

Allelopathic potential of 50 barley cultivars and the herbicidal effects of barley extract

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ABSTRACT

The potential allelopathic activity of 50 old barley (*Hordeum vulgare* L.) cultivars was determined in Perlite-based bioassay with barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.]. Besides, the effect of aqueous extract of most allelopathic cultivar on sterile oat (*Avena sterilis* spp. *sterilis* L.), hood canarygrass (*Phalaris paradoxa* L.), blackgrass (*Alopecurus myosuroides* Huds.) and wild mustard (*Sinapis arvensis* L.) was investigated. Barley aqueous extracts adversely affected the root length and fresh weight of barnyardgrass than germination. The inhibitory effects of 2- and 6-row barley cultivars extracts were similar. The Greek cv. 'Athinaida' proved most inhibitory to barnyardgrass. The germination, root length and total fresh weight of winter weeds were reduced with its increasing concentrations. Its aqueous extracts were more phytotoxic to canarygrass and blackgrass (81.1 to 86.8% inhibition index), than sterile oat or wild mustard (43 to 80.1% inhibition index). These results indicated that some barley cultivars were more allelopathic and could be used in sustainable cereal production systems or as source of allelopathic traits.

Key words: Allelopathy, *Alopecurus myosuroides* Huds., *Avena sterilis* spp. *sterilis* L., barley cultivar, barnyardgrass, blackgrass, dose-response, *Echinochloa crus-galli* (L.) P. Beauv., hood canarygrass, *Phalaris paradoxa* L., *Sinapis arvensis* L., sterile oat, wild mustard, whole-range assessment.

INTRODUCTION

Allelopathy is major component of weed-crop interference and it could supplement the integrated weed management (6,25,30,32). Most cereals are allelopathic (2,5,13,15,18,22,28). The cold water extracts of barley, rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.) straws as well as alcoholic extracts of roots contained phenolic compounds, toxic to plant growth (7).

Most of the plant chemicals associated with allelopathic activity are secondary metabolites from shikimic acid, acetate or terpenoid pathways (19,31). The alkaloids hordenine and gramine, released from the germinating seeds of barley are allelochemicals (28) and barley allelopathic potential varied among cultivars (2,5). The gramine and

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hordenine plays significant role in barley allelopathic potential and its defense against weeds, insects or pathogens (12,28). The knowledge of barley allelopathic potential is also essential to avoid its adverse effects in crop rotations (3). However, very limited plant breeding has been done to improve the allelopathic ability of crops (14), while selection and breeding of last decades have decreased the allelopathic ability of barley cultivars (5). Barley cultivars with high allelopathic potential would be most appropriate for situations, where herbicide usage may be limiting, such as organic or integrated production systems. The importance of a single trait may not be sufficient for weed suppression but integrating a number of traits, such as competition for light and nutrients and allelopathy would offer a potential to utilise the inherited competitive ability (6). Kim and Shin (20) reviewed the role of breeding allelopathic crops and introduced method to breed allelopathic crops.

Although the old barley cultivars with great allelopathic potential may be useful to develop new commercial cultivars but, they have not been well described. Furthermore, data on the effects of barley allelochemicals on winter weeds are limited. This research aimed (i) to assess the allelopathic potential of 50 old winter barley cultivars from 15 countries and (ii) to find inhibitory effects of most allelopathic cultivar aqueous extracts on 4 major weeds (sterile oat, hood canarygrass, blackgrass and wild mustard) in Greece.

MATERIALS AND METHODS

Plant material

A field experiment was conducted during 2005-2006 at Technological and Educational Institute of Thessaloniki, northern Greece (22°44'10'' E, 40°37'06'' N, altitude 0 to 2 m, total annual rainfall: 380 to 570 mm and temperatures below 0 °C are recorded during winter). The soil was sandy loam (Typic Xeropsamment), [clay 80 g/kg, silt 280 g/kg, sand 640 g/kg, organic matter 11 g/kg and pH 7.6]. Nitrogen and phosphorus were applied as ammonium sulpho-phosphate (20-10-0) at 100 and 50 kg/ha, respectively, and added to soil before barley sowing. Also, 20 kg N/ha as ammonium nitrate (33.5-0-0) were applied in early April. Thirty (6-row) and twenty (2-row) barley cultivars from 15 countries (Table 1) were sown at 170 kg seed/ha on 30 October, 2005. Experiment was done in randomized complete block with three replicates. Plot size was 2 x 3 m. The test cultivars were selected for wide range of competitiveness, based on previous field experiments. In all plots, broadleaf weeds were controlled with 0.9 + 0.3 kg a.i./ha of bromoxynil + mecoprop (Brominal H EC, 120 + 360 g a.i./L) applied post-emergence or by hand-weeding, while, grass weeds were observed in very low densities. Other cultural practices were done as per the recommended production practices.

Seeds from mature plants of barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], sterile oat [*Avena sterilis* spp. *sterilis* L.], hood canarygrass [*Phalaris paradoxa* L.], blackgrass [*Alopecurus myosuroides* Huds.] and wild mustard [*Sinapis arvensis* L.], were collected in Thessaloniki, at the end of the growing season in September, 2004. All collected seeds were dried in greenhouse, air-cleaned to remove unviable seeds and other plant residues and stored in refrigerator at 3–6 °C until used for experiment. The viability of barnyardgrass, sterile oat, hood canarygrass, blackgrass and wild mustard seeds before sowing was 50, 45, 25, 25 and 25%, respectively.

Barnyardgrass bioassay

The experimental treatments consisted of two factors: (I). Barley cultivars 50 and (II). Barley aqueous extracts concentrations: 3 (0, 2, 4%, w/v). The treatments were replicated thrice in a completely randomized design. Barley plants of each cultivar were harvested at the ear emergence growth stage [Feekes growth stage (FGS) 10.1] (23). These were chopped into 5-cm long pieces, dried at room temperature (24 °C) for 72 h and grounded in Wiley mill through 1-mm screen. Then, aqueous extracts (w/v) were prepared by adding 4 or 8 g powdered material in 200-mL deionized water in 400-mL glass jars and shaken on horizontal shaker for 4 h at 200 rpm. The solutions were filtered through four layers of cheesecloth to remove debris, centrifuged at 1750 g for 1 h and the supernatants were filtered through filter paper (Whatman No. 42). The extracts were stored for one week at 4 °C until bioassayed. We used water-extract method of Ben-Hammouda *et al.* (3) and Kim and Shin (20) to evaluate the allelopathic potential of barley, because this method is based on water soluble substances and reduces the environmental influences (27). Generally, allelopathic studies are done with biological assays, under laboratory or controlled conditions to overcome the problem of separating competitive and allelopathic effects under field conditions (17). We used two aqueous extract concentrations (2 and 4%) for each barley cultivar as per Ben-Hammouda *et al.* (3) and Chung *et al.* (11).

Petri dish bioassays were done to assess the allelopathic effects of 50 barley old cultivars aqueous extracts on germination, root length and fresh weight of barnyardgrass in Perlite. Barnyardgrass was used as a indicator plant owing to its high germination ability and high sensitivity to barley allelochemicals (10,13).

Fifty barnyardgrass seeds were placed in Petri dishes (8.5-cm dia) and were covered with 6 g Perlite. Each Petri dish was moistened with 15 mL barley extract from each barley cultivar as per treatment. Deionized water was used in control Petri dishes. The Petri dishes were covered and kept on shallow trays in growth chamber (16 h light: 8 h dark, at 27±2 °C) for 8 days. At the end of incubation period, germination, root length and total fresh weight of germinated seeds were recorded and expressed as % of water-control. The experiment was conducted twice. Fungal contamination was not observed during these experiments.

Winter weeds bioassay

The barley cultivar 'Athinaida' aqueous extracts proved most inhibitory to germination and seedling growth of barnyardgrass, hence these Petri dish bioassays were done. The experimental treatments consisted of two factors: (I). Barley 'Athinaida' aqueous extract concentrations: 5 (0, 0.63, 1.25, 2.5, 5%, w/v) and (II). Test weed spp. 4 (sterile oat, hood canarygrass, blackgrass, wild mustard). The treatments were replicated thrice in a completely randomized design. Aqueous extracts (w/v) of 'Athinaida' barley were prepared by adding 1.25, 2.5, 5.0 or 10.0 g powdered dry plant material in 200-mL deionized water in 400-mL glass jars and shaking on horizontal shaker for 4 h at 200 rpm. Afterwards, the extracts were prepared as described previously. It gave aqueous extract concentrations of 0.63, 1.25, 2.5 and 5.0%.

The test weeds (sterile oat, hood canarygrass, blackgrass or wild mustard) seeds at 50, 100, 100 or 100, respectively, were placed in Petri dishes (8.5-cm dia) and were covered with 6 g Perlite. The Petri dishes were moistened with 10 mL 'Athinaida' barley extract per Petri dish.

Deionized water was used as control. The Petri dishes were covered and kept on shallow trays in growth chamber (8 h light: 16 h dark, at 22 ± 2 °C) for 12 days. At the end of the incubation period, plants were removed from the Petri dishes, carefully washed free of Perlite, and germination, root length and total fresh weight (of germinated seeds only) were measured. Measured data were expressed as % of water-control. The experiment was conducted twice.

Inhibition indices

The allelopathic dose-response effects of barley ‘Athinaida’ aqueous extract on barnyardgrass germination, root length and fresh weight were assessed by the Whole-range assessment method (1). Inhibition index was calculated by Equation #1 used by Liu *et al.* (26) where concentrations tested ranged from 0 to D_n , D_c was the threshold dose at which response equaled the value of control and above which the responses were inhibitory, $R(0)$ was the response at 0 aqueous extract concentration (control) and $f(D)$ represented the response function. The germination, the root length or the total fresh weight inhibition areas across the whole range of barley ‘Athinaida’ aqueous extract concentrations and the corresponding Inhibition indices (I) were calculated separately for each replicate using the WESIA (Whole-range Evaluation of the Strength of Inhibition in Allelopathic-bioassay) software (26) and afterwards were subjected to a combined over repetition time ANOVA.

$$I = \int_{D_c}^{D_n} [R(0) - f(D)] dD / \int_0^{D_n} R(0) dD \quad [1]$$

Statistical analyses

For the barnyardgrass bioassay data, a combined over repetition time ANOVA was performed using a factorial approach (barley cultivar extract x aqueous extract concentration). Data were not transformed before the ANOVA as it was not needed. Also, single degree of freedom contrasts comparing the main effects of the two barley groups (2-row and 6-row) on germination, root length and total fresh weight of barnyardgrass were performed. For the winter weed bioassay data, a combined over repetition time ANOVA was also performed using a factorial approach (weed species x aqueous extract concentration). The programme SPSS (34) was used to conduct the analysis of variance and the LSD procedures were used to detect and separate mean treatment differences at $P=0.05$.

RESULTS AND DISCUSSION

Barnyardgrass bioassay

The aqueous extract concentrations, barley cultivar and barley cultivar x aqueous extract concentration interactions significantly affected the barnyardgrass germination, root length and total fresh weight (Table 1).

Germination

Barley cultivars inhibited the germination of barnyardgrass (Table 1). The 2- and 6-row barley cultivars extracts caused similar inhibition in barnyardgrass germination (14 and 13%, respectively) (Fig. 1). Barley cvs ‘Athinaida’, ‘Gloria’s-Copal’s (30B-4Y-22M)’

Table 1. Inhibitory effects (% of water-control) of 50 barley aqueous extracts on germination, root length and total fresh weight of barnyardgrass. Means are averaged across two experiments

Barley cultivars (Country)	Germination		Root length		Fresh weight	
	Aqueous extract concentration (g dry matter per 100 mL)					
	2	4	2	4	2	4
6-Row spike	-% of control					
Athinaida (Greece)	68.7	36.3	58.5	31.2	42.7	16.3
Pistacho (Mexico)	86.0	72.0	91.9	56.7	90.3	71.2
Pistacho (2B-1Y-1B) (Mexico)	89.0	76.4	67.1	39.9	70.7	62.6
Trompilo (Mexico)	96.2	80.9	43.0	14.0	85.5	45.0
Gloria's-Come's (6B-2Y-8M) (Mexico)	91.8	78.8	63.6	40.7	87.8	69.3
Gloria's-Come's (5B-5Y-1M) (Mexico)	98.0	94.2	58.5	22.1	90.3	62.9
Gloria's-Come's (4Y-1H-6Y-2M) (Mexico)	93.5	85.9	73.5	38.2	93.0	59.4
Gloria's-Come's (17Y-1B-1Y) (Mexico)	88.6	83.4	69.7	40.6	87.6	61.8
Gloria's-Come's (4Y-1H-6Y-4M) (Mexico)	95.7	88.9	63.6	33.2	88.7	66.1
Gloria's-Copal's (6B-2Y-1M) (Mexico)	100.2	96.5	61.2	23.7	97.1	65.7
Gloria's-Copal's (30B-4Y-20M) (Mexico)	92.0	84.0	56.7	24.8	89.7	57.0
Gloria's-Copal's (30B-4Y-1M) (Mexico)	94.3	83.5	56.7	22.2	84.0	46.4
Gloria's-Copal's (30B-4Y-23M) (Mexico)	88.5	88.6	56.2	21.1	82.7	55.9
Gloria's-Copal's (30B-4Y-12M) (Mexico)	95.7	74.0	77.3	28.9	99.2	57.6
Gloria's-Copal's (30B-4Y-22M) (Mexico)	81.4	69.2	66.8	31.2	83.4	55.1
Caco's (Mexico)	92.6	95.4	70.9	47.7	89.2	69.2
Robust (USA)	90.0	75.0	69.8	43.4	85.1	63.5
Karla (USA)	93.8	92.6	70.9	37.6	90.7	62.4
Arupo's (Mexico)	98.0	88.7	76.9	38.1	97.6	74.6
Bedford (Canada)	100.5	89.7	71.1	42.8	97.4	76.3
Karan 280 (India)	92.0	84.9	76.1	27.2	85.2	52.4
Heatland (Canada)	85.6	72.2	74.2	36.8	91.0	60.7
Mantman's-EH 165 (Mexico)	79.4	72.0	66.6	29.6	81.2	49.7
Paico's (Mexico)	92.7	90.9	60.2	31.2	98.7	63.4
M 9724 B (Mexico)	90.6	81.4	76.0	30.9	96.1	66.1
Lignee 640 (France)	95.5	94.0	78.6	32.0	89.7	76.5
Galt-Brea's (Mexico)	98.7	92.8	80.6	47.8	98.6	88.3
Ruda's (Mexico)	99.9	86.1	86.8	64.9	96.4	85.4
Bulk Resist. Desc-Sutter (Mexico)	94.9	80.1	70.2	38.3	94.6	61.5
Malebo (Australia)	86.9	77.9	69.8	40.0	95.6	58.3
2-Row spike						
ND 4994.16 (USA)	88.8	84.1	64.7	49.2	92.4	73.6
Antartica (Brazil)	71.9	71.2	83.0	53.1	68.7	46.8
Cerise (UK)	82.7	76.2	85.7	79.5	79.8	61.5
Mata (New Zealand)	86.3	86.0	76.4	44.2	83.3	65.2
Manapou (New Zealand)	96.1	95.4	63.2	26.4	90.1	67.8
Ideal (German)	99.6	96.1	56.6	25.6	92.4	60.9
Bowman (USA)	91.4	87.1	70.5	52.0	82.6	81.4
Cunhild (Denmark)	91.2	78.0	66.4	31.8	78.8	55.6
Patty (France)	78.8	79.6	69.4	37.4	79.7	58.1
Stirling (Australia)	94.7	85.7	81.6	38.6	97.1	79.5
Guanajuato (Mexico)	96.0	87.7	59.0	23.0	86.8	53.1
Koral (German)	93.1	76.5	72.1	25.2	86.2	50.0
Shanon (Australia)	81.3	68.6	73.3	37.3	78.9	62.4
Helena (The Netherlands)	73.2	62.6	71.2	35.5	76.5	50.7
Lignee 640-Kober-Teran 78 (Mexico)	91.1	69.7	63.4	15.4	91.6	41.1
Teran 78 (Ecuador)	95.7	81.1	59.9	22.1	98.0	57.2
Roland (Sweden)	95.6	86.1	67.5	33.2	88.8	65.3
Legia (France)	88.2	86.8	99.0	70.8	89.5	90.0
Goldmarker (UK)	98.5	96.7	75.6	49.5	96.2	87.2
Goldmarker-Ark Royal-G. Promise (UK)	92.6	77.8	73.0	26.2	97.0	62.2
LSD (0.05)	14.1		8.6		14.7	
CV (%)	14.3		14.2		17.2	

and 'Helena' at both aqueous extract concentrations caused maximum inhibition in germination of barnyardgrass (31 to 64, 19 to 31 and 27 to 37%, respectively). Whereas cvs 'Gloria's-Copal's (6B-2Y-1M)', 'Bedford', 'Galt-Brea's', 'Manapou', 'Ideal' and 'Goldmarker' were least inhibitory to germination (0 to 3, 0 to 10, 1 to 7, 4 to 5, 0 to 4 and 1 to 3%), respectively.

Seedling growth

Barley cultivars also inhibited the root length and total fresh weight of barnyardgrass. However, at both aqueous extract concentrations (2 and 4%), barnyardgrass germination was affected less than root length or total fresh weight (Table 1). The 2- and 6-row barley cultivars extracts caused similar inhibition in barnyardgrass growth (Fig. 1). Six-row barley cultivars extracts caused 48 or 25% inhibition, in root length or total fresh weight of barnyardgrass, respectively, while the corresponding inhibition from 2-row barley cultivars extracts was 45 or 25%. The higher concentration of 6-row barley cultivars extracts caused greater inhibition in root length and total fresh weight than extracts of 2-row cultivars.

The higher aqueous extract concentration (4%) inhibited the barnyardgrass root length (29 to 86%), while, 2% concentration caused 1 to 57% inhibition (Table 1). The 4% extract concentration of barely cvs 'Trompilo' and 'Lignee 640-Kober-Teran 78' caused maximum inhibition (86 and 85%, respectively) in root elongation of barnyardgrass, while the extracts of cvs 'Legia' and 'Cerice' caused lower inhibition (29 and 20%, respectively). The 2% aqueous extract of 'Trompilo' caused 57% inhibition in root length of barnyardgrass, while the lowest inhibition was by 'Legia' (1%), 'Ruda's' (13%) and 'Cerice' (14%).

Total fresh weight of barnyardgrass at both aqueous extract concentrations was adversely affected most by 'Athinaida' (57 to 84%) and least by 'Galt-Brea's' (1 to 12%). However, total fresh weight inhibition caused by aqueous extracts of the other barley cultivars ranged from 3 to 31% at the lower aqueous extract concentration and from 10 to 59% at the higher aqueous extract concentration (Table 1).

The greatest inhibitory effect on barnyardgrass, as averaged across germination, root length and total fresh weight inhibition, was caused by the cultivar 'Athinaida' (58%), followed by 'Trompilo' (39%), 'Helena' (38%), 'Lignee 640-Kober-Teran 78' (38%) and 'Mantnan's-EH 165' (37%) (data not shown).

The variabilities in inhibitory effects of 50 barley cultivars on barnyardgrass germination, root length or total fresh weight inhibition could be attributed to their differences in kind, total amount and physicochemical characteristics of allelochemicals produced by different barley cultivars. Similar results were reported by Bertholdsson (5) who found that barley cultivars originated from different countries had different allelopathic activity on ryegrass (*Lolium perenne* L.). Burgos *et al.* (9), Chung *et al.* (11), Dhima *et al.* (13), Hanson *et al.* (16) and Lemerle *et al.* (24) also found similar results working with extracts taken from different rye, barley, wheat, triticale and rice (*Oryza sativa* L.) cultivars. Regarding the chemistry of substances involved in allelopathy, Liu and Lovett (28) reported that the allelopathic effects of barley could be attributed to the alkaloids hordenine and gramine released from germinating seeds. Baghestani *et al.* (2) and

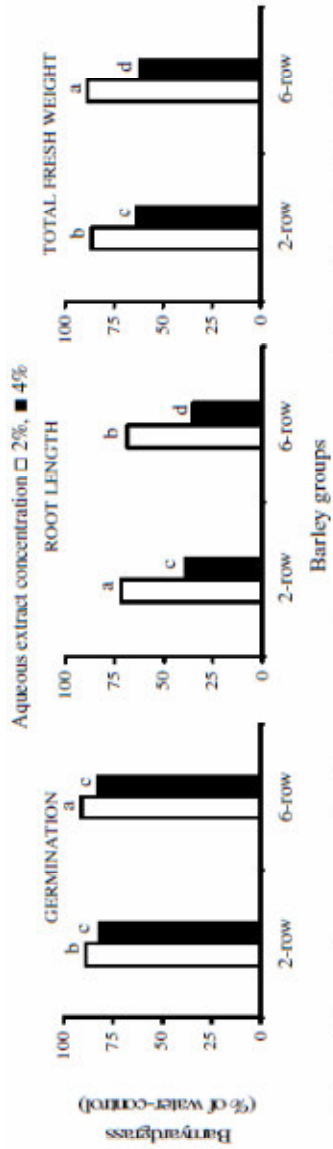


Figure 1. Inhibitory effects (% of water-control) of two barley groups (2- and 6-row) on germination, root length and total fresh weight of barnyardgrass. Columns within each graph with different letters indicate significant differences at $P < 0.05$.

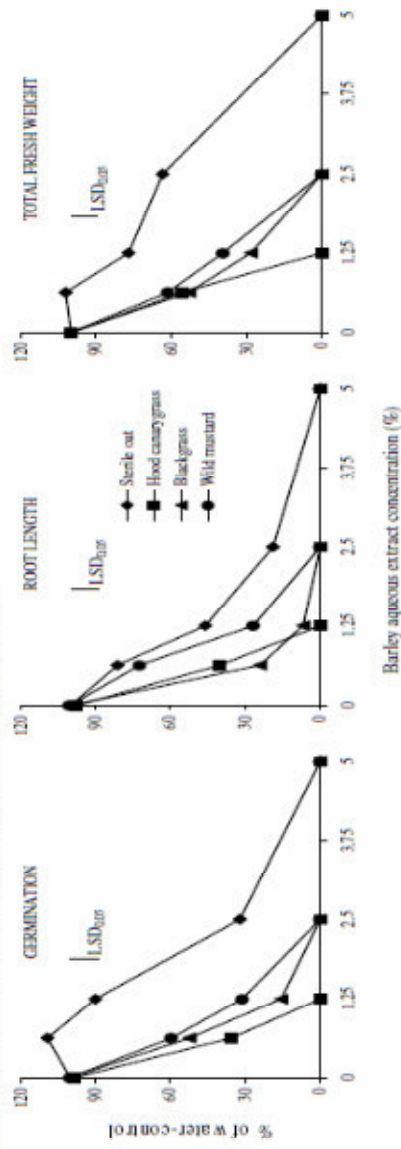


Figure 2. Inhibitory effect (% of water-control) of 'Athinada' barley aqueous extracts on germination, root length and total fresh weight of sterile out, hood canarygrass, blackgrass or wild mustard as affected by concentration. Means are averaged across two experiments.

Ma *et al.* (29) reported that barley germplasm contained higher phenolic concentrations than wheat germplasm, while Ben-Hammouda *et al.* (4) found that leaves were the most important source of allelopathic substances in barley. However, in contrast to these findings, Kudsk *et al.* (22) found minor differences in hordenine and gramine concentrations among the wild types and tested barley cultivars, which could not explain their observed variations in the allelopathic activity.

The increased inhibition of barnyardgrass germination, root length and total fresh weight with increasing aqueous extract concentration agrees with results of Burgos and Talbert (8), Chon and Kim (10) and Dhima *et al.* (13). In addition, the similar germination, root length or total fresh weight inhibition of barnyardgrass by extracts (averaged across concentrations) taken from both 6- and 2-row barley cultivars is in agreement with the results of Bertholdsson (5), who found that the Finnish 2- and 6-row barley cultivars had similar inhibitory effects on ryegrass used as bioassay plant indicator.

Winter weeds bioassay

The germination, root length and total fresh weight were significantly affected by weed species, 'Athinaida' aqueous extract concentration and weed species x 'Athinaida' aqueous extract concentration interactions. As the ANOVA did not indicate in most cases significant repetition time or repetition time x treatments interaction, the weed species x 'Athinaida' aqueous extract concentration interaction means are presented (Fig. 2).

I. Germination

The 'Athinaida' aqueous extracts significantly inhibited the germination of sterile oat, hood canarygrass, blackgrass or wild mustard (Fig. 2). However, Ben-Hammouda *et al.* (3) reported that extracts of barley plants did not affect the seed germination of either durum (*Triticum durum* L.) or bread wheat cultivars. The tolerance differences among the plant indicators used could account for the inhibition differences caused by test cultivars.

The increasing Athinaida aqueous extract concentration reduced the germination of test weeds but not proportionally (Fig. 2). Regarding susceptibility differences between the four weeds examined, the comparison of estimated inhibition indices (*I*) showed that the susceptibility order for germination was hood canarygrass = blackgrass = wild mustard > sterile oat (Table 2). The estimated 50% growth inhibition of sterile oat, hood canarygrass, blackgrass or wild mustard indicated that 50% germination inhibition was caused by Athinaida aqueous extract of 0.36 and 2.17% (data not shown).

II. Seedling growth

The 'Athinaida' aqueous extracts also inhibited the root growth and fresh weight of sterile oat, hood canarygrass, blackgrass or wild mustard (Fig. 2). In particular, although most of the weed seeds germinated in the presence of 'Athinaida' aqueous extracts, but their development was abnormal and finally did not survive. These results are in agreement with Liu and Lovett (28) who found that barley extracts inhibited the white mustard (*Sinapis alba* L.) emergence and initial growth. Overland (33) also reported that both gramine and hordenine, in barley extracts, inhibited the growth of common chickweed [*Stellaria media* (L.) Cyr.] and shepherd spurge [*Capsella bursa-pastoris* (L.) Medic.].

Table 2. Inhibitory effects of *Athinaida* cvr aqueous extracts on growth of sterile oat, hood canary grass, blackgrass or wild mustard as per inhibition indices (*I*) estimated from WESIA (Whole-range Evaluation of the Strength of Inhibition in Allelopathic-bioassay) software

Weed species	Germination	Root length	Total fresh weight
		Inhibition index ¹	
Sterile oat	52.8 b	68.1 c	43.0 c
Hood canarygrass	86.4 a	85.8 a	85.2 a
Blackgrass	83.0 a	86.8 a	81.1 ab
Wild mustard	80.1 a	79.8 b	79.0 b
CV (%)	7.7	2.3	6.7

¹Means within each column followed by the same letter are not significantly different according to the LSD test at the 5% level

The increasing concentrations of *Athinaida* aqueous extract reduced the root length or total fresh weight of test weeds but not proportionally (Fig. 2). These results agree with Ko *et al.* (21) and Ma *et al.* (30) who found that increasing concentration of rice water-extracts (0.63 to 5.0 g/100 mL) decreased both root and shoot growth of barnyardgrass. Regarding susceptibility differences between the four weeds examined, the comparison of estimated inhibition indices (*I*) showed that the susceptibility order was hood canarygrass = blackgrass > wild mustard > sterile oat for root length and hood canarygrass ≥ blackgrass ≥ wild mustard > sterile oat for total fresh weight (Table 2).

The estimated 50% growth inhibition of sterile oat, hood canarygrass, blackgrass or wild mustard indicated that the 50% root length or total fresh weight inhibition was caused from *Athinaida* aqueous extract concentrations between 0.31 and 1.20 or 0.50 and 2.50%, respectively (data not shown). These results are in agreement with Ma *et al.* (29) who found that rice water-extracts concentrations of 1.75 to 3.50%, caused 50% inhibition root or shoot growth of barnyardgrass.

To determine the inhibitory effects of barley aqueous extracts, we used the soil-free Petri dish bioassay for greater reliability. Because the inert material used (perlite instead of soil) in the bioassay eliminates the possible differences in inhibitory effects related to barley aqueous extracts adsorption on soil colloids and consequently increases the accuracy and reproducibility of the method to determine the inhibitory effects.

Our results indicate that barley cultivars like '*Athinaida*', '*Trompilo*', '*Gloria*'s-Copal's (30B-4Y-22M)', '*Helena*' and '*Lignee 640-kober-Teran 78*' produce high amounts of substances with increased allelopathic potential on barnyardgrass. Besides, the aqueous extracts of cv '*Athinaida*' significantly reduced the germination and initial growth of sterile oat, hood canarygrass, blackgrass and wild mustard. Therefore, these allelopathic cultivars could be used in sustainable crop production systems and could reduce the amount of herbicides used to control these four winter weeds species. Furthermore, efforts in allelopathy studies should be continued (i) incorporate these allelopathic traits into the commercial barley cultivars, (ii) determine the chemistry of allelochemicals found in these cultivars and (iii) examine their potential to develop as natural herbicides.

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