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Comparisons of Phytotoxicity of Barley Parts Extracts in Three Growth Stages on Annual Ryegrass

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Abstract: Problem statement: The importance of allelopathy in nature and in agroecosystem has attracted researcher's attention with the main goal of using the phenomenon in biological control of weeds. currently, active involvement of scientists from different disciplines made allelopathy a multidisciplinary subject, and transformed the research from basic to applied, enabling use of allelopathy in agriculture and forestry. Screening accessions of allelopathic crops and natural vegetation for their ability to reduce weeds is the basic approach for utilizing the phenomenon. **Approach:** Phytotoxicity of barley extracts (*Hordeum vulgare* L.) on Annual ryegrass (*Lolium rigidum* L.) was investigated. Water extracts of barley, four varieties were bioassayed on germination and seedling growth of *Lolium rigidum* to: (i) test the heterotoxicity of barley on *Lolium rigidum*, (ii) study the dynamics of allelopathic potential over three growth stages and (iii) identify the most allelopathic plant part of barley. Roots, stems and leaves were extracted at three growth stage separately. (iv) indicated which variety has the highest allelopathic potential. **Results:** Seedling growth bioassays demonstrated that the *Lolium rigidum* responded differently to the allelopathic potential of barley. For *Lolium rigidum* radicle growth and germination were more depressed than coleoptile growth, though. The allelopathic potential of barley plant parts was not stable over its life cycle for *Lolium rigidum*. Leaves were the most phytotoxic barley plant parts for *Lolium rigidum* in the all stages. Leaves extract of barely at stage 11 had the highest inhibition on germination. The most inhibition of coleoptile growth when treated with leaves extract at stage 11.At stage 8 the leaves extract of Jonob variety had the highest inhibition on radicle growth of *Lolium rigidum*. **Conclusions:** Results suggested that the response by *Lolium rigidum* varied depending on the source of allelochemicals (plant part) and the growth stage of the barley plant and kind of variety.

Key words: Allelopathy, *Hordeum vulgare* L., *Lolium rigidum* L., Heterotoxicity

INTRUDUCTION

Since the 1950, 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[2,16], but the application of biological weed control has often proved difficult in practice[19]. 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[13].

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[1,8-10]. Nevertheless, crop plants with superior weed suppressive ability under field conditions would be highly desirable in agriculture[18].

Globally, over 295 weed biotypes have now been reported to have acquired resistance to important herbicides. At least 177 weeds species, including 106 dicots and 71 monocots, have evolved resistance to herbicides[6]. Annual ryegrass (*Lolium rigidum*), one of the most widespread and troublesome weed, has developed resistance to 9 major herbicide groups, including glyphosate[6].

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The ineffectiveness of herbicides on resistant weed species and environmental imperatives, have prompted the search for non-herbicidal innovations to manage weed populations[23].

A number of studies have shown that there are large differences between crop cultivars in their ability to suppress weeds and these differences have been explained in part by means of variable capacity to secrete chemical substances affecting weed growth, i.e., allelopathy[19,22,].

Studies have shown that many species have allelopathic potential, such as Eucalyptus[4,15], Acacia[20], Pine[5], Sunflower[3], Alstonia[21] and .Allelopathy may be an important feature of barely varieties that allows them to compete with other plants.

Bioassays of germination, radicle growth and coleoptile growth are used to test the allelopathic potential of a crop species[16]. The allelopathic potential can be observed in the form of heterotoxicity as in the case of tall fescue (*Festuca arundinacea* L.)[11].

Since the allelopathy of small grain cereals has been little studied, the present work aimed to: (i) test the heterotoxicity of barley on *Lolium rigidum*, (ii) study changes in allelopathic potential over three growth stages on *Lolium rigidum* (iii) identify the most allelopathic plant part in stage, (iv) indicated which variety has the highest allelopathic potential.

MATERIALS AND METHODS

Four barley (*Hordeum vulgare* L.) varieties namely Jonob, Kavir, Karoon and Eizeh were grown at 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. Destructive sampling of barley plants were made at its three growth stages[12]:

Stage 8 = last leaf just visible
Stage 10 = in boot
Stage 11 = grain development

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 panicles were used to prepare water extracts. Water extracts of barley plants were prepared by following the methods described by[16]. 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 *Lolium rigidum*.

To determine the allelopathic effect of barley extracts, Annual ryegrass (*Lolium rigidum* L.) seeds were collected in October 2005, cleaned and stored at-35°C. Before the start of experiments for the determination of allelopathic effect, the *Lolium rigidum* 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 *Lolium rigidum* seeds were placed on a filter paper in sterilized 9 cm diameter Petri dishes. The experiment was designed under Completely Randomied Design (CRD) with four replications. A non-amended treatment was included as a control. For germination bioassays, 25 seeds were placed in a PD. Each experimental unit consisted of two PD. For radicle or coleoptile bioassays, an average across a cluster of 10 growths TT with one pre-germinated seed each was used as a single observation for each treatment. Analysis of variance was conducted using Dunkan program of MSTATC[21].

RESULTS

Extract of all growth stages of barley significantly affected the germination percentage of Annual ryegrass (Table 1). Maximum seed germination percentage was recorded was noted at stage 11 showing a considerable allelopathic effect on seed germination .In case of variety of barley there were non significant difference were recorded among the barley varieties(Table 2).

Table1: Analysis of variance table

		Mean^2		
Source	Freedom	Germination (%)	Root length (cm)	Shoot length (cm)
Replication	3	245.833	136.901	85.918
FA (Growth stage)	2	1159.028*	569.562*	46.539
FB (Variety)	3	68.519	251.567*	112.584
FC (Plant partition)	2	2912.674*	165.765	5502.763*
AB (Growth stage×variety)	6	1358.796*	5215.480*	64.819
AC (Growth stage×plant partition)	4	284.809	122.718	89.267
BC (Variety×plant partition)	6	677.720	113.258	139.362
ABC (Growth stage×variety×plant partition)	12	270.341	113.885	118.911
Error	105	329.286	84.827	83.206

*: Values are the means of four replications and variants are statistically significant at p<0/05 level, according to duncan s multiple range test

Table 2: Germination, radicle and coleoptile growth of annual ryegrass (*Lolium rigidum* L.) treated with water extract of plant parts, four variety and three growth stage of barley

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)
Stage8	86.46ab	11.79b	13.761
Stage 10	90.63a	13.62b	13.871
Stage 11	80.83b	18.46a	15.519
Kavir variety	84.306	16.89a	15.673
Karoon variety	86.250	16.75a	16.023
Jonob variety	85.694	11.51b	13.529
Eizeh variety	87.639	13.36ab	12.309
Stem	83.54b	13.29b	11.32b
Root	94.69a	25.65a	26.29a
Leaf	79.69b	4.935c	5.545c

*: Values are the means of four replications. Variants possessing the same letters (a, b, c and d) are not statistically significant at p<0/05 level, according to duncan s multiple range test

Table 3: Germination, radicle and coleoptile growth of Annual ryegrass (*Lolium rigidum* L.) treated with interaction of growth stages and varieties of water extract of barely

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)
Stage 8 of kavir variety	68.333c	10.58bc	11.898
Stage 8 of karoon variety	90.833ab	16.68b	18.006
Stage 8 of jonob variety	88.750ab	7.134c	13.843
Stage 8 of eizeh variety	97.917a	12.78bc	11.297
Stage 10 of kavir variety	92.083ab	14.77bc	15.476
Stage 10 of karoon variety	91.667ab	17.86ab	14.786
Stage 10 of jonob variety	92.500ab	10.41bc	12.288
Stage 10 of eizeh variety	86.250ab	11.45bc	12.935
Stage 11 of kavir variety	92.500ab	25.31a	19.647
Stage 11 of karoon variety	76.250bc	15.71b	15.277
Stage 11 of jonob variety	75.833bc	16.97b	14.457
Stage 11 of eizeh variety	78.750bc	15.86b	12.696

*: Values are the means of four replications. Variants possessing the same letters (a, b and c) are not statistically significant at p<0/05 level, according to duncan s multiple range test

Water extract of plant parts (roots, stems, leaves) of barely at stage 8, 10 and 11 significantly affected the germination percentage of Annual ryegrass. The allelopathic effect of leaf extract on germination percentage of *Lolium rigidum* also considerable.

Interaction effect of growth stages and different varieties of barley significantly inhibited germination of *Lolium rigidum*.

With regarded to seed germination percentage significant allelopathic effect of barley was recorded in stage 8 of Kavir variety and stage11 of Jonob, Karoon and Eizeh varieties, respectively (Table 3).

Interaction of different vareities with plant parts and Interaction of growth stage with plant parts there were non significant difference were recorded in seed germination of *Lolium rigidum* .

For radicle growth bioassay, extract from different growth stage and kind of barley variety significantly affected the root length of *Lolium rigidum*. The compare of mean value showed that maximum root length was inhibited by extract of stage 8 and Jonob variety.

Interaction effect of growth stage and different variety of barley significantly affected the root length of *Lolium rigidum*. Maximum root inhibitory effected was noted in the stage 10 of Jonob variety, very closely followed by stage 8 of Kavir variety.

In case of shoot length bioassay, only plant partition significantly affected the shoot length of *lolium rigidum* plant and maximum inhabitation was noted by leaf extract.

DISCUSSION

Germination bioassays of barley at three different phenological stages were sensitive enough to detect the heterotoxicity potential of any plant component of barley. Leaves extract of barely at stage 11 had the highest inhibition on germination. Results of bioassay are in agreement with the results reported by[7]. The present of allelochemicals like phenolic acids may be the reason for poor germination of the weeds[24]. The decrease in germinability was well correlated with increased membrane deteriotion, assayed as electrical conductivity and enhanced lipid peroxidation, detected as increased malondialdehyde content. Leaves were the most phytotoxic barley plant parts for Annual ryegrass (*Lolium rigidum* L) in the 4 variety at the all stages, these results are in agreement with the results reported by[16,24]. For *Lolium rigidum* radicle growth were more depressed than coleoptile growth. Its reported by[16] that seedling growth bioassays were sensitive to allelopathic effects with the radicle being relatively more sensitive than the coleoptile (Table 1 and 2).

Irrespective of the wheat species, radicle growth was generally reduced by barley extracts[16].

The allelopathic potential of a barley plant on Lolium rigidum varied according to the source of extracts as was found with sorghum and white mustard[3,10,16]. 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[12]. Seedling growth bioassays demonstrated that the *Lolium rigidum* responded differently to the allelopathic potential of barley. These inhibitory effects on the growth of radicles and coleoptile might be associated with a direct molecular alteration or as a specific growth are orientation in order to avoid allelochemicals[5].

The most inhibition of coleoptile growth when treated with leaves extract at stage 11.

At stage 8 the leaves extract of Jonob variety had the highest inhibition on radicle growth of wild oat.

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 varieties, response differences in the current study were attributed to genetic differences between varieties. It is possible that cultivars may produce different amounts of one of more allelopathic substances at a given extract concentration.

CONCLUSION

The results suggest that all four varieties of barely have the potential to be allelopathic in some ways, although the level of effects might differ. This indicates that allelochemicals produced by barley species have a strong potential to be inhibit other plants. Barely varieties are widely known to have secondary chemicals that might act as allelopathic agents. It can be concluded that barely have the potential to be allelopathic, although the level of their allelopathy varies. This potential then will benefit barely because they can compete strongly with other species to survive[24].

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 another crop species. This study suggests that the allelopathic compounds may serve as a potential natural herbicide by inhibiting seed germination and growth of *Lolium rigidum*. If these varieties are used to contribute to the control of *Lolium rigidum*, they may also be used as genetic markers to identify allelopathic varieties.

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