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Prospects of using Fungal Metabolites for the Management of *Rumex dentatus*, a Problematic Weed of Wheat

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Abstract

Rumex dentatus L. is a very common weed of wheat (*Triticum aestivum* L.) in Pakistan. Present laboratory and pot trials were carried out to evaluate metabolites of *Drechslera holmii, Drechslera biseptata* and *Drechslera australiensis* as natural herbicides against *R. dentatus.* Fungal metabolites were prepared in M1D medium. In laboratory bioassays, original and 50% diluted metabolites of different *Drechslera* spp. significantly reduced seed germination, shoot biomass and root biomass of *R. dentatus* seedlings by 5–56%, 15–83% and 68–88%, and that of wheat seedlings by 8–20%, 16–38% and 28–38%, respectively. Metabolites of *D. biseptata* and *D. australiensis* exhibited greater herbicidal activity than metabolites of *D. holmii.* In foliar spray repeated pot trials, original metabolites of the three *Drechslera* spp. significantly reduced shoot biomass of one- and two-week old plants of *R. dentatus* by 54–60% and 27–46%, respectively. By contrast, the effect of metabolites of all the three *Drechslera* spp. was non-significant on growth of wheat plants. Metabolites of *D. australiensis* showed the highest herbicidal activity in pot trials. It is concluded that metabolites of Pakistani *Drechslera* spp. possess herbicidal potential. © 2013 Friends Science Publishers

Keywords: *Drechslera* species; Fungal metabolites; Herbicidal activity; *Rumex dentatus*

Introduction

Rumex dentatus (family Polygonaceae) is native to southern and eastern Asia viz. China, India, Japan and Korea (Anonymous, 2013). Ecological surveys of various wheat growing areas of Pakistan have shown that *R. dentatus* is one of the most frequently occurring weeds of wheat (Qureshi *et al*., 2009; Qureshi and Bhatti, 2001; Siddiqui and Bajwa, 2001). It is an annual herb and is highly competitive causing remarkable reduction in yield of wheat (Siddiqui *et al*., 2010). Anjum and Bajwa (2010) reported 83% reduction in grain yield of wheat when crop and weed were grown in 1:1 ratio. *R. dentatus* is known for its inherent allelopathic activity, producing compounds that inhibit the growth of neighboring plants (Hussain *et al*., 1997). The weed not only reduces yield of wheat but also deteriorate quality of the produce (Memon *et al*., 2003).

Chemical herbicides are used to control *R. dentatus*. Although chemical herbicides have been proved very effective in controlling *R. dentatus* (Cheema *et al*., 2006; Usman *et al*., 2010; Naseer-ud-Din, 2011), However, we are now facing new challenges regarding weed control with these synthetic agro-chemicals such as emergence of weeds resistant to herbicides (Yuan *et al*., 2007; Llewellyn *et al*., 2009; Farooq *et al*., 2011), and concerns about herbicide effects in food, soil, ground water and atmosphere (Marín *et al*., 2003; Rial-Otero *et al*., 2005). Keeping in mind these shortcomings of herbicides and overwhelming demand for organic farming in the world, scientists are in search of alternative environmental friendly weed management strategies. In this regards use of crude and pure natural herbicidal constituents of plants and fungi has gained much attention in current years as an alternative to synthetic herbicides (Javaid and Adrees, 2009; Javaid, 2010; Javaid *et al.,* 2013). There are many successful stories to control weeds by the use of fungal metabolites (Evidente *et al*., 2008; Javaid and Ali, 2011; Vurro *et al*., 2012).

Many fungal species of the genus *Drechslera* are plant pathogen (Jorgensen and Olsen, 2007; Rabbani *et al.,* 2011). Metabolites of some species of *Drechslera* have also been reported to have herbicidal effects (Evidente *et al*., 2005). Evidente *et al.* (2006) reported that *Drechslera gigantea*, a fungal pathogen isolated from *Digitaria sanguinalis* produced phytotoxins in liquid and solid cultures which were potential mycoherbicide of grassy weeds. Earlier, Shukla *et al.* (1990) isolated a phytotoxin Drechslerol-C that was highly effective against an invasive plant species *Costus speciosus.* Javaid *et al.* (2011) reported that metabolites of some *Drechslera* species from Pakistan exhibited potential herbicidal activity against noxious parthenium weed (*Parthenium hysterophorus* L.). Likewise, Akbar and Javaid (2012a, b) found that metabolites of *Drechslera* species were very effective against two problematic grassy weeds of wheat namely *Avena fatua* and

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Phalaris minor. However, data concerning the herbicidal effect of *Drechslera* species against a problematic broad-leaf weed of wheat *R. dentatus* is lacking. Therefore, the present study was designed to evaluate herbicidal potential of metabolites of three *Drechslera* species from Pakistan against *R. dentatus.*

Materials and Methods

Preparation of Culture Filtrates of Test Fungi

Three *Drechslera* spp. viz. *D. australiensis*, *D. holmii* and *D. biseptata* were obtained from Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, Punjab University Lahore. Mineral medium (M1D) was prepared in distilled water as described by Evidente *et al*. (2006). This medium comprised of 1.2 mM $Ca(NO3)_2$, 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 H_3BO_3 and 4.5 µM KI. To adjust 5.5 pH of the medium, 0.1 M HCl was used. In each 500 mL conical flask, 200 mL M1D medium was autoclaved at 121°C for 30 min. After cooling at room temperature, flasks were inoculated with actively growing fungal discs of 5-mm diameter and incubated at 25±2ºC for 28 days. Fungal cultures were filtered using sterilized muslin cloth, centrifuged at 4000 × *g* for ten minutes followed by filtration through sterilized Whatman filter paper No. 1. Dilutions of 50% were prepared by addition of sterilized distilled water to the original metabolites (100%) following Javaid *et al.* (2013).

Laboratory Bioassays

The effect of original and diluted metabolites of the three test *Drechslera* species was assessed on germination as well as on early seedling growth of *R. dentatus* and a wheat var. Uqab 2000 in 9-cm diameter Petri plates. Seeds of wheat and *R. dentatus* were surface sterilized with 1% sodium hypochlorite for 10 min. Twenty seeds of each of the test weed species and wheat were arranged on sterilized filter paper beds in Petri plates. To each Petri plate, 3 mL of fungal metabolites were poured. Treatments with 3 mL of M1D medium (Original and 50% diluted) served as positive control while distilled water was used in negative control treatment. All the treatments were performed in quadruplicate. Plates were arranged in a completely randomized design at 16°C with 10 h light period daily in a growth room. After 15 days, seed germination, and length and fresh weight of root and shoot were recorded. To determine dry biomass, materials were placed in an electric oven for 72 h at 60°C (Javaid and Ali, 2011).

Foliar Spray Pot Trials

These experiments were conducted during November-December 2010 in University of the Punjab, Lahore, Pakistan. Mean daily temperature and relative humidity in the months of November and December were 20°C and 15°C, and 62% and 67%, respectively. Plastic pots (12-cm deep, 8-cm diameter), each containing 350 g soil having sandy loam texture, 0.69% organic matter, pH 7.8, available phosphorus 6.3 mg kg^{-1}, exchangeable potassium 100 mg kg^{-1} and 350 mg kg^{-1} nitrogen content. Seeds of wheat and *R. dentatus* (10 in each pot) were sown in these pots on November 20, 2010. Each treatment was replicated four times. Pots were placed in open under natural environmental conditions. Pots were divided into two groups after germination so that foliar spray can be executed on 1-week and 2-week old plants. Experiment was conducted using completely randomized design. Experiment regarding the effect of foliar spray on *R. dentatus* was repeated to confirm the herbicidal potential of fungal metabolites against the target weed species.

Foliar spray with original metabolites of the three *Drechslera* spp. was carried out on 1-week and 2-week old weed and wheat plants four times with intervals of four days. About 5 mL of fungal metabolites were sprayed on each pot to completely moist the plants. Spray with distilled water was carried out on plants of negative control whereas M1D medium without fungal inoculation was used for spray on plants of positive control treatment. All the sprays were carried out during evening hours. After 50 days growth, weed and wheat plants were carefully uprooted and washed thoroughly under tap water to remove soil. Then these plants were allowed to remain under fan, set on a water absorbent at room temperature until excess moisture was evaporated. Various plant vegetative growth parameters viz. shoot length, and dry biomass of root and shoot were recorded (Javaid *et al*., 2011).

Statistical Analysis

All the data of laboratory and pot bioassays were subjected to analysis of variance (ANOVA). Treatment means were separated by applying Duncan's Multiple Range Test at 5% level of significance (Steel *et al.,* 1997).

Results

Laboratory Bioassays

The effect of M1D broth was non-significant on germination of *R. dentatus* in Petri plate laboratory bioassays. Metabolites of all the three *Drechslera* spp. significantly reduced germination to variable extents. The highest reduction in germination was due to original metabolites (100%) of *D. biseptata* (up to 56% reduction) followed by those of *D. australiensis* and *D. holmii*, respectively. The original metabolites of *D. australiensis* and *D. holmii* reduced germination significantly by 12% and 40%, respectively (Table 1).

Original M1D growth medium exhibited significant effect on shoot growth of *R. dentatus* resulting in 15%, 17%

and 15% reduction in length, and fresh and dry weight of shoot, respectively. However, the effect of diluted M1D medium was non-significant on different shoot growth parameters. Negative effect of the different fungal metabolites was significant on various shoot growth parameters as compared to control. Original metabolites of *D. australiensis* showed the highest toxicity and reduced shoot length and dry biomass of the weed by 85% and 88%, respectively. Similarly, original metabolites of *D. biseptata* caused 81% and 83% decline in shoot length and dry biomass, respectively. Metabolites of *D. holmii* showed the least herbicidal activity resulting in 73% and 72% reduction in length and dry weight of shoot over control, respectively (Table 1). Root growth of *R. dentatus* exhibited more susceptibility to fungal metabolites application than shoot growth. There was 82–94% and 77–88% decline in root length and biomass, respectively due to various fungal metabolite treatments. Metabolites of *D. australiensis* showed the highest activity in inhibiting various root growth parameters of *R. dentatus*. This species caused 94% and 88% reduction in root length and root dry weight, respectively (Table 1).

The effect of growth medium on the germination of wheat was non-significant when compared with control. Different fungal metabolites reduced germination of wheat by 8–15% over control. However, the effect of all the metabolites except 100% metabolites of *D. holmii* was non-significant on germination of wheat (Table 2). Original growth medium had significant adverse effect on various shoot and root growth parameters. However, the effect was far low as compared to fungal metabolites. There was 18% and 10% suppression in shoot length and dry biomass because of original growth medium, and 30–38% and 28–38% reduction in these parameters over control due to original concentration of different fungal metabolites, respectively. Similarly, original growth medium significantly reduced root length and dry biomass by 17% and 15% while various fungal metabolites suppressed these growth parameters by 38–67% and 33–38%, respectively, over control (Table 2).

Foliar Spray Pot Trials

Data presented in Table 3 shows that foliar spray with growth medium had non-significant effect on length as well as dry biomass of *R. dentatus* shoot. Shoot length of 1-week old weed plants was significantly reduced by foliar spray with metabolites of each of the three fungal species. Similarly, spray with metabolites of *D. australiensis* and *D. bisepta* significantly reduced shoot length of 2-week old weed plants. Metabolites of *D. australiensis* caused the highest reduction in shoot length (42%) of 1-week old weed plants. Metabolites of *D. holmii* and *D. biseptata* showed comparatively less pronounced herbicidal activity against the test weed species causing 33% and 23% reduction in shoot length of 1-week old plants, respectively. In general, the adverse effect of metabolites of various test fungal species on shoot biomass was comparable to that of their effect on shoot length. The highest decline of 60% in shoot biomass of 1-week old plants was recorded due to application of metabolites of *D. australiensis* followed by *D. biseptata* (56%) and *D. holmii* (54%). Negative effect of foliar spray on shoot biomass was more pronounced in 1-week than in 2-week old plants. Root biomass of the weed was significantly suppressed in 1-week old plants by 68–82% due to foliar spray of different *Drechslera* species. Metabolites of *D. australiensis* proved to be the most effective, inhibiting root biomass by 82% followed by 74% and 68% reduction in root biomass due to metabolites of *D. holmii* and *D. biseptata*, respectively. Herbicidal activity of metabolites of various fungal species on 2-week old plants was comparatively less pronounced, where 58-73% reduction in root biomass was recorded.

The effect of all the fungal metabolite treatments on shoot and well as root biomass of wheat var. Uqab 2000 was non-significant both on 1-week and 2-week old plants (Table 4).

Discussion

In the recent years, there is a growing trend from synthetic compounds towards natural ecofriendly compounds. In search of natural herbicidal compounds based upon fungal metabolites, a number of highly successful attempts have been taken (Evidente *et al*., 2006; Berestetskiy, 2008). However, studies about the herbicidal activity of metabolites of Pakistani *Drechslera* species are scarce. The present study was, therefore, designed to investigate the herbicidal potential of metabolites of *Drechslera* spp. from Pakistan against a problematical weed of wheat. In general, metabolites of all the three test *Drechslera* spp. namely *D. australiensis, D. biseptata* and *D. holmii*, exhibited herbicidal activity to variable extent against *R. dentatus*.

In laboratory bioassays, generally original concentration of the M1D growth medium reduced germination, length as well as fresh and dry biomass of *R. dentatus* seedlings. However, this effect was far less pronounced as compared to the effect of fungal metabolites. Original growth medium reduced germination, shoot length, shoot dry biomass, root length and root biomass by 5%, 15%, 15%, 14% and 6%, respectively. On the other hand, original metabolites of various *Drechslera* species suppressed germination, shoot length, shoot dry biomass, root length and root biomass by 12–56%, 73–85%, 72–88%, 82–94% and 77–88%, respectively. Although contents of the original growth medium exhibited adverse effect on germination and seedlings growth to some extent, however, it is very likely that most of the medium contents were used during the 28 days growth period of the test fungal species, and the effect of the medium components was probably negligible in the fungal metabolite treatments.

Table 1: Effect of metabolites of three *Drechslera* species on germination and growth of *Rumex dentatus* 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	19.0 a	5.4 a	1.20 a	18.8 a	0.87 a	0.155 a
Growth medium	50	97 a	17.7 a	4.9 ab	1.15 ab	18.3 a	0.78 a	0.140 b
	100	95 a	16.2 b	4.5 b	1.02 b	16.1 b	0.77 a	0.145 ab
D. holmii	50	95 a	7.1 c	1.7 cd	0.47 c	5.6 c	0.25 b-d	0.050 cd
	100	88 b	5.2 de	1.2 de	0.34 c-e	3.4 d	0.36 b	0.035 de
D. biseptata	50	48 d	4.9 e	1.1 de	0.27 d-f	2.7 de	0.13 cd	0.034 de
	100	44 d	3.6 f	0.7 e	0.21 ef	1.8 ef	0.08 d	0.019 f
D. australiensis	50	59 c	6.5 cd	1.1 de	0.28 d-f	3.1 d	0.15 cd	0.032 ef
	100	60 c	2.9 f	0.7 e	0.14 f	1.2 f	0.08 d	0.019 f

Table 2: Effect of metabolites of three *Drechslera* species on germination and growth of wheat variety Uqab 2000 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	131 a	90 a	5.0 a	109 a	65 a	9.6 a
Growth medium	50	97 ab	113 b	86 ab	4.8 ab	101 a	56 b	8.9 a
	100	94 a-c	107 c	81 bc	4.5 bc	91 b	51 bc	8.1 b
D. holmii	50	90 b-d	100 d	76 c	4.0 de	81 c	42 de	6.6 cd
	100	85 de	75 h	51 e	3.1 h	49 e	36 ef	6.4 cd
D. biseptata	50	92 b-d	96 de	67 d	3.9 d-f	76 c	43 de	6.7 cd
	100	88 c-e	91 f	54 e	3.4 gh	68 d	39 ef	6.4 cd
D. australiensis	50	89 b-d	100 d	80 c	4.2 cd	82 c	48 cd	6.9 c
	100	80 f	81 g	54 e	3.6 e-g	35 f	34 f	6.0 d

Values with different letters in a column show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test
Note: 100% means original growth medium and fungal metabolites

Table 3: Effect of foliar spray of metabolites of three *Drechslera* spp. on growth of 1-week and 2-week old plants of *Rumex dentatus*

Treatment time	Treatments	Shoot length (cm)	Shoot dry biomass (g)	Root dry biomass (g)
1-week old Plants	Control	6.44 a	0.072 a	0.066 a
	Growth medium	6.34 a	0.070 a	0.065 a
	D. holmii	4.30 c	0.033 e	0.017 d
	D. biseptata	4.96 c	0.032 e	0.021 cd
	D. australiensis	3.72 d	0.029 e	0.012 e
2-week old plants	Control	6.44 a	0.072 a	0.066 a
	Growth medium	6.34 a	0.070 a	0.065 a
	D. holmii	6.16 a	0.044 c	0.023 c
	D. biseptata	5.62 b	0.052 b	0.028 b
	D. australiensis	4.15 c	0.039 d	0.018 d

Table 4: Effect of foliar spray of metabolites of three *Drechslera* spp. on growth of 1-week and 2-week old plants of wheat

Treatment time	Treatments	Shoot length (cm)	Shoot dry biomass (g)	Root dry biomass (g)
1-week old Plants	Control	37.10 a	0.39 a	0.210 a
	Growth medium	37.08 a	0.37 a	0.202 a
	D. holmii	35.82 a	0.36 a	0.183 a
	D. biseptata	36.25 a	0.34 a	0.180 a
	D. australiensis	36.17 a	0.37 a	0.202 a
2-week old plants	Control	37.10 a	0.39 a	0.210 a
	Growth medium	37.08 a	0.37 a	0.202 a
	D. holmii	36.20 a	0.34 a	0.192 a
	D. biseptata	35.50 a	0.34 a	0.195 a
	D. australiensis	35.75 a	0.37 a	0.203 a

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

In laboratory bioassays, metabolites of all the test *Drechslera* spp. reduced seed germination of *R. dentatus* by 12–56%. Earlier, Idrees and Javaid, (2008) have reported 23% decrease in germination of *P. hysterophorus* seeds due to metabolites of *D. hawaiiensis*. In a similar study, Javaid and Adress (2009) reported 20%, 30% and 93% decline in germination of *P. hysterophorus* seeds due to metabolites of *D. biseptata, D. australiensis* and *D. rostrata*, respectively.

In the present study, metabolites of *D. australiensis* exhibited the best herbicidal activity against shoot groth of *R. dentatus.* Recently, Akbar (2013) isolated a herbicidal constituent namely holadysenterine from culture filtrates of this fungal species which may be responsible for herbicidal activity against the target weed species. Other herbicidal metabolites isolated from various *Drechslera* spp. so far include de-O-methyldiaporthin, drazepinone, Ophiobolin E and 8-epi-ophiobolin J (Hallock *et al.*, 1988; Evidente *et al.*, 2005, 2006).

Earlier, Akbar and Javaid (2010) studied the herbicidal effect of metabolites of the presently tested *Drechslera* species using malt extract as growth medium instead of M1D medium used in the present. The results of the two studies reveal that M1D is comparatively better growth medium than malt extract for the preparation of fungal metabolites for management of weeds of wheat. Recently, Javaid *et al*. (2013) have also reported similar differential herbicidal effects of metabolites of *Trichoderma* spp. prepared in M1D and malt extract growth media against *P. hysterophorus.* The variable herbicidal potential of the fungal metabolites prepared in different growth media could be due to the formation of different quantities of metabolites in different growth media (Zonno *et al*., 2008). In the present study, seedling growth of weed species was also adversely affected by metabolites of the test *Drechslera* species. Similar inhibition in seedling growth of other weed species such as *P. hysterophorus* has also been reported due to metabolites of *Drechslera* and other fungal species (Javaid and Adrees, 2009; Javaid *et al*., 2011). Findings of the present study reveal that metabolites of different test *Drechslera* spp. showed variable herbicidal activity against the germination and seedling growth of the weed. Metabolites of *D. biseptata* were found more effective in suppressing germination of the test weed species than the metabolites of other two fungal species. Dissimilarity in herbicidal activity of different *Drechslera* spp. has also been reported against growth of *P. hysterophorus* seeds (Javaid and Adrees 2009; Javaid *et al*., 2011). Differential herbicidal activity of metabolites of different *Drechslera* spp. could be attributed to the variation in chemical constituents of different fungal species (Evidente *et al*., 2005; Eneyskaya *et al*., 2009; Yang *et al*., 2009). In general, root growth was more susceptible than shoot growth to different fungal metabolites in laboratory bioassays. It is due to the fact that roots are the first to absorb herbicidal constituents from the surrounding, thus they show greater susceptibility to the applied materials than shoots (Noor and Khan, 1994).

In pot experiments, the effect of foliar spray with M1D medium on growth of the weed as well as wheat plants was nonsignificant. Although metabolites of all the *Drechslera* species exhibited pronounced herbicidal activity against *R. dentatus*, however, *D. australiensis* was found to be the most effective fungal species causing 42% reduction in shoot length of 1-week old weed plants. Adverse effects of foliar spray on shoot biomass of *R. dentatus* were more pronounced in 1-week old than in 2-week old plants. Although roots were not directly exposed to foliar spray application, however, root biomass in *R. dentatus* was also severely suppressed in 1-week old plants by 68–82% due to foliar spray of different *Drechslera* species. Previously, various studies conducted regarding the effect of foliar spray of metabolites of different pathogenic fungi including species of *Fusarium, Alternaria* and *Drechslera* against *P. hysterophorus* support the findings of the present study and suggested that fungal metabolites can be exploited as herbicides (Idrees and Javaid, 2008; Javaid and Adrees, 2009; Javaid *et al*., 2011).

In laboratory as well as pot trials, the effect of metabolites of various test *Drechslera* species was more severe on germination and growth of weed than that of wheat. Differential response of wheat and *R. dentatus* to the same fungal metabolites could be ascribed to different morphological and anatomical characteristic of the two plant species as wheat is monocotyledonous while *R. dentatus* is dicotyledonous. The differential response of *R. dentatus* and wheat to fungal metabolites can be best exploited in the management of *R. dentatus* by the metabolites of *Drechslera* spp.

The present investigation concludes that metabolites of all the three *Drechslera* species possess herbicidal potential against *R. dentatus*. Metabolites of *D. australiensis* exhibited the highest herbicidal activity. Further studies are needed to isolate and identify the effective herbicidal constituents from culture filtrates of *D. australiensis* to be used as structural lead for the synthesis of natural product based herbicides for management of *R. dentatus*.

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