

Full Length Research Paper

Exploring fungal-derived compounds for weed management: A focus on *Phalaris minor*

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In the present study, herbicidal activity of metabolites of four fungi namely *Drechslera hawaiiensis* M.B. Ellis, *D. biseptata* (Sacc. and Roum.) Richardson and Fraser, *D. holmii* (Luttr.) Subramanian and Jain, and *D. australiensis* (Bugnicourt) Subramanian and Jain., was evaluated against *Phalaris minor* Retz., a problematic monocotyledonous weed of wheat. Fungal metabolites were prepared by incubating the fungal species in M-1-D medium for 28 days at 25°C. In laboratory bioassays, the effect of original (100%) and diluted (50%) fungal metabolites was studied on germination and seedling growth of the target weed species. All the four fungal metabolites significantly reduced seed germination, and shoot and root growth of seedlings of the test weed species. The highest activity against seed germination was exhibited by metabolites of *D. australiensis* resulting in up to 94% reduction in the studied parameter. Similarly the best herbicidal activity against shoot growth was due to metabolites of *D. hawaiiensis* where 65% and 64% reduction in length and dry biomass of shoot was recorded. In foliar spray pot bioassays, fungal metabolites were sprayed on 1-week and 2-week old weed plants four times with 4 days intervals. In general, the effect of the fungal metabolites on various shoot and root growth parameters was insignificant. The present study concludes that metabolites of *D. australiensis* can be used as potent pre-emergence herbicides for the management of *P. minor*.

Keywords: Alternative herbicides, fungal metabolites, littleseed canarygrass, *Phalaris minor*, weed of wheat.

INTRODUCTION

Wheat is an important staple food crop of Pakistan. The yield of wheat in Pakistan is severely affected by weeds (Siddiqui et al., 2010). There have been reported more than 40 weed species from various wheat growing areas of Pakistan (Siddiqui and Bajwa, 2001; Qureshi and Bhatti, 2001). Weeds compete with the crop plants not only by occupying the space (Wright et al., 2001) but also compete for other resources such as water (Thakur, 1984), light and nutrients (Tollenaar et al., 1997). In addition, inhibitory effects of weeds on crop plants through the release of allelochemicals are also well documented (Javaid et al., 2007).

Littleseed canarygrass (*Phalaris minor* Retz.) is one of the most problematic, frequently occurring and densely populated weed. It is a narrow-leaf highly competitive

weed that caused 33–68% reduction in yield in different wheat varieties in Pakistan when there is 1:1 ratio of wheat and weed (Siddiqui et al., 2010). Depending upon the density of this weed, other workers have reported 10–65% yield losses in wheat (Mehra and Gill, 1988; Chhokar et al., 2008). It is also a troublesome weed of wheat fields in India, USA, Canada, Africa, Australia, France, Iran, Iraq and Mexico (Holm et al., 1979). Due to introduction of high-yielding dwarf wheat varieties along with increased irrigation and fertilizer facilities, *P. minor* has become aggravated pest. In addition, rice–wheat crop rotation also stimulated its emergence, growth and development (Balyan and Malik, 1989; Chhokar and Malik, 1999; Chhokar et al., 1999; Singh et al., 1995, 1999).

Although synthetic herbicides are very effective in controlling weeds of wheat (Cheema et al., 2006; Bibi et al., 2008), however, the use of these agro-chemicals has increased consumer's concern due to environmental pollution and other health related issues (Marin et al.,

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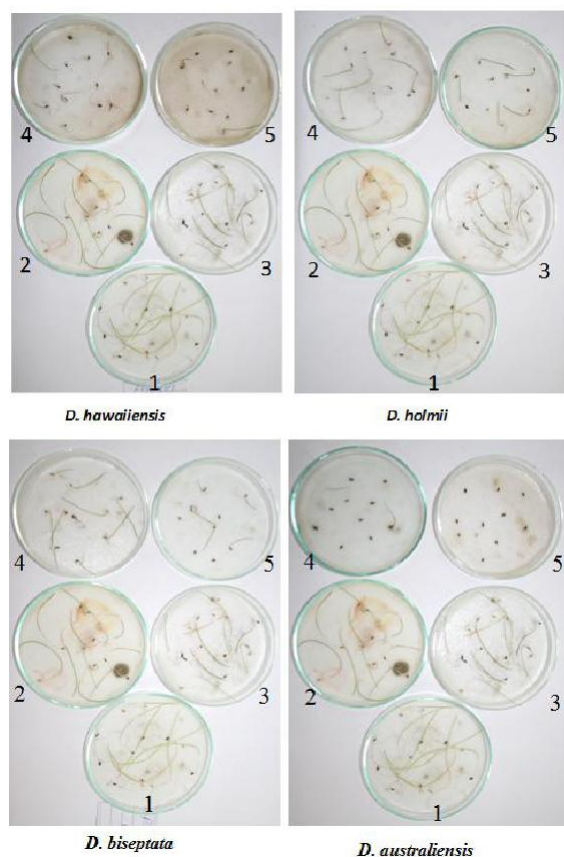


Figure 1. Effect of culture filtrates of four *Drechslera* species on germination and growth of *Phalaris minor* in laboratory bioassays. 1: Control (water), 2: 50% Medium, 3: 100% Medium, 4: 50% Fungal culture filtrates (FCF), 5: 100% FCF.

2003; Rial-Otero et al., 2005), as well as numerous cases of herbicide resistance in many weeds world over (Devine and Shukla, 2000; Yuan et al., 2007). For more sustainable systems, there is an increasing trend towards the search for alternatives of these chemicals which are based on natural compounds (Cuthbertson and Murchie, 2005). Use of fungal metabolites with herbicidal activity is one of the alternative strategies to manage the weeds (Palmer et al., 2005; Javaid and Adrees, 2009; Javaid and Ali, 2011). The present study was carried out to investigate the herbicidal activity of metabolites of various *Drechslera* species for the management of *P. minor*.

MATERIALS AND METHODS

Preparation of fungal culture filtrates

Cultures of four fungal species *Drechslera hawaiiensis* M.B. Ellis, *D. biseptata* (Sacc. and Roum.) Richardson and Fraser, *D. holmii* (Luttr.) Subramanian and Jain, and *D. australiensis* (Bugnicourt) Subramanian and Jain were obtained from Fungal Culture Bank of

Pakistan, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Minimal medium (M-1-D) was prepared in distilled water as described by Evidente et al. (2006). This medium consisted of 0.79 mM KNO₃, 1.2 mM Ca(NO₃)₂, 3.0 mM MgSO₄, 0.87 mM KCl, 87.6 mM sucrose, 0.14 mM NaH₂PO₄, 22 μM H₃BO₃, 7.4 μM FeCl₃, 27.1 mM ammonium tartrate, 8.7 μM ZnSO₄, 30 μM MnSO₄, and 4.5 μM KI. HCl (0.1 M) was used to maintain pH at 5.5. This medium was poured into 500 mL flasks at the rate of 200 mL medium in each flask. Flasks were autoclaved at 121°C for 20 min. After cooling at room temperature, flasks were inoculated with 5 mm discs of each of the four test fungal species and incubated at 25–2 °C in an incubator for 28 days. Cultures were filtered through four layers of muslin cloth and centrifuged at 4000 rpm for ten minutes. These filtrates were filtered through sterilized Whatman filter paper No. 1. and stored at 4°C in a refrigerator. Dilutions (50%) of these original (100%) filtrates were prepared by adding autoclaved distilled water following Javaid and Adrees (2009). Filtrates were generally used within a week to avoid any contamination or alteration.

Laboratory bioassays

Seeds of *P. minor* were collected during May 2009 from wheat fields of University of the Punjab, Lahore, Pakistan. Experiment was conducted in November 2009. Weed seeds were surface sterilized with 1% sodium hypochlorite for 10 min followed by numerous washings with autoclaved water. Twenty seeds of the test weed species were arranged at equal distance in sterilized 9 cm diameter Petri plates lined with sterilized filter papers. Three milliliters of original as well as diluted fungal metabolites of each test species were poured in the Petri plates. Original and diluted M-1-D medium was used as positive control and distilled water as negative control. Each treatment was replicated four times. Completely randomized design (CRD) was followed in a growth room maintained at 16°C with 10 h light period daily. Data regarding germination of seeds was recorded after 15 days. Plants were thinned and 10 uniform seedlings were selected for measurement of different root and shoot growth parameters (Figure 1). Materials were dried at 60°C in an electrical oven till constant weight (Javaid and Ali, 2011).

Foliar spray bioassays

Pot experiment was conducted during November-December 2009 in University of the Punjab, Lahore, Pakistan. Plastic pots of 8-cm diameter and 12-cm deep were filled with sandy loam soil (450 g) having organic matter 0.69%. Initially ten weed seeds were sown in each pot and thinned to six uniform seedlings after germination. For the performance of foliar spray, pots were arranged in two sets, 1-week and 2-week old seedlings. Each treatment was replicated four times. All the pots were set in a completely randomized design under natural environmental conditions of light, humidity and temperature. Original metabolites of the four fungal species were sprayed on 1-week and 2-week old weeds plantlets. Both the sets were sprayed 4 times with 4 days intervals in between. Negative control treatment was sprayed with distilled water whereas M-1-D medium without fungal inoculation was used as positive control. Sprays were conducted during evening hours. Plants were uprooted after 50 days of sowing. Data regarding various shoot and root growth parameters were recorded (Javaid et al., 2011).

Statistical analysis

Analysis of variance followed by Duncan's Multiple Range Test

Table 1. Effect of culture filtrates of four *Drechslera* species on germination and growth of *Phalaris minor* in laboratory bioassays.

Fungal species	Conc. (%)	Germination (%)	Shoot length (mm)	Shoot fresh wt. (mg)	Shoot dry wt. (mg)	Root length (mm)	Root fresh wt. (mg)	Root dry wt. (mg)
Control	0	100 a	52 a	5.17 a	0.53 a	58 a	5.31 a	0.78 a
Growth medium	50	96 a	44 b	4.70 ab	0.45 b	44 b	4.31 b	0.63 b
	100	96 a	42 b	4.35 b	0.41 bc	37 c	3.72 c	0.53 c
<i>D. hawaiiensis</i>	50	81 c	26 e	2.65 d	0.27 d	25 d	2.27 d	0.35 d
	100	56 e	18 h	1.90 e	0.19 e	10 f	0.88 g	0.18 f
<i>D. holmii</i>	50	88 b	29 d	3.45 c	0.39 c	19 e	1.90 de	0.35 d
	100	56 e	25 ef	2.62 d	0.29 d	11 f	0.96 g	0.19 f
<i>D. biseptata</i>	50	77 c	35 c	3.05 cd	0.38 c	12 f	1.40 f	0.28 e
	100	65 d	23 fg	2.62 d	0.29 d	11 f	0.86 g	0.17 f
<i>D. australiensis</i>	50	13 f	21 g	3.62 c	0.37 c	16 e	1.50 ef	0.34 d
	100	6 g	21 g	2.75 d	0.29 d	6 g	0.65 g	0.15 f

In a column, values with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test. Note: 100% means original fungal culture filtrates.

(Steel and Torrie, 1980) was utilized to analyze different growth parameters.

RESULTS AND DISCUSSION

Laboratory bioassays

Data regarding the effect of metabolites of the four *Drechslera* spp. against germination of *P. minor* seeds is presented in Table 1 and Figure 1. The effect of both 100% as well as 50% of M-1-D medium was insignificant ($P \leq 0.05$) on seed germination. Both original as well as diluted metabolites of all the four test fungal species significantly reduced germination by 12-94%. Adverse effect of the original metabolites on germination was more pronounced as compared to the diluted ones. Metabolites of *D. australiensis* were found the most effective where 87% and 94% suppression in seed germination was recorded due to diluted and original metabolites, respectively. Recently, Akbar and Javaid (2010) reported that metabolites of *D. australiensis* prepared in malt extract medium were also effective against germination of *P. minor*. In the present study, fungal metabolites prepared in M-1-D medium exhibited greater herbicidal activity than those prepared in malt extract medium by Akbar and Javaid, (2010). The results of these studies clearly indicate that type of growth medium also has an effect on herbicidal potential of fungal culture filtrates.

Shoot length of seedlings was significantly reduced by metabolites of all the four test fungal species. Although shoot length was also significantly reduced in original and

diluted growth medium treatments, however, the effect was not as much pronounced as in case of fungal metabolites. In contrast to the fungal metabolites where 33–63% reduction in shoot length was recorded over control, there was only 15–19% reduction in shoot length due to growth medium. Moreover, it is likely that most of the ingredients present in growth medium were utilized during the fungal growth, and hence these ingredients could have very little effect in fungal metabolites as compared to pure growth medium. Metabolites of *D. hawaiiensis* were found to be the most effective in suppressing the shoot length followed by metabolites of *D. australiensis* (Table 1). The effect of growth medium and fungal metabolites on fresh and dry biomass of shoot was generally similar to that of their effect on shoot length. All the fungal metabolites significantly reduced the fresh and dry biomass of shoot by 30–63% and 24–64% over control, respectively (Table 1). Findings of the present study are in agreement with the results of earlier studies reporting that the culture filtrates of other *Drechslera* species exhibit herbicidal activity (Evidente et al., 2006; Javaid and Adrees, 2009; Javaid et al., 2011). Drazepinone, a trisubstituted tetrahydronaphthofuroazepinone with herbicidal activity against monocot weeds have been reported from *D. siccanis* (Evidente et al., 2005). Similarly, Sugawara et al (1987) isolated an herbicidal compound ophiobolin I from *D. sorghicola* and *D. maydis*.

Pure growth medium adversely and significantly reduced length as well as fresh and dry biomass of the seedlings. However, the effect was not as much pronounced as that of fungal metabolites. Metabolites of

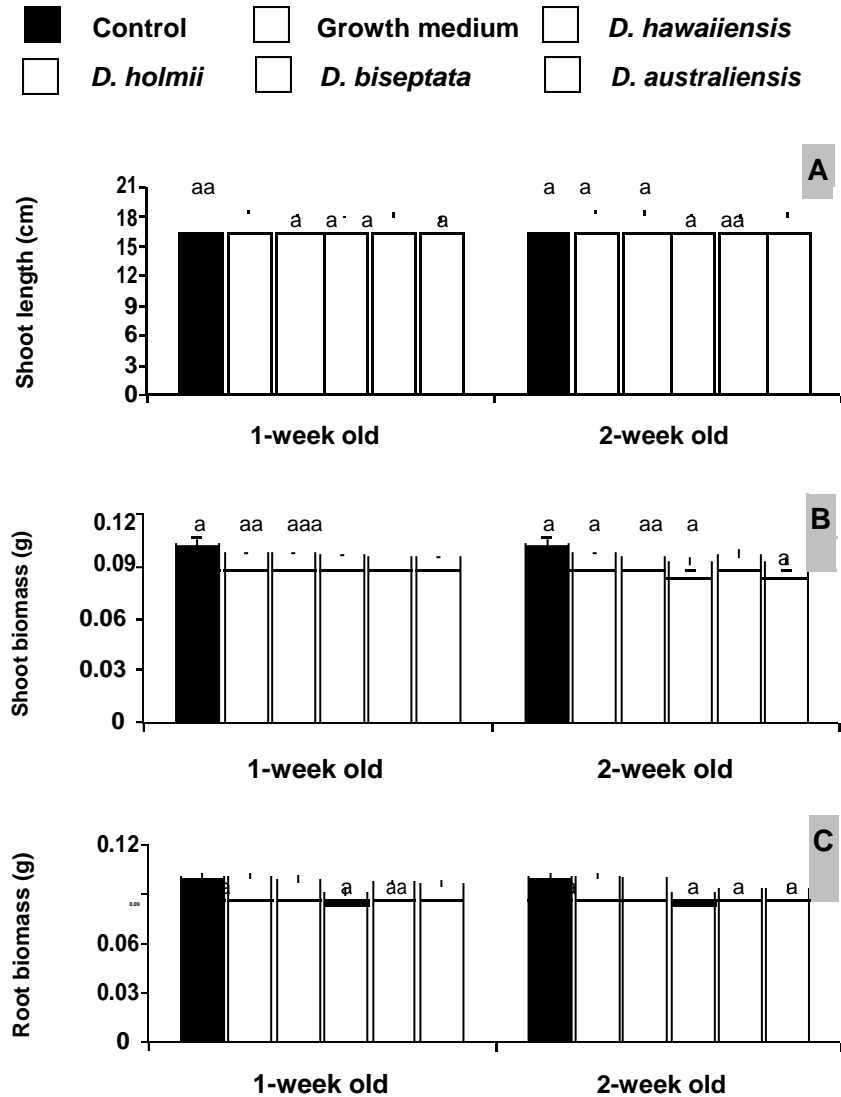


Figure 2. Effect of foliar spray of culture filtrates of four species of *Drechslera* on growth of 1-week and 2-week old *Phalaris minor* plants. Vertical bars show standard errors of means of four replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test at $P \leq 0.05$.

all the four test fungal species significantly reduced length as well as fresh and dry biomass of root as compared to control and growth medium treatments. There was 56–89% reduction in root length due to different concentrations of the various culture filtrates as compared to control. Similarly, different fungal metabolite treatments reduced fresh and dry biomass of root by 57–88% and 55–81% over control. The highest adverse effect on root growth was exhibited by metabolites of *D. australiensis* followed by metabolites of *D. hawaiiensis* (Table 1).

Pot trials

Data regarding the effect of foliar spray of various fungal

metabolites on shoot length, and shoot and root biomass of *P. minor* is presented in Figure 2. The effect of various foliar spray treatments was insignificant both on 1-week and 2-week old *P. minor* plants. The non-significant effect of the fungal metabolites on pot grown *P. minor* plants could be attributed to the very small quantities of the herbicidal constituents present in these metabolites. The results of the laboratory bioassays clearly indicate that the herbicidal constituents present in these fungal culture filtrates are highly effective against very young and delicate seedlings. It is likely that if these metabolites are used in concentrated form, these may be effective against pot grown *P. minor* plants. Moreover, laboratory bioassays also revealed that metabolites of *D. australiensis* can suppress germination of *P. minor* seeds up to 94%. These metabolites can be used as

pre-emergence herbicides for the management of *P. minor*. Further studies are required to isolate and identify these herbicidal constituents.

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