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The Heterotoxicity of *Hordeum vulgare* L. Extracts in Four Growth Stages on Germination and Seedlings Growth of *Avena ludoviciana*

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Abstract: Phytotoxicity of barley extracts (*Hordeum vulgare* L.) on wild oat (*Avena ludoviciana* Durieu) was investigated. Water extracts five varieties of barley were bioassayed on germination and seedling growth of wild-oat to test the heterotoxicity of barley on wild-oat, study the dynamics of allelopathic potential over four growth stages and identify the most allelopathic plant part of barley in each stage. Whole barley plants were extracted at growth stage 4 (stems not developed enough), whilst for the following growth stages roots, stems, panicles and leaves were extracted separately. Seedling growth bioassays demonstrated that the wild-oat responded differently to the allelopathic potential of barley. For wild-oat radical growth and coleoptile growth were more depressed than germination, though. The allelopathic potential of barley plant parts was not stable over its life cycle for wild-oat. Leaves and stems were the most phytotoxic barley plant parts for wild-oat in the all stages. Among the varieties Eizeh appeared as the best one showing toxicity to seed germination of wild oat at its stage 4 and 8. Results suggested that the response by wild-oat varied depending on the source of allelochemicals (plant part) and the growth stage of the barley plant and kind of variety. The results led to conclude that Eizeh variety of barley was good to grow as it has good check on seed germination of wild oat plants as well as it also retarded the growth of root and shoot length of oat.

Key words: Allelopathy, phytotoxicity, barley, wild-oat, germination

INTRODUCTION

Allelopathy as a mechanism of plant interference in agro-ecosystems (Kimber, 1973) offers an opportunity to manage weeds in a crop sequence (Aldrich and Kremer, 1997) that could lead to reduced labor costs and increased efficiency (Chung *et al.*, 2003). Previous studies have shown that sorghum (*Sorghum bicolor* L.) vegetation possess a variety of potent inhibitors such as dhurrin, acyanogenic glycoside (Chung *et al.*, 2003) and phenolics (Inderjit, 2006) which are potentially allelopathic to weeds (Alsaadawi *et al.*, 1986; John and Nelson, 1998) with a maximum of inhibitory activity at harvest (Bogatek *et al.*, 2004). This was not the case for all grasses, some exhibited higher toxicity to wheat seedling growth when their residues were still green (Hedge and Miller, 1990). Straw extracts of Rice have the highest inhibition rate on Barnyardgrass (Chung *et al.*, 2003). The inhibition of barnyardgrass germination and seedling growth by rice hull extracts may reflect the allelopathic potential of individual rice culextivars.

Bioassays of germination, radicle growth and coleoptile growth are used to test the allelopathic potential of a crop species (Moncef *et al.*, 2001). The allelopathic potential can be observed in the form of autotoxicity as in the case of alfalfa (*Medicago sativa* L.) (Haskins *et al.*, 1984) or heterotoxicity as in the case of tall fescue (*Festuca arundinacea* L.) (Joung and Chung, 2000).

Since the allelopathy of small grain cereals has been little studied, the present work aimed to test the heterotoxicity of barley on wild-oat, study changes in allelopathic potential over four growth stages on wild-oat identify the most allelopathic plant part in each stages.

MATERIALS AND METHODS

Five barley (*Hordeum vulgare*) varieties namely Jonob, Kavir, Karoon, Tropy and Eizeh were grown at Research Institute of Forests and Rangelands, Ahwaz, Iran, in 2005 to 2007. 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 were observed. Destructive sampling of barley plants were made at its four growth stages (Kimber, 1973):

- Stage 4 = Leaf sheaths lengthening
- Stage 8 = Last leaf just visible
- Stage 10 = In boot
- Stage 11 = Grain development

At stage 4, whole plant of barley was used to prepare water extracts. Whereas at stage 8 and stage 10 roots, stem and leaves of barley plants were used to prepare water extracts and at stage 11 roots, stem, leaves and penicles were used to prepare water extracts. Water extracts of barley plants were prepared by following the methods described by Moncef *et al.* (2001). All of the water extracts prepared at different growth stages of barley were used to determine the allelopathic effect on seed germination, root length (cm) and shoot length (cm) of wild oat (*Avena ludoviciana*).

To determine the allelopathic effect of barley extracts wild-oat seeds were collected in October 2005, cleaned and stored at -35°C. Before the start of experiments for the determination of allelopathic effect, the wild oat 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 wild-oat seeds were placed on a filter paper in sterilized 9 cm diameter petri dishes. The experiment was designed under Completely Randomized Design (CRD) with four replications. Ten milliliter of the barley plant extract was added to each Petri dish and distilled water was used as

a control. All the Petri dishes were placed in a lighted growth chamber at 24°C. Germination percentage, seedling growth, root and shoot length were regularly recorded. The data was analyzed by using Analysis of Variance method and means were compared by using Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS

Extracts of whole plant (stage 4) of all barley varieties significantly affected the germination percentage, root and shoot length of wild oat plants (Table 1). As compared to control, maximum seed germination percentage was recorded in extracts of barley variety Karoon, very closely followed by Jonob variety, indicating very less allelopathic effect on seed germination of wild oat (Table 1). Minimum seed germination percentage recorded was noted in extracts

Table 1: Germination percentage, root length and shoot length wild-oat with water extract of barley varieties at stage 4

Source	df	Germination (%)	Root length (cm)	Shoot length (cm)
Analysis of variance				
Replication	3	87.153	4.802	109.687
Factor A	5	1164.375*	976.010*	711.795*
Error	15	412.153	20.723	55.589
Compare of treatment means				
Control		100.00a	39.530a	37.360a
Jonob		93.75a	3.538b	7.150b
Kavir		85.00ab	1.125b	6.600b
Karoon		97.50a	0.820b	7.625b
Tropy		75.00ab	1.375b	5.650b
Eizeh		55.00b	0.000b	0.000b

*Significantly different from control (p<0.05) assessed by Duncan's multiple range test. 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

Table 2: Germination percentage, root length and shoot length of wild-oat with water extract of different plant parts of barley varieties at stage 8

Source	df	Germination (%)	Root length (cm)	Shoot length (cm)
Analysis of variance				
Replication	3	877.564	160.791	217.147
Factor A	12	964.103*	590.540*	630.600*
Error	36	301.175	93.819	66.434
Compare of treatment means				
Control		100.0a	39.53a	37.36a
Root extract of Jonob		70.00bc	1.669f	3.563cde
Root extract of Eizeh		87.50ab	25.87ab	29.72a
Leaf extract of Eizeh		47.50c	4.207def	2.417de
Root extract of Karoon		80.00ab	19.28bcd	14.17bcd
Stem extract of Eizeh		96.25ab	25.44ab	25.03ab
Leaf extract of Jonob		91.25ab	5.330cdef	14.83bcd
Leaf extract of Karoon		72.50abc	1.000f	0.750e
Leaf extract of Kavir		100.0a	17.68bcde	30.29a
Stem extract of Karoon		91.25ab	2.728ef	1.900de
Stem extract of Jonob		93.75ab	15.98bcdef	15.84bc
Stem extract of Kavir		100.0a	20.35bc	16.25bc
Root extract of Kavir		97.50ab	2.017f	1.805de

*Significantly different from control (p<0.05) assessed by Duncan's multiple range test. 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

Table 3: Germination percentage, root length and shoot length wild-oat with water extract of different plant parts of barley varieties at stage 10

Source	df	Germination (%)	Root length (cm)	Shoot length (cm)
Analysis of variance				
Replication	3	77.587	219.908	137.845
Factor A	15	148.879 ^{NS}	498.640*	602.763*
Error	45	105.865	94.609	68.815
Compare of treatment means				
Control			24.31bc	37.36a
Root extract of Kavir			7.592def	6.575e
Root extract of Karoon			30.88ab	34.54a
Root extract of Jonob			5.843ef	5.313e
Root extract of Tropy			22.48bcd	13.21cde
Root extract of Eizeh			39.34a	31.15ab
Stem extract of Karoon			0.000f	0.000e
Leaf extract of Eizeh			6.265ef	6.758e
Stem extract of Eizeh			17.61bcde	26.39ab
Leaf extract of Tropy			7.360def	3.720e
Stem extract of Jonob			8.885cdef	6.853e
Stem extract of Kaveer			18.95bcde	20.67bcd
Leaf extract of Kavir			6.525def	11.29de
Leaf extract of Karoon			4.625ef	4.280e
Leaf extract of Jonob			18.44bcde	25.27abc
Stem extract of Tropy			2.578ef	6.805e

^{NS}No significantly different. *Significantly different from control ($p < 0.05$) assessed by Duncan's multiple range test, 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

Table 4: Germination percentage, root length and shoot length wild-oat with water extract of different plant parts of barley varieties at stage 11

Source	df	Germination (%)	Root length (cm)	Shoot length (cm)
Analysis of variance				
Replication	3	518.552	21.855	12.059
Factor A	20	1467.173*	521.472*	430.010*
Error	60	642.093	102.096	95.360
Compare of treatment means				
Control		100.0a	39.53a	37.36a
Stem extract of Kaveer		100.0a	27.92abcde	25.03abcdef
Root extract of Kavir		100.0a	34.50ab	32.28abc
Root extract of Jonob		77.50ab	13.50defgh	9.832fghi
Root extract of Eizeh		82.50ab	33.42abc	26.38abcde
Panicle extract of Kavir		78.75ab	18.34bcdefg	11.99efghi
Panicle extract of Eizeh		73.75ab	21.34bcdef	17.11cdefgh
Leaf extract of Karoon		78.75ab	7.485fgh	6.963ghi
Stem extract Karoon		60.00abc	11.97efgh	9.535fghi
Leaf extract of Kavir		67.50ab	16.71cdefgh	13.64defghi
Stem extract of Eizeh		100.0a	30.09abcd	31.48abc
Root extract of Tropy		60.00abc	4.125gh	6.270hi
Root extract of Karoon		63.75abc	22.61bcdef	14.95defghi
Stem extract of Tropy		77.50ab	19.13bcdefgh	14.83defghi
Stem extract of Jonob		93.75a	28.32abvde	22.94abcdefg
Leaf extract of Eizeh		65.00abc	0.1300h	0.1930i
Panicle extract of Jonob		82.50ab	41.03a	29.85abcd
Leaf extract of Jonob		68.75ab	21.63bcdef	17.73bcdefgh
Panicle extract of Karoon		76.25ab	31.00abc	33.51ab
Panicle extract of Tropy		25.00c	10.60fgh	9.060fghi
Leaf extract of Tropy		42.50bc	13.06efgh	17.58bcdefgh

*Significantly different from control ($p < 0.05$) assessed by Duncan's multiple range test. 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

of barley variety Eizeh showing a considerable allelopathic effect on seed germination. Maximum root length of wild oat plants was retarded by the extracts of barley variety Jonob. In case of shoot length of wild oat there were non significant differences were recorded among the extracts of barley varieties, however maximum allelopathic effect was recorded in the extracts of barley variety Eizeh.

At stage 8, the extracts of barley varieties significantly inhibited the seed germination of wild oat (Table 2). Maximum seed germination was reduced by the leaf extracts of barley variety Eizeh (Table 2). Root length of wild oat was strongly affected by the leaf extracts of Karoon variety, root extract of Jonob and root extract of Kavir variety. The allelopathic effect of leaf extract of Eizeh and Jonob barley varieties on root length of wild oat

was also considerable. With regard to shoot length significant allelopathic effect of barley was recorded in leaf extract of Karoon variety, root extract of Kavir variety, stem extract of Karoon variety, leaf extract of Eizeh and root extract of Jonob variety.

Allelopathic effect of different plant parts extracts of barley varieties at stage 10 showed non-significant differences on germination percentage while significant differences with regard to root length and shoot length of the plant (Table 3). Maximum root inhibitory effect was noted in the stem extract of Karoon variety, very closely followed by the stem extract of Tropy, leaf extract of Karoon, root extract of Jonob and leaf extract of Eizeh variety. Shoot length of wild oat was inhibited by stem extract of Karoon variety leaf extract of Tropy Leaf extract of Karoon root extract of Jonob root extract of Kavir and stem extract of Tropy variety of Barley.

Water extracts of different plant parts of barley varieties at stage 11 showed significant inhibitory effects in seed germination, root length and shoot length of the plant (Table 4). The compare of mean values showed that maximum seed germination was inhibited by panicle extract of Tropy variety (Table 4). With regard to root length of plant maximum inhibitory effect was showed by the leaf extract of Eizeh variety, closely followed by root extract of Tropy and leaf extract of Karoon varieties. Maximum shoot length inhibitory effect was showed by leaf extract of Eizeh variety, very closely followed by root extract of Tropy variety.

DISCUSSION

Results of present study suggested that the response by wild-oat (*Avena ludoviciana*) varied depending on the source of allelochemicals (plant part) and the growth stage of the barley plant and kind of the variety. Germination bioassays of barley at four different phenological stages were sensitive enough to detect the heterotoxicity potential of any plant component of barley. However, seedling growth bioassays were sensitive to allelopathic effects with the radicle being relatively more sensitive than the coleoptile (Table 1). Results of both types of bioassay are in agreement with the findings reported by Hedge and Miller *et al.* (1990), Kimber (1973), Panasiuk *et al.* (1986) and Weston *et al.* (1989).

The allelopathic potential of a barley plant on wild oat varied according to the source of extracts as was found with sorghum and white mustard by Bogatek *et al.* (2004), Guenzi *et al.* (1967), Inderjit (2006) and Sebile and Karaman (2007). In addition, the allelopathic potential of barley was unstable over the life cycle of the barley plant. This potential was at maximum near physiological maturity

as was for sorghum plant (Kimber, 1973). Seedling growth bioassays demonstrated that the wild-oat responded differently to the allelopathic potential of barley. For wild-oat radicle growth and coleoptile growth were more depressed than germination, though.

During all the growth stages leaves and stems were appeared as the most phytotoxic part of barley plant for seed germination, root and shoot length of oat, these results are in agreement with the findings of Moncef *et al.* (2001) and Yansen (2007).

These results support the use of seedling bioassays as a tool to screen for tolerance or sensitivity of a crop species to the allelopathic potential of wild oat and other crop species. This study suggests that the allelopathic compounds may serve as a potential natural herbicide by inhibiting seed germination and growth of oat. These varieties could be re-used to contribute to the control of wild oat in the barley fields and they may also be used as genetic markers to identify allelopathic varieties.

Overall the Eizeh barley variety showed allelopathic effects at its growth stages 4, 8, 10 and 11 by retarding the seed germination, root length and shoot length. Karoon and Jonob varieties showed toxicity only to root and shoot length of wild oat at their growth stages 8 and 10. Whereas, variety Kavir appeared as toxic to root length of wild oat at its growth stage 8, root and shoot length at its growth stages 10 and 11. These findings are also in conformity with the results of Moncef *et al.* (2001).

The results showed that Eizeh barley variety was the only whose growth stage 4 and 8 retarded the seed germination of oat whereas the other varieties retarded the root and shoot length of wild oat. These results were leading to conclude that Eizeh variety of barley is good to grow as it has good check on seed germination of wild oat plants as well as it also retarded the growth of root and shoot length of oat.

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