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MANAGEMENT OF SOME PROBLEMATIC WEEDS OF WHEAT BY METABOLITES OF *Drechslera* sp. PREPARED IN MALT EXTRACT MEDIUM

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ABSTRACT

Present study was designed to evaluate the herbicidal activity of metabolites of four Drechslera species viz. D. australiensis, D. hawaiiensis, D. biseptata and D. holmii (prepared in malt extract broth) against some problematic weeds of wheat namely Rumex dentatus, Phalaris minor and Avena fatua in 2009. Metabolites of Drechslera spp. were employed in 100% (original) and 50% concentrations. These metabolites wrought appreciable reduction in the germination of test weed's seeds by 3-72%. Original metabolites of all the fungal species significantly reduced shoot length and biomass by 39-72% and 30-70%, respectively. Metabolites of D. australiensis D. hawaiiensis and D. biseptata showed pronounced phytotoxic activity against all tested weeds, D. holmii appeared to be least effective. Root growth was more susceptible to metabolites than shoot growth. The metabolites of Drechslera spp. reduced 56-97% and 27-92% in root and shoot biomass, respectively. The present study concludes that metabolites of all the tested Drechslera spp. contain phytotoxic constituents that can be used as benign method of weed control alternative to synthetic chemical herbicides for management of some weeds of wheat. Further studies regarding the isolation of effective ingredients are in progress.

Keywords: Alternate herbicides, *Avena fatua, Drechslera, Phalaris minor, Rumex dentatus,* weeds of wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is regarded as the staple food of Pakistan. It occupied an area of 8.14 m ha during the year 2005-2006 with an average grain yield of 2278 kg ha^{-1} (MINFAL, 2007), which is very low as compared to yield potential possessed by most of its cultivars. Among the reasons for this low yield, weeds are the most important. Siddiqui and Bajwa (2001) and Qureshi and Bhatti (2001) reported 31 and 45 weed species in wheat growing areas of Punjab and Sindh, respectively. In these studies, *Phalaris minor, Avena fatua,*

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Medicago polymorpha, Coronopus didymus, Melilotus parviflora, Rumex dentatus and Chenopodium album were found to be the most frequently occurring and densely populated weeds. These weeds are known to cause 20-60% yield losses in different wheat cultivars (Siddiqui, 2005).

Several methods of the weed control and weed eradication have been devised. Among these, chemical method is the common one. Various chemical herbicides such as Topik, Puma Super, Affinity and Buctril Super etc. are very effective in controlling weeds of wheat in wheat fields of Pakistan (Bibi *et al.*, 2005; Cheema *et al.*, 2006). However, in recent years, the use of chemicals has increased consumer's concern and their use is becoming more restrictive due to carcinogenic effects, residual toxicity problems, environmental pollution, occurrence of microbial resistance and high inputs (Marin *et al.*, 2003; Rial-Otero *et al.*, 2005). For more sustainable, eco-friendly integrated disease management strategies, there is a growing trend toward alternatives to synthetic chemical herbicides, which are less pesticide dependant or based on naturally occurring compounds (Cuthbertson and Murchie, 2005). One such alternative strategy to manage the weeds is the isolation of natural herbicidal constituents from plants (Batish *et al.*, 2007) and fungi (Evidente *et al.*, 2008; Javaid and Adrees, 2009). The present study was carried out to evaluate the herbicidal activity of culture filtrates of four species of *Drechslera* against some problematic weeds of wheat.

MATERIALS AND METHODS

Selection and procurement of test fungal species

Four plant pathogenic fungal species viz. *Drechslera australiensis*, *D. biseptata*, *D. hawaiiensis*, and *D. holmii* were selected to be evaluated for their herbicidal potential against three weeds of wheat. These test species were procured by Fungal Culture Bank of Pakistan, Institute of Plant Pathology, University of the Punjab Lahore, Pakistan.

Preparation of culture filtrates of the test fungi

All the tested fungal species were sub-cultured on Malt Extract Agar (MEA) medium in 9 cm diameter Petri plates and stored at 4 °C. Two percent (w/v) malt extract broth was prepared in distilled water, poured into 250 ml conical flasks @ 100 ml medium in each flask. These flasks were then autoclaved at 121 °C for 30 minutes and were inoculated with 5 mm agar discs of each of the four test fungal species from the margins of actively growing fungal colonies. Inoculated flasks were incubated at 25±2 ºC in an incubator for 20 days. The grown cultures were filtered through sterilized Whatman filter paper No.1. These extracts were stored at 4 ºC in a refrigerator as original concentrations. Sterilized distilled water was added to the

original filtrates (100%) to prepare dilution of 50% (Javaid and Adrees, 2009).

Laboratory bioassays

In laboratory bioassays, the effect of different concentrations of culture filtrates of the four selected fungal species was evaluated on germination and early seedling growth of test weed species. For this seeds of weeds were surface sterilized with 1% sodium hypochlorite for 10 minutes, 10 seeds of each test weed species were placed in sterilized 9 cm diameter Petri plates lined with a filter paper, moistened with 3 ml of different concentrations of fungal culture filtrates. Treatment in a similar manner but with 2 and 1% malt extract broth served as positive control. Similar treatment with distilled water was also made which served as negative control. Each treatment was replicated thrice. Petri plates were arranged in a completely randomized design in a growth room maintained at 25 °C and 10 h light period daily. After 20 days seed germination, root and shoot length and their fresh biomass were determined.

Statistical analysis

The data were analyzed by analysis of variance followed by Duncan's Multiple Range Test using computer software SPSS and COSTAT.

RESULTS AND DISCUSSION

Effect of fungal metabolites against *P. minor*

Data regarding the effect of culture filtrates of four *Drechslera* spp. against germination and seedling growth of *P. minor* are presented in Table-1. The effect of both 1% and 2% malt extract medium was insignificant on seed germination. Culture filtrates of both 50% and 100% concentration of all the four tested fungal species significantly reduced germination by 51-72%. Adverse effect of 100% culture filtrates on germination was more pronounced as compared to 50% concentration. The effect of the two concentrations of malt extract broth was insignificant on length as well as biomass of shoot. Shoot length was significantly reduced by culture filtrates of the test fungal species except 50% filtrates of *D. biseptata.* This concentration was also ineffective in reducing the shoot biomass while other treatments suppressed the shoot biomass to variable extents. Adverse effect of 100% culture filtrates of *D. biseptata* and *D. holmii* was significant as compared to control and two concentrations of malt extract broth. Root length was significantly suppressed by culture filtrates of all four *Drechslera* species. There was 50–97% reduction in root length due to different concentrations of the various culture filtrates as compared to control. Culture filtrates of *D. biseptata* were comparatively less effective against root length of *P. minor* as compared to filtrates of other fungal species. Root biomass was decline

by 11–54% due to various culture filtrates treatments. The effect of all the treatments except 50% filtrates of *D. biseptata* was significant.

Table-1. Effect of original (100%) and diluted (50%) culture filtrates of four *Drechslera* spp. against germination and seedling growth of *Phalaris minor*.

Fungal species	Conc. (%)	Germination (%)	Shoot length (mm)	Shoot biomass (mg)	Root length (mm)	Root biomass (mg)
Control (water)	0	83 a	61 a	5.7 ab	72 b	4.6 a
Malt Extract Broth	1	85 a	60 a	5.4 ab	78 a	4.6 a
	2	77 a	64 a	5.9 a	77 a	5.0 a
D. australiensis	50	41 b	44 cd	4.0 abc	3 e	2.9 cd
	100	23 cde	35 d	4.0 abc	3 e	2.4 cd
D. biseptata	50	41 b	56 ab	5.7 ab	36 b	4.1 ab
	100	23 e	40 cd	3.0 c	13 c	3.3 bc
D. hawaiiensis	50	40 b	48 bc	4.4 abc	4 e	2.8 cd
	100	23 e	44 cd	3.7 bc	3 e	2.2 d
D. holmii	50	36 bcd	43 cd	4.2 abc	3 e	2.5 cd
	100	26 de	35 d	3.3 c	2 e	2.1 d

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

Effect of fungal metabolites against *A. fatua*

Data regarding the effect of culture filtrates of four *Drechslera* spp. against germination and seedling growth of *A. fatua* are demonstrated in Table-2. The effect of both 1% and 2% malt extract broth on germination and various shoot/root growth parameters was insignificant. Different culture filtrate treatments reduced the germination by 8–47%. All except 50% culture filtrates of *D. hawaiiensis* and *D. holmii* significantly suppressed germination. All the culture filtrate treatments except 50% *D. biseptata* significantly reduced shoot growth of *A. fatua* in terms of length and biomass. Root growth was more susceptible and suppressed by culture filtrates of all the four *Drechslera* species. There was 14–65%, 2–43%, 44–95% and 29–92% reduction in shoot length, shoot biomass, root length and root biomass due to various culture filtrate treatments, respectively.

Effect of fungal metabolites against *R. dentatus*

Data regarding the effect of culture filtrates of four *Drechslera* spp. against germination and seedling growth of *R. dentatus* are summarized in Table-3. The effect of 1% as well as 2% of malt extract broth was insignificant on germination the target weed species. Original (100%) culture filtrates of *D. australiensis*, and *D. holmii* significantlt reduced germination while the effect of all other treatments was insignificant on studied parameter. Shoot length was reduced by 8-72% due to different culture filtrate treatments. Effect of all filtrate treatments except 50% *D. biseptata* was significant as

compared to control. Original filtrates of *D. australiensis* were found most effective in suppressing shoot length of *R. dentatus.* Shoot biomass showed a response to different filtrate treatments similar to that of shoot length. Root growth was more susceptible to the application of culture filtrates of four *Drechslera* species. Root length and biomass were significantly reduced by 47–97% and 30–88% due to different fungal culture filtrate treatments.

Table-2. Effect of original (100%) and diluted (50%) culture filtrates of four *Drechslera* spp. against germination and seedling growth of *Avena fatua.*

Fungal species	Conc. (%)	Germination (%)	Shoot length (mm)	Shoot biomass (mg)	Root length (mm)	Root biomass (mg)
Control (water)	0	85 ab	115 a	48 a	163 a	51 a
Malt Extract Broth	1	88 a	107 a	46 a	165 a	48 a
	2	95 a	106 a	43 ab	158 a	51 a
D. australiensis	50	58 cd	66 bc	32 c	19 c	9 c
	100	58 cd	40 d	27 c	11 c	5 c
D. biseptata	50	63 cd	99 a	46 a	91 b	36 b
	100	55 cd	56 bcd	26 c	20 c	11 c
D. hawaiiensis	50	78 bcd	57 bcd	31 c	14 c	8 c
	100	58 cd	46 cd	27 c	14 c	7 c
D. holmii	50	65 bcd	73 b	33 c	17 c	10 c
	100	45 d	60 bcd	29 bc	8 c	4 c

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

Table-3. Effect of original (100%) and diluted (50%) culture filtrates of four *Drechslera* spp. against germination and seedling growth of *Rumex dentatus.*

Fungal species	Conc. (%)	Germination (%)	Shoot length (mm)	Shoot biomass (mg)	Root length (mm)	Root biomass (mg)
Control (water)	0	98 a	15.4 a	10.5 b	34 b	2.6 a
Malt Extract Broth	1	93 ab	13.4 bc	14.7 a	42 a	2.9 a
	2	90 abc	15.2 a	15 a	40 a	2.7 a
D. australiensis	50	85 abc	6.4 f	6.5 de	1 e	0.9 c
	100	75 c	4.3 g	3.1 g	1 e	0.7 c
D. biseptata	50	93 ab	14.1 ab	9.2 bc	18 c	1.9 b
	100	85 abc	9.3 de	7.2 de	1 e	0.6 c
D. hawaiiensis	50	90 abc	12.3 c	8.1 cd	12 e	0.9 c
	100	95 ab	9.1 de	6.0 ef	1 e	0.4 c
D. holmii	50	95 ab	10.1 d	6.6 de	1 e	0.3 c
	100	80 bc	7.9 ef	4.7 fg	1 e	0.3 c

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

Results of the present study showed 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 Tokousbalides, 2000; Evidente *et al.*, 2005, 2006a; Javaid and Adrees, 2009). 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. In another study, Evidente *et al.*, (2005) reported Drazepinone, a trisubstituted tetrahydronaphthofuro azepinone from *Drechslera siccans* with herbicidal activity against monocot weeds. Earlier, Sugawara *et al.*, (1987) islated ophiobolin I from Drechslera maydis and Drechslera sorghicola that possessed herbicidal activity. Kastanias and Tokousbalides (2000) isolated pyrenophorol isolated from a *Drechslera avenae* pathotype that exhibited herbicidal potential against weeds. Further studies regarding the isolation of potential herbicidal constituents from the *Drechslera* species used in the present study, are in progress.

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