



Full Length Article

Management of *Rumex dentatus* (Toothed Dock) by Fungal Metabolites under Field Conditions

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Abstract

A field trial was conducted to investigate the herbicidal potential of culture filtrates of four *Drechslera* spp. viz. *D. australiensis* (Bugnicourt) Subramanian and Jain., *D. biseptata* (Sacc. and Roum.) Richardson and Fraser, *D. hawaiiensis* M.B. Ellis and *D. holmii* (Luttr.) Subramanian and Jain, against toothed dock (*Rumex dentatus* L.), a problematic dicotyledonous weed of wheat (*Triticum aestivum* L.). These culture filtrates were used either alone or in combination with half dose of a commercial herbicide Bromoxynil+MCPA (2-methyl-4-chlorophenoxyacetic acid). Herbicidal application either alone or in combination with fungal culture filtrates completely controlled the weed growth. All the four fungal culture filtrates when used alone significantly reduced the weed biomass by 30–58% over weedy check. Consequently, grain yield in wheat was increased by 6–22%. Culture filtrates of *D. australiensis* and *D. hawaiiensis* exhibited the highest herbicidal activity resulting in 58 and 57% reduction in weed biomass over weedy check. Application of culture filtrates of *D. australiensis* and *D. hawaiiensis* resulted in Rs. 11632 (28%) and Rs. 10413 (25%) more profit than conventional weedy check, respectively. However, the highest net profit of Rs. 70100 was obtained in hand weeding treatment followed by Rs. 69128 in half dose of herbicide, which was Rs. 28444 (68%) and Rs. 27472 (66%) higher than weedy check, respectively. It is concluded that culture filtrates of *D. australiensis* and *D. hawaiiensis* contain herbicidal metabolites and can be used as alternatives to herbicides for the management of *R. dentatus* in organic wheat production. © 2015 Friends Science Publishers

Keywords: Alternative management; *Drechslera* spp.; *Rumex dentatus*; Weed of wheat

Introduction

In agro-ecological systems, weeds are important because these compete for the same resources as for cultivated crop and ultimately deteriorate the quality and yield of crops (Khan *et al.*, 2012). *R. dentatus* is an important weed growing in wheat field in Pakistan (Siddiqui and Bajwa, 2001). Yield losses in wheat have been documented as high as 55% when *R. dentatus* was growing in 1:1 ratio with wheat (Siddiqui *et al.*, 2010). At present, the most reliable mean to control such weeds is the use of chemical herbicides such as Bromoxynil+MCPA, fluroxypyr +MCPA and traisulfuran+terbutryn (Abbas *et al.*, 2009; Naseer-ud-Din *et al.*, 2011). However, there are some environmental consequences of these synthetic agro-chemicals such as evolution of herbicide resistance in weeds and health hazards (Rashid *et al.*, 2010; Singh *et al.*, 2012). There is thus need to replace these synthetic agro-chemicals with eco-friendly herbicides based on natural compounds from plants and microbes (Javaid, 2010).

The literature abounds with examples of herbicidal fungal metabolites used as weed control agents. These fungal metabolites have been proved as potential herbicides

both in crude and purified forms (Javaid, 2010). Culture filtrates of different species of *Trichoderma* are known to exhibit herbicidal activity against little seed canary grass (*Phalaris minor* Retz.) and *R. dentatus* (Javaid and Ali, 2011). Similarly, culture filtrates of various fungal species exhibited significant herbicidal activity against parthenium (*Parthenium hysterophorus* L.) weed (Javaid and Adrees, 2009; Javaid *et al.*, 2011). Phytotoxic fungal metabolites namely leptosphaerodione, elsinochrome A and cercosporin have been isolated from different isolates of phytopathogenic fungal species *Stagonospora convolvuli* Dearn. & House. These metabolites have been shown to be toxic to *Convolvulus arvensis* L. and *Calystegia sepium* (L.) R. Br. (Ahonsi *et al.*, 2005). Evidente *et al.* (2008) isolated four oxazatricycloalkenones namely phyllostictines A-D from *Phyllosticta cirsii* Desm. and tested for their herbicidal activity. Phyllostictine A was proved highly phytotoxic against canada thistle [*Cirsium arvense* (L.) Scop.].

Some species of genus *Drechslera* are known for the production of herbicidal metabolites. Evidente *et al.* (2006a, b) isolated four herbicidal constituents viz. ophiobolin A, 6-epi-ophiobolin A, anhydro-6-epi-ophiobolin A and ophiobolin I from *Drechslera gigantea* Heald and Wolf,

which were very effective against several grassy and dicotyledonous weeds including animated oat (*Avena ludoviciana* Dur.), annual canarygrass (*Phalaris canariensis* L.), lambsquarters (*Chenopodium album* L.) and common sowthistle (*Sonchus oleraceus* L.). In the recent past, few studies conducted in Pakistan showed that metabolites of *Drechslera* species have the potential to be used against problematic broad-leaf weeds namely *R. dentatus* and parthenium (*Parthenium hysterophorus* L.) as well as grassy weeds of wheat namely *Avena fatua* and *Phalaris minor* (Javaid *et al.*, 2011; Akbar and Javaid, 2012a, b, 2013). However, all these earlier studies regarding the herbicidal activities of fungal metabolites were carried out either in Petri plates or in pots. Such studies under field conditions are lacking. The present study was therefore, carried out to evaluate the herbicidal effects of culture filtrates of four *Drechslera* spp. from Pakistan namely *D. australiensis*, *D. biseptata*, *D. hawaiiensis* and *D. holmii* against a problematic broad-leaf weed of wheat namely *R. dentatus* under field conditions.

Materials and Methods

Selection of Test Fungal Isolates

Four species of *Drechslera* viz. *D. australiensis*, *D. hawaiiensis*, *D. biseptata* and *D. holmii* were obtained from Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Single spore isolates of all these fungal species were maintained on malt extract agar medium.

Selection of Weed of Wheat and Wheat Variety

Seeds of the test weed species were collected from wheat fields of Lahore, Pakistan, at the end of growing season of wheat in May 2009. A commonly cultivated wheat variety in Punjab, Pakistan namely Sehar 2006 was selected for the present study. Certified seeds of the wheat variety were procured from Federal Seed Certification Department, Lahore, Pakistan.

Preparation of Culture Filtrates of the Test Fungi

Minimal medium (M-1-D) was prepared in distilled water as described by Evidente *et al.* (2006b). This medium consisted of 1.2 mM $\text{Ca}(\text{NO}_3)_2$, 0.79 mM KNO_3 , 0.87 mM KCl, 3.0 mM MgSO_4 , 0.14 mM NaH_2PO_4 , 87.6 mM sucrose, 27.1 mM ammonium tartrate, 7.4 μM FeCl_3 , 30 μM MnSO_4 , 8.7 μM ZnSO_4 , 22 μM H_3BO_3 and 4.5 μM KI. The pH was adjusted to 5.5 with 0.1 M HCl. Medium was poured into 500 mL conical flasks at 200 mL medium in each flask. Flasks were autoclaved at 121°C and 103425 P cm^{-2} pressure for 20 min and cooled to room temperature. Flasks were individually inoculated with agar discs (5 mm) of each of the four test fungal species from the margins of actively growing fungal colonies. Inoculated flasks were

incubated at $25 \pm 2^\circ\text{C}$ 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°C in a refrigerator. Sterilized distilled water was added to the original filtrates (100%) to prepare dilution of 50% (Javaid and Adrees, 2009).

Field Preparation

Field trial was carried out during the wheat growing season of 2009–2010 at Dhang Shah District Qasur, ($30^\circ 56' \text{N}$ and $74^\circ 13' \text{E}$), 80 kilometers from Lahore, Pakistan. All the recommended agronomic practices from preparation of field till harvesting were followed. Soil was sandy loam in texture having available potassium 43 mg kg^{-1} , EC 2.4 mScm^{-1} 2.4, pH 7.7, organic matter 0.84% and available phosphorous 4.5 mg kg^{-1} . Experiment was laid out in randomized complete block design with three replications. Each plot measured $1.54 \times 1.54 \text{ m}^2$. Nitrogen (N) was applied at 160 kg ha^{-1} as urea, P_2O_5 at 110 kg ha^{-1} as single super phosphate and K_2O at 60 kg ha^{-1} as sulphate of potash. Full doses of P_2O_5 and K_2O , and half-dose of N were applied as basal, while rest of the N was top-dressed at initiation of flowers.

Sowing of Seeds

Three wheat seeds per hill were sown at 22 cm inter and intra row spacing accommodating seven rows in each plot with 7 plants per row. Wheat seeds were sown on November 15, 2010. After germination, thinning was carried out at the stage of full emergence of first leaf to maintain one wheat seedling at one place for uniformity in different treatments. First irrigation was carried out twenty days after sowing and subsequent irrigations were carried out according to the requirement of the crop. A total of six irrigations with tube well water were carried out as dry spell was observed through out course of the present study. Seeds of *R. dentatus* were sown in the field at the time of first irrigation. Weed species belonging to different plant genera along with *R. dentatus* emerged in the field after first irrigation. All weed species except *R. dentatus* were removed manually and 1:1 ratio of *R. dentatus* and wheat plants was maintained.

Treatments and Experimental Layout

There were twelve treatments comprising of a weed free control, a weedy check, four treatments with the application of culture filtrate of *D. hawaiiensis*, *D. holmii*, *D. biseptata* and *D. australiensis*, two treatments comprised of application of synthetic herbicide (Bromoxynil+MCPA) at recommended and half dose. Remaining four treatments comprised application of culture filtrates of all the four test *Drechslera* species mixed in 1:1 ratio with half dose of synthetic herbicide.

Schedule of Foliar Sprays

A total of three sprays were carried out with fungal culture filtrates. First spray was carried out when *R. dentatus* was at three to four leaves stage (40 days after sowing of wheat). Two subsequent sprays with fungal culture filtrates were carried out with intervals of 7 days. Culture filtrates were sprayed at 100 L ha⁻¹. Only a single spray of synthetic herbicide Bromoxynil+MCPA 200/200EC, either alone or mixed with fungal culture filtrates was carried out at three to four leaf stage of the weed using Knapsack hand sprayer with 4T-jet nozzle.

Harvesting and Data Collection

Wheat crop was harvested at maturity on April 15, 2011. Data regarding plant height, dry biomass, number of fertile tillers per plant, total grain yield and hundred grains weight were recorded. Whole weed biomass from each plot was harvested at the time of harvesting of wheat. Weed plants were cut at the soil surface level and completely dried in an electric oven at 60°C and dry biomass was measured. Wheat dry biomass and grain yield as well as weed biomass were calculated on per hectare basis.

Statistical and Economic Analyses

All the data were subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to separate the treatment means at 5% level of significance using computer software COSTAT. In order to find out comparative benefits of different treatments, economic and marginal analyses were carried out on the basis of mean values of grain yield in different treatments following the procedure given by CIMMYT (1988).

Results

Effect of Fungal Culture Filtrates on Weed Biomass

Both recommended and half doses of herbicide Bromoxynil+MCPA completely checked the growth of the target weed species. Similarly, weed growth was also completely checked when half dose of the herbicide was used in combination with culture filtrates of different *Drechslera* spp. In general, culture filtrates of all the four *Drechslera* spp. significantly reduced biomass of *R. dentatus* as compared to weedy check. However, variability among the herbicidal activity of the culture filtrates of different *Drechslera* spp. was evident. Among the four *Drechslera* spp., culture filtrates of *D. australiensis* and *D. hawaiiensis* were found to be more effective against *R. dentatus* than the culture filtrates of the other two *Drechslera* species. There was 58, 57, 31 and 30% reduction in dry biomass of *R. dentatus* due to foliar application of culture filtrates of *D.*

australiensis, *D. hawaiiensis*, *D. biseptata* and *D. holmii*, respectively (Table 1).

Effect of Fungal Culture Filtrates on Wheat Growth and Yield

Maximum and significant reduction of 14% in height of wheat plants was recorded due to *R. dentatus* in weedy check as compared to weed free control. Plant height was also significantly reduced due to presence of the weed in treatments where only culture filtrates of different *Drechslera* species were used as foliar spray. There was 6.3, 7.7, 10.7 and 13.3% reduction in plant height due to foliar spray of *D. australiensis*, *D. hawaiiensis*, *D. biseptata* and *D. holmii*, respectively as compared to weed free control. The effect of weed competition on height of wheat plants was insignificant over control where synthetic herbicide Bromoxynil+MCPA was sprayed either alone or in combination with the culture filtrates of the four *Drechslera* species (Table 2). The response of number of tillers and total above ground dry biomass to the weed competition and foliar spray applications was generally similar to that of the response of plant height. Maximum and significant reduction of 30% and 28% in tillering and above ground dry matter, respectively was recorded in weedy check as compared to control. Similarly, there was 9–21% and 12–23% reduction in number of tillers and above ground dry matter, respectively due to weed competition in various treatments where only the fungal culture filtrates were used as foliar spray. In contrast, in all the synthetic herbicide treatments, either alone or in combination with fungal culture filtrates, the effect of *R. dentatus* interference on the two studied parameters was nonsignificant as compared to control (Table 2).

Data regarding the effect of weed competition and various types of foliar spray applications on grain yield and 100 grains weight are presented in Table 2. Maximum grain yield (2.320 t ha⁻¹) was obtained in weed free treatment. The highest reduction in grain yield (43%) was recorded in weedy check over control. Similarly, significant reduction in grain yield was also recorded due to the weed competition over control in treatments, where only fungal culture filtrates were used in the foliar spray application. There was 21%, 22%, 35% and 36% reduction in grain yield over weed free control in treatments of foliar spray applications of culture filtrates of *D. australiensis*, *D. hawaiiensis*, *D. biseptata* and *D. holmii*, respectively. *R. dentatus* interference significantly reduced the weight of 100 grains by 30%, 25% and 27% in weedy check, and in treatments where culture filtrates of *D. biseptata* and *D. holmii*, respectively were used in the foliar spray application, respectively. The effect of *R. dentatus* on grain yield and 100 grains weight was non-significant in Bromoxynil+MCPA treatments, either alone or in combination with fungal culture filtrates.

Table 1: Effect of foliar spray of herbicide Bromoxynil+MCPA and metabolites of *Drechslera* spp. on biomass of *Rumex dentatus*

Treatments	Weed dry biomass (t ha ⁻¹)	Reduction over weedy check (%)
Weed free (Hand weeding)	0 d	-
Weedy check	3.557 a	0
<i>Drechslera hawaiiensis</i>	1.520 c	57
<i>D. holmii</i>	2.496 b	30
<i>D. biseptata</i>	2.449 b	31
<i>D. australiensis</i>	1.492 c	58
Bromoxynil+MCPA (Recommended dose)	0 d	100
Bromoxynil+MCPA (Half dose)	0 d	100
Bromoxynil+MCPA (Half dose) + <i>D. hawaiiensis</i>	0 d	100
Bromoxynil+MCPA (Half dose) + <i>D. holmii</i>	0 d	100
Bromoxynil+MCPA (Half dose) + <i>D. biseptata</i>	0 d	100
Bromoxynil+MCPA (Half dose) + <i>D. australiensis</i>	0 d	100

±, Indicates standard errors of means of three replicates

In a column, values with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test**Table 2:** Effect of foliar spray with recommended (RD) and half dose (HD) of herbicide Bromoxynil+MCPA, and metabolites of *Drechslera* spp. on growth and yield of wheat

Treatments	Plant height (cm)	No. of tillers/plant	Dry biomass (t ha ⁻¹)	Grain yield (t ha ⁻¹)	100 grains weight (g)
Weed free (Hand weeding)	90.0 a	11 a	5.637 a	2.320 a	4.12 a
Weedy check	77.1 d	8 d	4.082 c	1.333 e	2.89 b
<i>D. hawaiiensis</i> (Dh)	83.1 bc	10 b	4.926 a-c	1.799 cd	3.73 a
<i>D. holmii</i> (Dhl)	78.0 d	9 c	3.366 bc	1.477 de	3.01 b
<i>D. biseptata</i> (Db)	80.3 cd	9 c	4.498 bc	1.515 de	3.07 b
<i>D. australiensis</i> (Da)	84.3 b	10 b	4.966 ab	1.838 b-d	3.76 a
Herbicide (RD)	86.3 ab	11 a	5.392 a	2.137 a-c	3.99 a
Herbicide (HD)	89.3 a	11 a	5.493 a	2.254 ab	4.10 a
Herbicide (HD) + Dh	88.7 a	11 a	5.598 a	2.227 ab	4.11 a
Herbicide (HD) + Dhl	88.7 a	11 a	5.470 a	2.149 a-c	4.06 a
Herbicide (HD) + Db	88.3 a	11 a	5.365 a	2.203 a-c	3.96 a
Herbicide (HD) + Da	89.3 a	11 a	5.520 a	2.254 ab	4.11 a

In a column, values with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test

Economic and Marginal Analyses

All the treatments showed higher net profit than weedy check. Among the various fungal culture filtrate treatments, filtrates of *D. australiensis* gave the highest net profit of Rs. 53288 followed by Rs. 52069 due to spray of filtrates of *D. hawaiiensis*, which were Rs. 11632 (28%) and Rs. 10413 (25%) higher than the net profit in weedy check. The highest net profit of Rs. 70100 was provided by hand weeding followed by Rs. 69128 in by half dose of herbicide, which were Rs. 28444 (68%) and Rs. 27472 (66%) greater over weedy check, respectively (Table 3). Hand weeding was the best treatment according to marginal analysis with a 3077% marginal rate of return, followed by half dose of herbicides with a 2097% marginal rate of return. Rest of the treatments, were dominated because of either increasing cost that vary or lower net profits (Table 4).

Discussion

In the field study, herbicidal activity of culture filtrates of four *Drechslera* species was assessed and compared with a commercial herbicide Bromoxynil+MCPA. The chemical herbicide was used either alone as recommended and half dosages, or its half dose was applied in combination with

culture filtrates of various *Drechslera* species. *R. dentatus* reduced wheat grain yield by 43% over weed free control. Application of recommended dose of chemical herbicide completely killed the weed plants. Although none of the fungal culture filtrates treatments completely eliminated the weed, however, these markedly reduced the weed biomass by 30–58% over weedy check. Similar decline in weed biomass has also been reported by Javaid *et al.* (2011) where culture filtrates of *Drechslera* spp. were exploited to manage parthenium weed in pot experiments. Wheat grain yield losses in treatments where foliar spray of culture filtrates of *D. australiensis*, *D. hawaiiensis*, *D. biseptata* and *D. holmii* was carried out were 21, 22, 35 and 36%, respectively as compared to 43% losses in weedy check. This variation in herbicidal activity of culture filtrates of different *Drechslera* spp. could be attributed to the variation in chemical constituents of different fungal species (Evidente *et al.*, 2005; Zhou *et al.*, 2008; Eneyskaya *et al.*, 2009; Yang *et al.*, 2009). Previous studies conducted regarding the effect of foliar spray of culture filtrates of different pathogenic fungi including species of *Fusarium*, *Alternaria* and *Drechslera* against parthenium weed support the findings of the present study and suggest that fungal culture filtrates can be exploited as herbicides (Idrees and Javaid, 2008; Javaid and Adrees, 2009; Javaid *et al.*, 2011).

Table 3: Economic analysis for the use of fungal metabolites for management of *Rumex dentatus* in wheat

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	T ₁₁	T ₁₂	Remarks
Grain yield	2.320	1.333	1.799	1.477	1.515	1.838	2.137	2.254	2.227	2.149	2.203	2.254	t ha ⁻¹
Gross income (PKR ha ⁻¹)	72500	41656	56219	46156	47344	57438	66781	70438	69594	67156	68844	70438	wheat grain price@ PKR 1250 40 kg ⁻¹
Cost of labour for hand weeding	2400	—	—	—	—	—	—	—	—	—	—	—	PKR 300 man ⁻¹ (8 men day ⁻¹ ha ⁻¹)
Cost of fungal metabolites ha ⁻¹	—	—	3750	3750	3750	3750	—	—	1250	1250	1250	1250	PKR 5 L ⁻¹ of fungal culture filtrates
Cost of herbicide ha ⁻¹	—	—	—	—	—	—	1820	910	910	910	910	910	PKR 1820/560 g active ingredients
Spray application cost ha ⁻¹	—	—	1200	1200	1200	1200	400	400	400	400	400	400	PKR 400 man ⁻¹ (one man day ⁻¹ ha ⁻¹)
Cost that vary	2400	0	4150	4150	4150	4150	2220	1310	2560	2560	2560	2560	PKR ha ⁻¹
Net profit	70100	41656	52069	42006	43194	53288	64561	69128	67034	64596	66284	67878	PKR ha ⁻¹
T ₁	Weed free (Hand weeding)												
T ₂	Weedy check												
T ₃	<i>Drechslera hawaiiensis</i> @ 250 L ^{3S} ha ⁻¹												
T ₄	<i>D. holmii</i> @ 250 L ^{3S} ha ⁻¹												
T ₅	<i>D. biseptata</i> @ 250 L ^{3S} ha ⁻¹												
T ₆	<i>D. australiensis</i> @ 250 L ^{3S} ha ⁻¹												
T ₇	Bromoxynil+MCPA (Recommended dose) @ 560 g a.i. ^{1S} ha ⁻¹												
T ₈	Bromoxynil+MCPA (Half dose) @ 280 g a.i. ^{1S} ha ⁻¹												
T ₉	Bromoxynil+MCPA (Half dose) + <i>D. hawaiiensis</i> @ 280 g a.i. ^{1S} ha ⁻¹ + 250 L ^{1S} ha ⁻¹												
T ₁₀	Bromoxynil+MCPA (Half dose) + <i>D. holmii</i> @ 280 g a.i. ^{1S} ha ⁻¹ + 250 L ^{1S} ha ⁻¹												
T ₁₁	Bromoxynil+MCPA (Half dose) + <i>D. biseptata</i> @ 280 g a.i. ^{1S} ha ⁻¹ + 250 L ^{1S} ha ⁻¹												
T ₁₂	Bromoxynil+MCPA (Half dose) + <i>D. australiensis</i> @ 280 g a.i. ^{1S} ha ⁻¹ + 250 L ^{1S} ha ⁻¹												

^{1S} One spray 40 days after sowing; ^{3S} Three spray at 40 + 47 + 54 days after sowing; PKR = Pakistani Rupee (1 US Dollar = 100 PKR); a.i. = Active Ingredients; @ = at the rate of

Table 4: Marginal analysis for the use of fungal metabolites for management of *Rumex dentatus* in wheat

Treatments	Dose (ha ⁻¹)	Variable cost (PKR ha ⁻¹)	Net profit (PKR ha ⁻¹)	Marginal cost (PKR ha ⁻¹)	Marginal net profit (PKR ha ⁻¹)	Marginal rate of return (%)
T ₂ Weedy check	—	—	41656	—	—	—
T ₈ Bromoxynil+MCPA	280 g a.i. ^{1S}	1310	69128	1310	27472	2097
T ₇ Bromoxynil+MCPA	560 g a.i. ^{1S}	2220	64561	—	—	D
T ₁ Weed free (hand weeding)	—	2400	70100	180	5539	3077
T ₁₂ Bromoxynil+MCPA + <i>D. Australiensis</i>	280 g a.i. ^{1S} + 250 L ^{1S}	2560	67878	—	—	D
T ₉ Bromoxynil+MCPA + <i>D. hawaiiensis</i>	280 g a.i. ^{1S} + 250 L ^{1S}	2560	67034	—	—	D
T ₁₁ Bromoxynil+MCPA + <i>D. biseptata</i>	280 g a.i. ^{1S} + 250 L ^{1S}	2560	66284	—	—	D
T ₁₀ Bromoxynil+MCPA + <i>D. holmii</i>	280 g a.i. ^{1S} + 250 L ^{1S}	2560	64596	—	—	D
T ₆ <i>Drechslera australiensis</i>	250 L ^{3S}	4150	53288	—	—	D
T ₃ <i>D. hawaiiensis</i>	250 L ^{3S}	4150	52069	—	—	D
T ₅ <i>D. biseptata</i>	250 L ^{3S}	4150	43194	—	—	D
T ₄ <i>D. holmii</i>	250 L ^{3S}	4150	42006	—	—	D

^{1S} One spray 40 days after sowing; ^{3S} Three spray at 40 + 47 + 54 days after sowing; PKR = Pakistani Rupee (1 US Dollar = 100 PKR); a.i. = Active Ingredients; D = Dominated due to less profits than the preceding treatment

Moreover, a number of herbicidal compounds have also been isolated from different species of *Drechslera*. As an example, culture of *D. siccans* (Drechsler) Shoemaker is reported to yield a phytotoxin named as 6, 8-dihydroxy-3-(2'-hydroxypropyl) isocoumarin (de-o-methyldiaporthin) and found very effective against common oat (*Avena sativa* L.), barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and spiny amaranth (*Amaranthus spinosus* L.) (Hallock *et al.*, 1988). Capiro *et al.* (2004) isolated two phytotoxic compounds namely cytochalasin B and dihydrocytochalasins from culture filtrates of *D. wirreganensis* Wallwork, Lichon and Sivan. and *D. campanulata* (Lév.) B. Sutton. Similarly, another metabolite namely drazepinone with broad spectrum herbicidal activity has been isolated from *D. siccans*

(Drechsler) Shoemaker. This compound was characterized as 3,5,12a-trimethyl-2,5,5a,12a-tetrahydro-1H naphtha [20, 30:4, 5] furo [2,3-b] azepin-2-one (Evidente *et al.*, 2005). In the present study, original culture filtrates were applied as foliar spray. It is very likely that efficacy of these filtrates can be enhanced markedly if these are applied in a concentrated form because generally, quantity of active herbicidal constituents in fungal culture filtrates is very low (Evidente *et al.*, 2006a, b).

In the present field trials, half dose of synthetic herbicide also completely killed the *R. dentatus* plants. Consequently, the effect of combined application of fungal culture filtrates and half dose of herbicide could not be assessed. Further studies are required in this regard using

lower concentrations of the herbicide in combination with fungal culture filtrates. In the present study, no adverse effect of the fungal metabolites on growth of wheat was noted. This differential response of the weeds especially *R. dentatus* and wheat to fungal metabolites can be best exploited in the management of *R. dentatus* and possibly other broad-leaf weeds by the culture filtrates of *Drechslera* spp. under field conditions. To the best of our knowledge, the present study is the first report of using fungal metabolites as herbicidal agents in true field conditions. Generally, earlier experiments were carried out in Petri plates, trays or pots (Vurro *et al.*, 2001; Javaid *et al.*, 2011).

The usefulness of any treatment depends on the cost involved and its economic return as well as its impact on the environment. In the present study, an economic benefit of Rs. 11632 and 10413 was achieved by the application of culture filtrates of *D. australiensis* and *D. hawaiiensis* that was 28% and 25% higher than the net profit obtained in weedy check. Although the highest net profit and economic return was obtained with hand weeding followed by application of 280 g a.i. ha⁻¹ of Bromoxynil+MCPA, however, fungal culture filtrates are more environment friendly than herbicides and can be used as an alternative for weed management in organic farming systems.

Conclusion

The present study concludes that culture filtrates of *D. australiensis* and *D. hawaiiensis* contain potent herbicidal constituents for the management of *R. dentatus* as they reduced biomass of this weed by 58% and 57%, consequently increased the wheat grains yield by 22% and 20%, respectively, as compared to weedy check. Recently, Akbar *et al.* (2014) have identified holadysenterine as potent herbicidal metabolite from culture filtrates of *D. australiensis* that may be used as an alternative to chemical herbicides in production of organic wheat.

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