The Journal of Animal & Plant Sciences, 22(4): 2012, Page: 977-982
ISSN: 1018-7081

HERBICIDAL ACTIVITY OF FUNGAL CULTURE FILTRATES AGAINST *CHENOPODIUM ALBUM* L. AND *AVENA FATUA* L.

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ABSTRACT

Culture filtrates of four *Drechslera* spp. namely *D. hawaiiensis* M.B. Ellis, *D. holmii* (Luttr.) Subramanian and Jain, *D. biseptata* (Sacc. and Roum.) Richardson and Fraser and *D. australiensis* (Bugnicourt) Subramanian and Jain., were evaluated for their herbicidal activity against two cumbersome weeds of wheat (*Triticum aestivum* L.) namely *Chenopodium album* L. (dicot) and *Avena fatua* L. (monocot). Culture filtrates of these fungal species were prepared in M-1-D medium. In *in vitro* bioassays, the effect of original (100%) and diluted (50%) fungal culture filtrates were investigated in Petri plates against different growth parameters of the two test weed species and a wheat var. Inqlab- 91. Culture filtrates of all the four *Drechslera* species appreciably reduced germination as well as root/shoot growth of *C. album* and *A. fatua.* All the fungal culture filtrates also suppressed root/shoot growth of wheat; however, the effect was less intense as compared to the effect against the two weed species. In foliar spray bioassays, the effect of original culture filtrates was studied on the growth of 1-week and 2-weeks old weeds and wheat plants. Culture filtrate of *D. hawaiiensis* significantly reduced the shoot and root biomass of 1-week old plants of *C. album*. Similarly, the culture filtrates of *D. biseptata* and *D. australiensis* significantly suppressed shoot biomass of both 1-week and 2-weeks old *A. fatua* plants. None of the fungal culture filtrates exhibited significant effect on shoot and root biomass of wheat in foliar spray bioassays.

Keywords: *Avena fatua, Chenopodium album, Drechslera* spp. fungal culture filtrates, natural herbicides, weeds of wheat.

INTRODUCTION

Wheat, a globally important cereal crop and the staple food of Pakistanis, is cultivated on an area of 9046 thousands hectares with an average grain yield of 2657 kg ha^{-1} and having total yield equal to 24032 thousands tones (Anonymous, 2010). This yield is very low as compared to per hectare yield of advanced countries of the world as well as yield potential possessed by most of wheat cultivars. There have been reported 31 and 45 weed species from wheat growing areas of the province Punjab and Sindh, respectively (Qureshi and Bhatti, 2001; Siddiqui and Bajwa, 2001). In these studies, among others *Avena fatua* and *Chenopodium album* were also found to be the most frequently occurring weeds in the wheat fields of Pakistan. *C. album* reduced the grain yield by 24% and 65% in wheat varieties Punjab 96 and Inqlab-91, respectively, when there was a 1:1 ratio of the weed and wheat plants (Siddiqui *et al*. 2010). Use of synthetic herbicides is regarded as the most reliable and the common method for the management of weeds. Various chemical herbicides such as Topic, Puma Super, Affinity and Buctril Super have been proved to be very effective in controlling weeds of wheat in Pakistan (Bibi *et al*. 2008; Cheema *et al*. 2006; Usman *et al*. 2010). However, in recent years, the use of synthetic chemicals has increased consumer's liabilities due to health problems, occurrence of microbial resistance against a number of weeds and high inputs (Marin *et al*. 2003; Rial-Otero *et al*. 2005). There is an increasing trend towards sustainable, environment friendly integrated disease management strategies, on the basis of alternatives to synthetic chemical pesticides (Cuthbertson and Murchie, 2005).

Isolation of natural herbicidal constituents from culture filtrates of plant pathogenic and other fungi (Javaid and Adrees, 2009; Palmer, *et al*. 2005; Zhang *et al*. 2010; Javaid, 2010) is one of the alternative strategies to manage the weeds. Phytotoxins are usually isolated from *in vitro* cultures of the pathogen grown on either solid or liquid media (Berestetskiy, 2008; Strange, 2007). Phytotoxic compound trans-4 aminoproline have been isolated from culture filtrates of *Ascochyta caulina* and found to have herbicidal efficacy against *Chenopodium album* (Evidente *et al*. 2000). Aliferis and Chrysayi-Tokousbalides (2006) studied the herbicidal effect of phytotoxic fungal metabolite (8*R*, 16*R*)-(−)-pyrenophorin against *Avena sterilis.* The herbicidal activity of this compound at 70 μM was observed as bleaching in leaves accompanied by increase in electrolyte leakage, increased superoxide dismutase activity, lipid peroxidation, decline in total protein and loss of photosynthetic pigments. Here in this manuscript we present evaluation of the herbicidal activity of culture filtrates of different *Drechslera* species

against *C. album* and *A. fatua*, the two noxious weeds of wheat.

MATERIALS AND METHODS

Preparation of fungal culture filtrates: Cultures of four species of *Drechslera* viz. *D. australiensis*, *D. hawaiiensis D. holmii* and *D. biseptata*, were kindly provided by Fungal Culture Bank of Pakistan, Institute of Mycology and Plant Pathology, 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 1.2 mM $Ca(NO_3)_2$, 0.79 mM KNO_3, 0.87 mM KCI, 3.0 mM $MgSO_4$, 0.14 mM NaH_2PO_4, 87.6 mM sucrose, 27.1 mM ammonium tartrate, 7.4 µM $FeC1_3$, 30 µM $MnSO_4$, 8.7 µM $ZnSO_4$, 22 µM $H3BO_3$ and 4.5 µM KI. 0.1 M HCl 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 minutes and cooled to room temperature. Flasks were individually inoculated with 5 mm agar discs of each of the four fungal species grown in Petri plates. Seeded flasks were incubated at 25±2 ºC in an incubator for 28 days. Cultures were filtered through four layers of muslin cloth, centrifuged at 4000 rpm for ten minutes followed by filtration through sterilized Whatman filter paper No. 1. These filtrates were stored at 4 ºC in a refrigerator. 50% dilution of this original (100%) filtrate was prepared by adding autoclaved distilled water (Javaid and Adrees, 2009). Filtrates were generally used within a week to avoid any contamination or alteration.

Laboratory bioassays: Response regarding germination and early seedling growth of the two weed species namely *Chenopodium album* and *Avena fatua* as well as a wheat variety Inqlab-91 against different concentrations of culture filtrates of four *Drechslera* spp. was evaluated *in vitro*. Seeds of weeds and wheat were immersed in 1% sodium hypochlorite for 10 minutes followed by several washings with autoclaved water for surface sterilization. Twenty seeds of the test weed species and wheat were placed at equal distance in sterilized Petri plates (9-cm diameter) with the bottom having a sterilized filter paper. Three milliliters of each concentration of fungal culture filtrates were poured in each Petri plate. Original and diluted M-1-D medium was used as positive control and distilled water as negative control. All tests were performed in quadruplicate. Completely randomized design (CRD) was followed in a growth room maintained at 16°C with 10 h light period daily. Data regarding seed germination, root and shoot length and biomass were recorded after 15 days. For dry weight determination, materials were placed in an electrical oven maintained at 60 °C till constant weight (Javaid and Ali, 2011).

Foliar spray bioassays: Pot experiments were conducted during October- November 2009 in the Institute of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan. Plastic pots having dimensions, 8-cm diameter and 12-cm deep were filled with 450 g sandy loam soil having organic matter 0.69%, pH 7.8, available phosphorus and potassium 6.3 mg kg^{-1} and 100 mg kg^{-1}, respectively. Seeds of test weed species and wheat were sown in these pots. Pots were arranged in two sets to perform the foliar spray on 1-week and 2-weeks 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 filtrates of the *Drechslera* spp. were sprayed on 1-week and 2-weeks old weeds and wheat plantlets. Both the sets were sprayed 4 times with four days interval in between. Distilled water spray was carried out as negative control whereas M-1-D medium without fungal inoculation was used as positive control. All the sprays were carried out during evening hours. Data were collected after 50 days of sowing. Plants were uprooted and washed thoroughly under ordinary water to remove soil. Roots were cut from shoots with the help of scissor. Data regarding shoot length, fresh and dry biomass of root and shoot were recorded.

Statistical analysis: Analysis of variance followed by Duncan's Multiple Range Test (Steel and Torrie, 1980) was utilized to analyze different growth parameters.

RESULTS AND DISCUSSION

Laboratory bioassays

Effect of fungal culture filtrates on growth parameters of *C. album*: Table 1 depicts data regarding the effect of culture filtrates of different *Drechslera* spp. against growth parameters of *C. album*. The effect of 100% M-1-D broth was significant ($P \leq 0.05$) on germination and shoot growth of test weed species. Original culture filtrates of various *Drechslera* spp. significantly reduced germination by 28-50%. Original filtrate of *D. hawaiiensis* was found to be the most effective in reducing shoot length and biomass as well as root length and biomass of *C. album*. Shoot length was reduced by 54-91% due to different culture filtrate treatments followed by subsequent decrease in shoot biomass by 58-81%. Effect of all the culture filtrates was found significant as compared to control. Root growth similarly exhibited high susceptibility to the application of culture filtrates of four *Drechslera* species. Root length and biomass were significantly reduced by 66-88% and 56-65% due to different fungal culture filtrate treatments. Overall *D. hawaiiensis* appeared to be the most effective fungus in inhibiting shoot and root growth

of *C. album* while the least effective fungus remained *D. holmii.*

Effect of fungal culture filtrates on growth parameters of *Avena fatua:* Table 2 demonstrates data regarding the effect of culture filtrates of four *Drechslera* spp. against growth parameters of *A. fatua.* The effect of original as well as 50% M-1-D broth on the germination and various shoot/root growth parameters was significant ($P \leq 0.05$). Different culture filtrate treatments reduced the germination by 28- 54%. All culture filtrates of *Drechslera* spp. significantly suppressed germination of *A. fatua* seeds at both 100% as well as 50% concentration. All the culture filtrate treatments except 50% *D. biseptata* significantly reduced shoot growth of *A. fatua* in terms of length and biomass. Roots were more susceptible as compared to shoot growth. There was 27-67%, 27-57%, 55-86% and 47-77% reduction in shoot length, shoot biomass, root length and root biomass due to various culture filtrate treatments, respectively. In an investigation, Evidente *et al*. (2005) reported Drazepinone, a trisubstituted tetrahydronaphthofuroazepinone from *Drechslera siccans* having herbicidal activity against monocot weeds. Earlier, Sugawara *et al*. (1987) isolated ophiobolin I from *Drechslera maydis* and *Drechslera sorghicola* that possessed herbicidal activity. Recently, Akbar and Javaid (2010) carried out a similar study using culture filtrates of *D. hawaiiensis, D. holmii, D. biseptata* and *D. australiensis* prepared in malt extract broth. These culture filtrates were found to have herbicidal activity against some weeds of wheat viz*., Phalaris minor*, *Avena fatua* and *Rumex dentatus*

Table 1: Effect of culture filtrates of four *Drechslera* species on germination and growth of *Chenopodium album* in laboratory bioassays.

Fungal species	Conc. (%)	Germi-nation (%)	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	16.5 a	1.40 a	0.19 a	10.40 a	0.19 a	0.087 a
Growth medium	50	93 ab	14.4 b	1.05 b	0.16 b	9.15 b	0.18 a	0.090 a
	100	88 bc	13.7 c	0.97 bc	0.15 b	8.75 b	0.15 b	0.080 a
D. hawaiiensis	50	66 e	1.9 h	0.37 ef	0.04 gh	1.40 fg	0.09 de	0.040 c
	100	50 f	1.5 h	0.29 f	0.03 h	1.23 g	0.08 e	0.030 c
D. holmii	50	81 cd	12.4 d	0.83 cd	0.10 cd	7.90 c	0.12 c	0.054 b
	100	72 de	7.6 f	0.72 d	0.08 de	3.57 d	0.10 d	0.038 c
D. biseptata	50	82 bc	10.3 e	0.77 d	0.10 c	7.70 c	0.12 c	0.063 b
	100	72 de	7.5 f	0.71 d	0.08 c-e	2.35 e	0.10 d	0.037 c
D. australiensis	50	56 f	3.3 g	0.54 e	0.07 ef	2.42 e	0.10 d	0.036 c
	100	50 f	2.7 g	0.44 ef	0.06 fg	1.71 f	0.09 de	0.033 c

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.

Table 2: Effect of culture filtrates of four *Drechslera* species on germination and growth of *Avena fatua* in laboratory bioassays.

Fungal species	Conc. (%)	Germi-nation (%)	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	100 a	59 a	4.7 a	140 a	45 a	6.8 a
Growth medium	50	88 b	87 b	53 b	4.5 a	120 b	41 b	6.0 b
	100	84 b	84 b	49 c	4.3 ab	115 c	40 b	5.6 c
D. hawaiiensis	50	70 cd	51 g	34 f	3.1 d	54 g	19 e	3.4 f
	100	46 e	41 h	29 g	2.5 ef	20 j	8 f	1.6 g
D. holmii	50	76 c	59 f	37 e	3.2 d	50 g	17 e	3.3 f
	100	71 c	33 i	24 h	2.0 f	27 i	8 f	1.8 g
D. biseptata	50	71 c	80 c	41 d	3.9 bc	86 d	29 c	4.1 d
	100	64 d	73 d	38 e	3.4 cd	62 f	21 e	3.5 f
D. australiensis	50	70 cd	67 e	37 e	3.5 cd	67 e	26 cd	4.0 de
	100	52 e	48 g	31 g	2.6 e	35 h	23 d	3.6 ef

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.

Table 3: Effect of culture filtrates of four *Drechslera* species on germination and growth of wheat var. Inqlab- 91 in laboratory bioassays.

Fungal species	Conc. (%)	Germi-nation (%)	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	115 a	71 a	4.3 a	92.1 a	49 a	7.5 a
Growth medium	50	98 ab	104 b	70 ab	4.2 a	91.1 a	42 b	6.5 b
	100	96 a-c	95 c	66 ab	3.9 ab	79.1 b	39 b	6.2 b
D. hawaiiensis	50	95 a-c	79 ef	49 ef	2.8 de	31.3 f	24 ef	3.6 fg
	100	88 d	75 fg	46 f	2.6 de	28.0 f	21 f	3.3 d
D. holmii	50	94 a-d	89 cd	59 cd	3.4 bc	68.3 c	33 cd	4.7 cd
	100	88 d	71 g	43 f	2.3 e	40.2 e	29 de	4.1 ef
D. biseptata	50	95 a-c	92 c	63 bc	3.8 ab	77.2 b	37 bc	5.4 c
	100	92 cd	85 de	57 cd	3.1 cd	68.2 c	34 cd	4.9 cd
D. australiensis	50	94 a-d	92 c	63 bc	3.8 ab	65.0 c	32 cd	5.2 c
	100	93 b-d	79 ef	54 de	3.1 cd	50.3 d	30 d	4.3 d-f

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

Effect of fungal culture filtrates on growth parameters of wheat var. Inqlab-91: Table 3 illustrates data regarding the effect of culture filtrates of four *Drechslera* spp. against growth parameters of wheat var. Inqlab- 91. The effect of 100% as well as 50% M-1-D broth was non-significant (P ≤ 0.05) on germination and shoot growth of test wheat variety. Original culture filtrates of various *Drechslera* spp. significantly reduced germination by 7-12%. Whereas 50% concentration of all fungal metabolites failed to cause any appreciable effect on germination. Wheat variety Inqlab- 91 showed least resistance to *D. holmii as* original filtrates of *D. holmii* were found to be most effective in reducing shoot growth parameters causing 38, 39 and 46% reduction in shoot length, shoot fresh weight and shoot dry weight respectively. In case of root growth parameters root length and biomass were greatly reduced by 26-70% and 34-56% due to different fungal culture filtrate treatments.

Foliar spray bioassays: The two test weed species and the wheat responded differently to the foliar spray of various *Drechslera* species. Culture filtrates of *D. hawaiiensis* significantly reduced shoot length, and shoot and root biomass of 1-week old plants of *C. album* by 8%, 19% and 17%, respectively over control. Conversely, the effect of metabolites of all other *Drechslera* species on various shoot and root growth parameters of this weed species was insignificant (Fig. 1). None of the fungal metabolites showed significant effect on shoot length of *A. fatua.* However, metabolites of all the fungal species except *D. hawaiiensis* significantly reduced the shoot biomass of 1-week old plants of this weed species by 16–37%. Similarly, metabolites of *D. biseptata* and *D. australiensis* significantly reduced shoot biomass of 2-weeks old *A. fatua* plants by 42% and 32%, respectively, as compared to control. Metabolites of *D. hawaiiensis* significantly enhanced root biomass of 1-week old *A.*

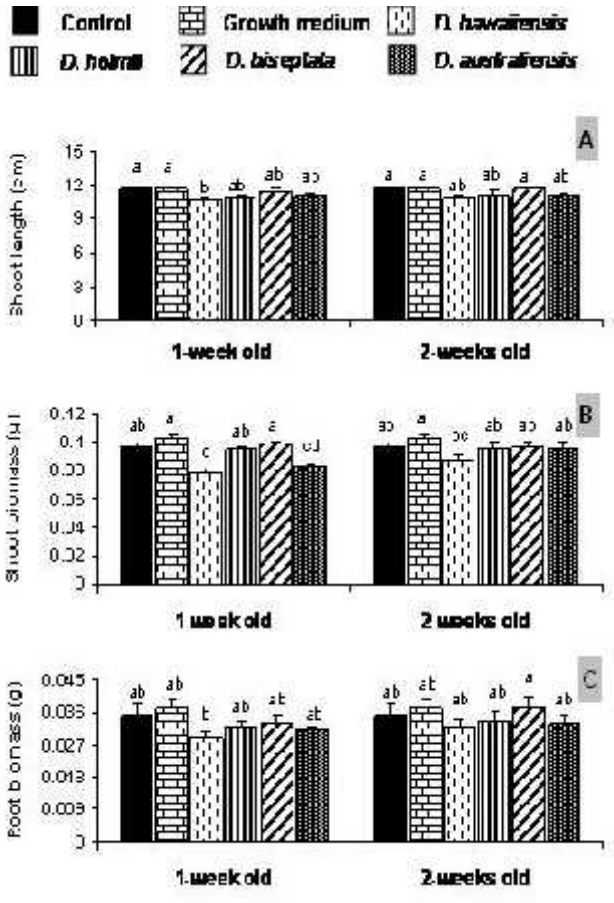

Fig. 1: Effect of foliar spray of culture filtrates of four species of *Drechslera* on growth of 1-week and 2-weeks old *Chenopodium album* plants. Vertical bars show standard errors of means of four replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test at P ≤ 0.05.

fatua plants by 16%. The effect of rest of the treatments was insignificant on this studied parameter (Fig. 2). Metabolites of various test fungal species reduced shoot length of 1-week and 2-week old wheat plants by 1–5% and 0.5–4%, respectively. Metabolites of all the four test fungi failed to exhibit any significant effect on shoot and root biomass of wheat (Fig. 3). Various herbicidal constituents have been identified from different *Drechslera* species. Evidente *et al*. (2006b) identified four herbicidal constituents from *Drechslera gigantea* viz. ophiobolin A, 6- epi-ophiobolin A, -anhydro-6- epi-ophiobolin A and ophiobolin I, which were very effective against several grass and dicotyledon weeds. Results of the present study show that culture filtrates of different *Drechslera* species contain herbicidal constituents for the management of some problematic weeds of wheat. These finding are in agreement with the results of some earlier studies where culture filtrates of other *Drechslera* species exhibited herbicidal activity against weeds (Kastanias and Chrysayi-Tokousbalides, 2000; Evidente *et al*., 2005, 2006ab; Javaid and Adrees, 2009; Javaid and Ali, 2011).

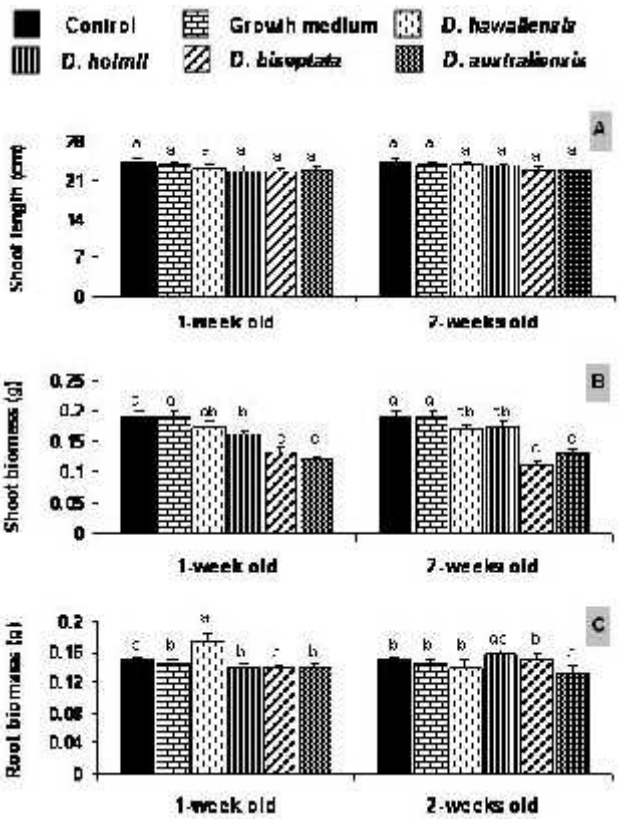

Fig. 2: Effect of foliar spray of culture filtrates of four species of *Drechslera* on growth of 1-week and 2-weeks old *Avena fatua* plants. Vertical bars show standard errors of means of four replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test at P ≤ 0.05.

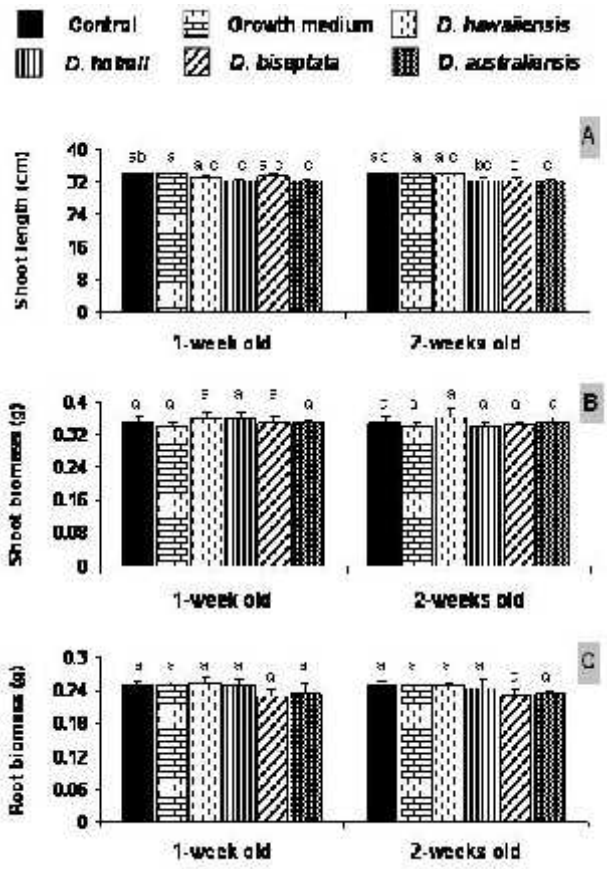

Fig. 3: Effect of foliar spray of culture filtrates of four species of *Drechslera* on growth of 1-week and 2-weeks old wheat var. Inqilab-91 plants. Vertical bars show standard errors of means of four replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test at P ≤ 0.05.

Conclusion: In both laboratory bioassays and pot trials, wheat was least affected by fungal metabolites. In laboratory bioassays, germination as well as early seedling growth of the two target weed species was severely affected by fungal metabolites. However, in pot trials, metabolites of *D. hawaiiensis* significantly reduced the growth of *C. album* and culture filtrates of rest of the fungal species significantly suppressed the shoot growth of *A. fatua*. In the present study, original culture filtrates were applied in the foliar spray bioassays. It is likely that if these culture filtrates are used in a concentrated form, these will be more toxic to these weed species. Further studies are required to isolate the active herbicidal constituents from these fungal culture filtrates.

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