.CHAPARSAR	-46	-25	-32	-34	-13	17
GHARIB	-51	-30	-27	-32	-9	18
HASANI	-46	-28	-17	-20	-22	10
MUSATAROM	-42	24	13	-19	-25	33
SEPIDROOD	-43	8	-6	-30	4	25
ALIKAZEMI	-43	-16	-16	-27	5	23
CV	3.4	16.8	16.9	8.5	10.5	10.1
HSD (0.5)	15.2	18	18.5	18.2	19.6	22.9

R= root, S= shoot, L= length, F= fresh, D= dry, W= weight, (-) indicates the inhibitory effect

Effect of Rice Cultivar's Origin

The comparison of the effects of hull extracts of local and foreign cultivars on barnyardgrass seedling growth is presented in Figure 4. IRRI's allelopathic cultivars (Dinorado, Taichung, Dollar and IR-29) had more inhibitory activities as compared to domestic cultivars. However, the differences were not significant. Five rice cultivars had the highest allelopathic properties on barnyardgrass seedling growth including Dinorado, Neda, Dashtisard and IR-60 with inhibitory activity and Line-229, with stimulating effect. Hull extracts of Dashtisard and Dinorado showed the highest inhibitory effect on root growth while Alikazemi and Sepidrood had the least

inhibitory effect. Dinorado and Neda also had the highest inhibitory effects on seedling shoot growth and hull extracts of Line-229 and USEN showed the highest stimulating effect on barnyardgrass shoot growth.

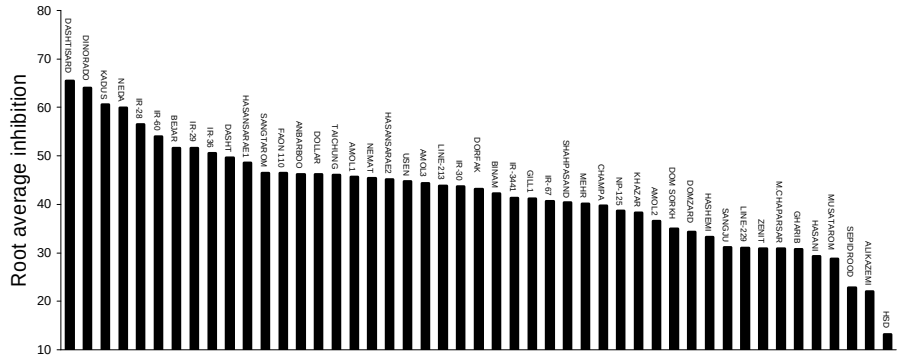

Figure 2. Average inhibitory effect of rice hull extracts on barnyardgrass seedlings root growth, (% of control).

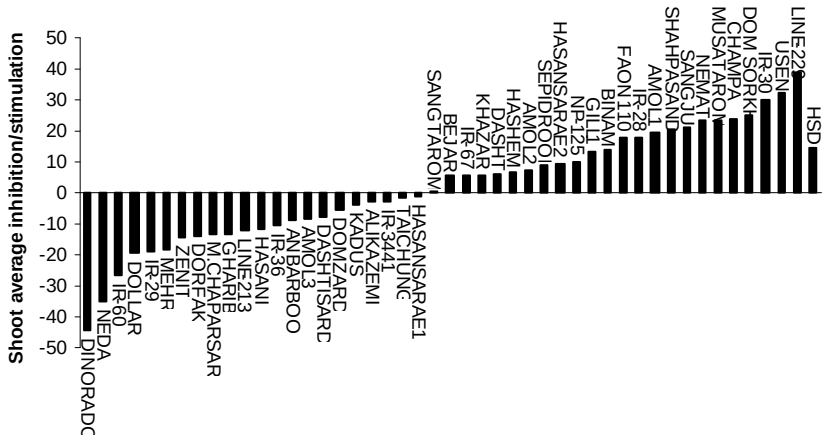

Figure 3. Average inhibitory/stimulatory effects of rice hull extracts on barnyardgrass seedlings shoot growth, (% of control). (+) indicates the stimulatory effects and (-) indicates the inhibitory effects of rice hull extracts.

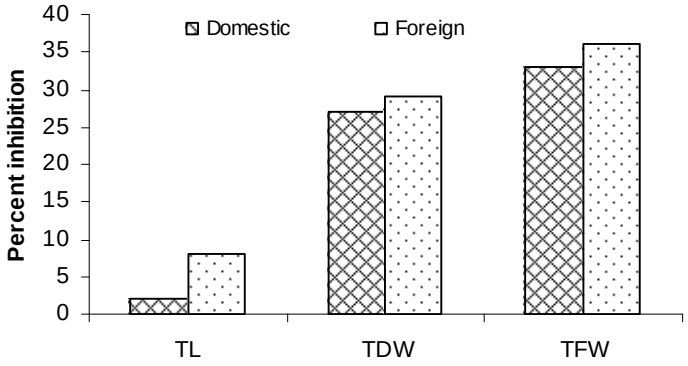

Figure 4. Comparison of allelopathic potential of rice hull extracts with different origin on barnyardgrass seedlings parameters TL=Total seedling length, TDW=Total Dry Weight, TFW=Total Fresh Weight

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DISCUSSION

The inhibitory effect of rice hull extracts on barnyardgrass seedling growth may reflect allelopathic potential of the rice cultivars. The magnitude of potential allelopathic effects varied between the studied rice cultivars. The results of this study are in agreement with those of Ahn *et al.* (2000), Chung *et al*. (2003), Asghari and Musavi (2002) and Dilday *et al*. (1991) who concluded that variation in allelopathic activity exists among cultivars. Among the 46 rice cultivars used in this study, Dinorado and Neda yielded extracts that reduced barnyardgrass total seedling length, total dry weight and total fresh weight more than other cultivars. These two cultivars may provide one of the most important gene resources for breeding rice cultivars with hulls high in allelopathic potential. Allelopathic potential can be a valuable trait to incorporate into rice cultivars to improve weed control. Comparing the effects of tested cultivars hull extracts on barnyardgrass seedling growth showed that the extracts could have either stimulatory or inhibitory effects on seedling growth. Rice (1984) reported stimulatory

effects at low concentrations of allelopathic substances, while inhibitory effects were reported at higher concentrations. Greater inhibitory effects on roots as compared with shoots (Figure 1) may be due to the direct contact of the root system to the extract solution in the growth media.

In this study, the inhibitory effects of hull extracts from Nemat and Dasht on barnyardgrass total seedling length, dry weight and fresh weight were (28, 22.5 and 35%) and (14, 30 and 32.5%), respectively. These results are in agreement with those reported by Asghari and Musavi (2002), on allelopathic activity of these cultivars in rice-barnyardgrass mixed in pots. Further investigations are needed to show a possible correlation between hull extracts and root exudates from an allelopathic rice cultivar.

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