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ABSTRACT

The allelopathic potential of the foliage tissues of eight barley cultivars was investigated using seedlings bioassays of Indian brown mustard (*Brassica juncea*) and green foxtail (*Setaria viridis*) as typical broadleaf and grass weeds of the croplands. The correlations of allelochemical compounds with seed germination of weeds were determined. The barley cultivars used were Jackson, Bronco, CDC Dolly, B1602, Harrington, UNA80, Stander, and TR251. Three dilutions of the extracts of each barley cultivar (20, 10 and 5 g/L) plus deionized distilled water, as a control, were assayed on the target weeds. The effects of barley cultivars and extract concentrations on seed germination and radicle and shoot lengths of the target weed seeds were highly significant (P<0.001). By increasing the concentration of the allelopathic extracts, the percent germination, radicle and shoot lengths of both target plants seedlings decreased. Seed germination and seedling growth of *B. juncea* were more sensitive than those of *S. viridis*. Relative to the germination inhibition of target weed seeds in comparison to the untreated control, the selected barley cultivars were categorized as being highly allelopathic, moderately allelopathic and of low allelopathic potential. Among the phenolic compounds detected, the cumulative effect of four small-quantity-compounds (vanillic, chlorogenic, *p*-coumaric, and ferulic acids) on average weed germination was very high (R² = 0.83), with *B. juncea* it was high (R² = 0.70) and with *S. viridis* it was very low (R² = 0.15). The two high concentration phenolic compounds of protocatechuic and *p*-hydroxybenzoic acid, when accumulated with the small-quantity phenolic compounds of barley cultivars, reduced the weed seeds germination correlation to r=−0.11.

**Keywords:** Allelopathy, Aqueous extracts, Barley, *Brassica juncea*, HPLC, Phenolic acids, *Setaria viridis*.

INTRODUCTION

Allelopathy is a mechanism of plant interference in agroecosystems that offers an opportunity to manage weeds in crop sequence but could also adversely affect crop yields and influence the choice of rotation. The allelopathic potential of many crop plants has been investigated and approved (Burgos *et al.*, 1999; Baghestani *et al.*, 1999; Wu *et al.*, 2001). Heavy use of herbicides in most integrated weed management (IWM) systems is a major concern since it causes serious threats to the environment, public health and increases costs of crop production. The degree of weed seed germination inhibition and growth suppression which can be attributed to crop allelopathy is highly important and worth while. This can be considered as a possible alternative weed management strategy (Macias, 1995).

Barley (*Hordeum vulgare* L.) is a smoother crop and possesses the allelopathic potential to suppress some weeds (Liu and Lovett,
In a modified bioassay system with reduced environmental influences, Liu and Lovett (1993b) found that root of barley released allelopathic chemicals which delayed germination and inhibited growth of white mustard (Sinapis alba L.). In further studies using HPLC they found that hordenine, as a major component of barley root which released up to a maximum of 2 µg/plant/day for up to 60 days in a hydroponics system, is responsible for the growth retardation. The mustard radicle tips exposed to hordenine and gramine showed cell wall damage, an increase in both size and number of vacuoles, autophagy, and disorganization of organelles (Liu and Lovett, 1993b). A linear relationship was found between peak area and concentration of authentic standards of both hordenine and gramine using the HPLC method for barley seedlings (Hoult and Lovett, 1993).

Baghestani et al. (1999) found that various concentrations of spring cereal cultivar root extracts inhibited Brassica kaber hypocotyls and root growth with no negative effects on germination. As cereal root exudate concentrations increased, B. kaber growth decreased. Benzoic, caffeic, o-coumaric, and vanillic acids, scopoletin and para-hydroxybezoic acid were all found in barley and other examined cereal seedlings using HPLC. Smith and Martin (1994) have found that the extract of leaf and stem tissue of Italian ryegrass (Lolium multiformum), little barley (Hordeum pusillum) and tall fescue (Festuca arundinacea) suppressed seed germination and seedling growth of alfalfa (Medicago sativa). They have estimated that concentrations of about 5.0 g/L of aqueous extracts from foliage tissue of little barley reduced seed germination and seedling growth in alfalfa and Italian ryegrass by 50%. Production of allelopathic compounds varies within the same species (Barnes et al., 1987). Understanding the allelopathic potential of crop cultivars is very important for weed management, use as cover crops in conservation tillage, breeding or engineering cultivars to enhance allelopathic production (Burgos et al., 1999).

The objectives of the present experiments were to find the potential allelopathic property of eight barley cultivar aqueous extracts on the germination and seedling growth of isolates and quantify the phenolic compounds of barley cultivars to determine whether or not these allelochemicals correlated with the target weed’s seed germination.

**MATERIALS AND METHODS**

**Seedbed Preparation and Barley Cultivation**

To provide an adequate seedbed for barley growth under greenhouse conditions, the silty loam provided was sterilized in an oven using 2.1 kg cm⁻² pressure under 132 °C for 1 hour, then mixed with pre-sterilized Mito-Mix 290 (a horticultural product) using machine mixture. Thirty-two 25×50×7 cm flat plastic culture trays were filled each with five kg of the prepared soil, then mixed with 30 gr of slow release 14:14:14 NPK fertilizer. The trays were placed in a greenhouse with 14 hours light per day, and a 22/16 °C day/night temperature regime. The trays were irrigated to field capacity and the soil pH was determined (pH=6.94). The seedbed was corrugated longitudinally in five rows, 4 cm apart. One hundred uniform seeds of each barley cultivar were sown in each tray (20 seeds per row). The trays were sprayed with 0.1 % of No-dump (Oxine benzoate 2.5%) fungicide to control soil-borne diseases.

Eight barley cultivar seeds were obtained from Alberta Agricultural Research Station (Canada) including Jackson, Bronco, CDC Dolly, B1602, Harrington, UNA80, Stander, and TR251. The trays were replicated four times. The trays were irrigated every other day and allowed to germinate and grow for 30 days. The foliar section of each plant was cut from the base and stored in labeled paper bags. The samples were freeze dried for three days, and ground with a Wiley mill to pass through a 40-50 mesh screen. The
ground tissues were used for allelochemical extractions.

**Aqueous Extracts Preparation**

Ten grams of each cultivar ground tissue were placed in a 1-L Erlenmeyer flask, and 500 ml deionized distilled water (DDI) was added to it. The flasks were covered with aluminum foil to protect them from photodecomposition, then placed on a rotary shaker (≈ 250 revolutions per min.) at laboratory temperature (≈ 22°C) for 10 hours. The mixtures were filtered through 4 layers of cheesecloth and two layers of Whatman No.1 using a vacuum pump. The pH and electrical conductivity of the extracts were determined using a digital pH meter and a conductivity meter. These filtrates were considered as stock solutions. A series of solutions including the stock solution (extract of 20 g dry weight per liter of water; (S1), and concentration dilutions of 10 (S2), and 5 g/L (S3), were developed from the stock solutions. The extra stock solutions were kept in −20°C for later use.

**Petri Dish Bioassays**

The barley cultivar young plant aqueous extract bioassay was conducted on seed germination, growth of radicle and shoot appearance of *B. juncea* (Indian brown mustard) and *S. viridis* (green foxtail) as typical broadleaf and grass cropland weeds. The seeds were pre-sterilized with 1% sodium hypochlorite for 5 minutes and washed with distilled water. Three dilute series (S1, S2 and S3) of each cultivar's aqueous extract plus the control (DDI water) were used for germination tests. Fifteen *B. juncea* or *S. viridis* seeds (pre-tested seeds with >97% germinabilities) were evenly distributed on two layers of Whatman No.1 filter papers in each 9-cm disposable sterile Petri dish. Five ml of a dilute series was added to each Petri dish, covered with a lid, sealed with parafilm, then incubated at 22°C for five days. The bioassay was replicated four times and the experiment was repeated once.

Seeds were considered germinated if the radicle had emerged 2 mm from the seed coat (An *et al.*, 1997). The number of germination and the length of the radicle and shoot of the germinated weed seedlings were compared with the control and expressed as a percentage of the control. In total, three factors including 1) Aqueous extracts of eight barley cultivars plus control, 2) Extract concentrations in three levels, and 3) The two target weed seeds (*B. juncea* and *S. viridis*), as bioassay plants, were used on a randomized complete block design with four replications.

The data were subjected to analysis of variance, and highly significant or significant differences were tested at least with 1% or 5% levels using LSMEAN, i.e. least significant mean difference comparisons. Regression between measured parameters was made if needed.

**HPLC Sample Preparation**

Hot water extraction was performed according to the Kajimoto *et al.*, (1999) method. Samples of barley cultivar aqueous extract stock solutions (S1) were prepared by heating 0.10 g of each in 2.0 mL of water for one hour at 100°C. The samples were then injected onto the HPLC. The HPLC was a Varian 5000 (Varian, Mississauga, ON, Canada) equipped with a Shimadzu SIL-9A autosampler (Shimadzu Corporation, Columbia, MD) and a WATERS 486 UV detector (Waters, Milford, MA) at 280 nm. Separation was performed with a Supelcosil LC-18, 5 μm, 4.6 mm×15 cm (Supelco, Oakville, ON, Canada) column. The data were integrated and analyzed using Shimadzu CLASS-VP Chromatography Laboratory automated Software System (Shimadzu Corporation, Columbia, MD). The mobile phase utilized a gradient composed of a 0.01 M sodium citrate buffer (A) pH=5.4 adjusted with 50% acetic acid, and methanol (B). The best separation was obtained using the fol-
lowing gradient: 0 min, 2% B; 12 min, 4% B; 20 min, 13% B; 22 min, 13% B and 26 min, 2% B. The running time was 30 minutes. The solvent flow rate was 1.0 ml/min and separation was performed at room temperature. Each phenolic acid standard (protocatechuic, p-hydroxybenzoic, vanillic, syringic, caffeic, chlorogenic, p-coumaric, and ferulic acids) displayed a linear response ($R^2=0.95$) over 8-40 µg calibration series. The concentrations of phenolic acids in samples were calculated using syringic acid as an internal standard. Results were expressed in µg/g dry matter of barley cultivar foliar parts.

### RESULTS AND DISCUSSION

The effects of three concentrations of eight barley cultivar leaf dry powder aqueous extracts on germination, radicle and shoot lengths of two target plant species (*B. juncea* and *S. viridis*) are shown in Table 1. According to both analytical Models I and III, the effects of target weed seeds (TWS), extract concentrations (Cs) and barley cultivars (BC) on seed germinations were highly significant ($P<0.001$), but the three variables interaction (TWS * Cs * BC) on seed germination were also significant ($P=0.05$). The radicle lengths of target weed seedlings did not show significant differences ($P>0.05$) but the shoot did ($P=0.027$). Effects of other sources of variation on radicle and shoot lengths were the same as germination, except that the interaction of target weed seedlings with barley cultivars on radicle was not significant.

The barley cultivar aqueous extracts pH did not have a meaningful correlation with weed seed germination inhibition (Figure 1A; $R^2=0.057$). This indicates that the inhibitory property of the extracts is independ-
The negligibility of osmotic effects of aqueous extracts is supported by several investigators (An et al., 1997; Mason-Sedum, 1986).

There was an indirect relationship between the concentrations of barley cultivar aqueous extracts with percentage germination, radicle, and shoot lengths of target weeds’ seedlings (Table 2).

As the concentrations increased, the percentage germination, radicle and shoot lengths of the target weed seedlings significantly decreased and reached the lowest percentage compared with the control. This is in agreement with the findings of other researchers (Chaves et al., 2001; An et al., 2001; Wu et al., 2001).

The percentage germination and shoot length of *S. viridis* seedlings were significantly higher than the *B. juncea*, but their radicle lengths were not different (Figure 2). This indicated that barley cultivar allelochemicals were more inhibitorier on *B. juncea* germination and seedling growth than those of *S. viridis*. The sensitivity of plants to allelopathic compounds differs among species and genotypes within a species. Quayyum et al. (1999) have shown that lettuce seedlings were more sensitive to the aqueous extracts than wild rice. Overland (1966) has shown that the inhibitory activity of barley cultivars was selective and *Stellaria media* was more sensitive than *Capsula bursa-pastoris*. The importance of using adequate target weed species as a bioassay plant has been emphasized by Inderjit and Dukshini (1995).

![Figure 1](image)

**Figure 1.** Dynamics of (A) pH and (B) electrical conductivity of barley cultivar aqueous extracts on percentage germination inhibition of *B. juncea* and *S. viridis* seeds.

| Table 2. Effects of three levels of concentrations of barley cultivars allelochemicals on germination, root length, and shoot length of *B. juncea* and *S. viridis* seeds in Petri dishes. (Data are based on percentage of control and mean of barley cultivar aqueous extracts). |
|---|---|---|---|---|---|---|
| Barley extract Concentration (mg ml⁻¹) | *Setaria viridis* |  |  |  |  |  | *Brassica juncea* |
|  | Germination | Radicle length | Shoot length | Germination | Radicle length | Shoot length |
|  | Percent of control |  |  | Percent of control |  |  |
| 5 | 95.13 a | 60.36 a | 101.72 a | 72.93 a | 73.92 a | 99.67 a |
| 10 | 84.02 b | 22.71 b | 54.94 b | 24.85 b | 17.05 b | 33.32 b |
| 20 | 10.92 c | 11.24 c | 11.06 c | 15.18 c | 8.84 c | 11.20 c |

* Within columns, means followed by the same letter are not significantly different at the 0.05 levels as determined by least significant difference.
The degree of influence of barley cultivars aqueous extracts on the seed germination of target weeds can be simplified into three categories: 1) Cultivars with the highest allelopathic potential - Stander, CDC Dolly, 2) Cultivars with a moderate allelopathic potential - Harrington, UNA80, TR251, and 3) Cultivars with the lowest allelopathic potential - Bronco, B1602, Jackson (Figure 3). Extracts of barley cultivars reduced radicle length, seed germination and shoot length of target weeds in 76, 57 and 50% of non-treated controls, respectively (Figure 4). In other words, the seedling growth of the target weeds was more suppressed than the germination. Smith (1991), Smith and Martin (1994) and Ben-Hammouda et al. (1995) found aqueous leaf tissue extracts of several species have suppressed seedling growth in target plants more than seed germination. Indeed, the radicle growth was more sensitive to allelochemicals than the coleoptile growth. This is in agreement with Ahn and Chung (2000) who found that the length and dry weight of roots of Echinochloa crusgalli were more affected by hull extract than the shoots. An et al. (2001), on evaluation of Vulpia (Vulpia myuros) allelochemicals, also found all phenolic compounds caused greater inhibition on root elongation than on shoot length.

The interaction of barley cultivars with the target weeds’ seed germination, radicle and shoot length were highly significant (Table 1).
The aqueous extract of cultivar Stander by reducing seed germination of *B. juncea* to 16.83%, was the most influential inhibitor and the extract of cultivar Bronco at 36.63% was the least influential on seed germination. Significant differences were not observed in the shoot length of *B. juncea* treated with aqueous extract of various barley cultivars. The Stander extract by reducing the radicle length of *B. juncea* to 15.84% of the control was the most effective, and the extract of Bronco by reducing the radicle length of *B. juncea* to 36.02% was the least. In contrast to *B. juncea*, the germination, radicle and shoot length of *S. viridis* treated with aqueous extracts of barley cultivars were not significant. In other words, the phytotoxicity of barley cultivar aqueous extract on *S. viridis* as a grass weed is much lower than that of *B. juncea*. Similarly, Anaya *et al.* (1999), on the allelochemicals bioassay of *Metopium brownie*, have shown that the radicle growth inhibition of *Amaranthus hypochondriacus* as a broad leaf weed was much more severe than that of *Echinochloa crus-galli*, a grass weed.

Compared with the non-treated control, the percentage germination of target plant seeds decreased as concentrations of barley cultivars extracts increased (Figure 5). The germination suppression decreased from Stander, CDC Dolly, Harrington, UNA80, TR251, Bronco, B1602, to Jackson, respectively. By decreasing the ratio of extraction from 20 g/L to 5 g/L, the range of germination variation increased and the ability of individual cultivars' allelochemical potential in germination suppression became clearer.

Ahn and Chung (2000) have found an inverse relationship between rice hull water extract concentration and target weed seed germination. This finding also agrees with the work of Chaves *et al.* (2001) who have found that by increasing the concentration of *Cistus ladanfer* leaf aqueous extracts, the percentage germination, root and cotyledon length of *Rumex crispus* decreased.

Six phenolic compounds (protocatechuic, p-hydroxybenzoic, vanillic, chlorogenic, p-coumaric, and ferulic acids) were obtained from each barley cultivar foliar aqueous extract. None of the cultivars contained syringic, caffeic, or chlorogenic acid when

### Table 3. Effect of various barley cultivar aqueous extract allelochemicals on *B. juncea* and *S. viridis* seed germination, radicle, and shoot length in a petri dish (data mean of concentrations).

<table>
<thead>
<tr>
<th>Barley cultivars</th>
<th><em>Setaria viridis</em></th>
<th><em>Brassica juncea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Germination</td>
<td>Radicle length</td>
</tr>
<tr>
<td></td>
<td>Percent of control</td>
<td>Percent of control</td>
</tr>
<tr>
<td>Jackson</td>
<td>60.23 a</td>
<td>23.96 a</td>
</tr>
<tr>
<td>Bronco</td>
<td>55.56 a</td>
<td>20.02 a</td>
</tr>
<tr>
<td>CDC Dolly</td>
<td>54.97 a</td>
<td>19.63 a</td>
</tr>
<tr>
<td>B1602</td>
<td>63.74 a</td>
<td>24.34 a</td>
</tr>
<tr>
<td>Harrington</td>
<td>57.89 a</td>
<td>24.70 a</td>
</tr>
<tr>
<td>UNA80</td>
<td>61.40 a</td>
<td>19.50 a</td>
</tr>
<tr>
<td>Stander</td>
<td>54.73 a</td>
<td>25.92 a</td>
</tr>
<tr>
<td>TR251</td>
<td>59.65 a</td>
<td>24.84 a</td>
</tr>
</tbody>
</table>

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Effect of barley cultivars, aqueous extract allelochemicals on the average germination, root length and shoot length of *B. juncea* and *S. viridis* seeds in comparison to a non-treated control.
compared with the standard (Table 4). The concentrations of each phenolic acid differed among cultivars. The content of protocatechuic acid was the highest and chlorogenic acid was the lowest among the detected allelochemicals. The correlation coefficient \((r=-0.11)\) of total concentration of phenolic compounds with percentage germination inhibition was very low (Figure 6). This indicates that none of the phenolic compounds affect negatively or equally on germination. Both the inhibitory and stimulatory responses of the aqueous extracts are significant for assessing allelopathic properties (Rice, 1995).

In contrast to total phenolic compounds, there was a high correlation between the percentage germination reduction of \(B. \) juncea with the accumulation of four small-quantity-phenolic-compounds (vanillic, chlorogenic, p-coumaric, and ferulic acids) of barley aqueous extracts \((R^2 = 0.70)\) as shown in Figure 7. These results indicate that allelochemicals present in large quantities possessed low activity, while those present in small quantities possessed a strong inhibitory activity. An et al. (2001) on the basis of evaluation of the biological activity of identified allelochemical from \(Vulpia myuros\) have found that individual compounds

Table 4. Barley cultivar aqueous extract allelochemicals (Protocatechuic, p-Hydroxybenzoic, vanillic, chlorogenic, p-coumaric and ferulic acid) isolated using HPLC(Micro gr/L).

<table>
<thead>
<tr>
<th>Barley cultivar</th>
<th>Protocatechuic acid</th>
<th>p-Hydroxybenzoic acid</th>
<th>Vanillic acid</th>
<th>Chlorogenic acid</th>
<th>p-Coumaric acid</th>
<th>Ferulic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackson</td>
<td>9221.91</td>
<td>606.02</td>
<td>36.06</td>
<td>82.15</td>
<td>366.38</td>
<td>216.44</td>
</tr>
<tr>
<td>Bronco</td>
<td>2287.74</td>
<td>593.45</td>
<td>43.55</td>
<td>26.73</td>
<td>280.51</td>
<td>131.85</td>
</tr>
<tr>
<td>CDC Dolly</td>
<td>9752.91</td>
<td>701.60</td>
<td>460.84</td>
<td>29.64</td>
<td>414.75</td>
<td>120.27</td>
</tr>
<tr>
<td>B1602</td>
<td>6982.5</td>
<td>652.90</td>
<td>108.66</td>
<td>66.28</td>
<td>437.18</td>
<td>99.69</td>
</tr>
<tr>
<td>Harrington</td>
<td>11261.87</td>
<td>510.03</td>
<td>367.11</td>
<td>33.69</td>
<td>325.85</td>
<td>168.99</td>
</tr>
<tr>
<td>UNA80</td>
<td>11268.35</td>
<td>767.92</td>
<td>144.18</td>
<td>31.65</td>
<td>391.39</td>
<td>187.88</td>
</tr>
<tr>
<td>Stander</td>
<td>14466.18</td>
<td>680.58</td>
<td>472.18</td>
<td>29.17</td>
<td>368.82</td>
<td>201.68</td>
</tr>
<tr>
<td>TR251</td>
<td>16127.75</td>
<td>653.86</td>
<td>414.17</td>
<td>28.08</td>
<td>334.04</td>
<td>17.59</td>
</tr>
</tbody>
</table>
were not equally inhibitory to tested plants; allelochemicals present in large quantities possessed low activity, while those present in small quantities possessed a strong inhibitory activity. They have concluded that the exploration of the relative composition of a cluster of allelochemicals is more important than simply focusing on the identification of one or two compounds with strong biological activity. In contrast, the small-quantity-phenolic-compounds showed lower allelopathic effects on *S. viridis* germination ($R^2=0.15$), while the average two weeds germination correlation was also very high ($R^2=0.83$). The low effect of barley cultivar aqueous extract on seed germination of *S. viridis* indicates the weak influence of barley phenolic compounds on grass weeds.

This research has shown that barley cultivar shoot tissue aqueous extract for a specific weed is not constant, but is related to the target species, aqueous concentrations, and the proposed cultivars. Broadleaf weeds are more sensitive than grass weeds to barley extracts. The allelochemicals present in shoot tissues may greatly contribute to plant residue in the soil. The residue will interfere with the growth of weeds in that soil and will also affect the germination and vigor of weeds in the vicinity of the crop. Under-
standing the allelopathic potential of barley cultivars will help weed control management of the croplands by lowering the production costs due to herbicide application and hand-weeding. Another use of allelopathic cultivars is incorporating the genes carrying the allelopathic potential into varieties with high quantity and quality crops, which will benefit farmers and consumers as well as the environment.

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REFERENCES


رویاهی سبز بسیار ناچیز بود (15/0 = R²). تجمع دو ترکیب دگرآسیب فنولی دارای غلظت زیاد (اسید بروتوکاتکونیک و اسید هیدروکسی پرنیک) با ترکیب‌های فنولی غلظت کم ارقام جو سبب ضریب همبستگی منفی در جوانه زنی بذور علفهای هرز مذکور شد (11/0 - r).