

## HERBICIDAL POTENTIAL OF *Drechslera* SPP. CULTURE FILTRATES AGAINST *Parthenium hysterophorus* L.

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Herbicidal activity of culture filtrates of four *Drechslera* spp., namely *D. australiensis* (Bugnic.) Subram. & B.L. Jain, *D. biseptata* (Sacc. & Roum.) M.J. Richardson & E.M. Fraser, *D. hawaiiensis* Bugnic. ex M.B. Ellis, and *D. holmii* (Luttr.) Subram. & P.C. Jain, prepared in malt extract broth was investigated against parthenium weed (*Parthenium hysterophorus* L.) in both laboratory bioassays and pots. In laboratory bioassays, the effect of original (100%) and diluted (50%) culture filtrates of the four *Drechslera* spp. was studied on parthenium germination and seedling growth in 90 mm diameter Petri plates. Original culture filtrate of all the four *Drechslera* species significantly reduced germination, shoot length, shoot fresh biomass, root length, and root fresh biomass of parthenium seedlings by 43 to 77%, 77 to 82%, 69 to 82%, 90 to 92%, and 67 to 83%, respectively, as compared to the control. In pot trials, foliar application of original fungal culture filtrates was carried out on 1-wk and 2-wk old parthenium seedlings. Culture filtrates of all the four *Drechslera* spp., except *D. holmii*, markedly reduced parthenium shoot dry weight. Two-week-old plants were more susceptible to foliar spray than the 1-wk old plants. There was a 13 to 55% and 28 to 65% reduction in shoot dry weight of 1-wk and 2-wk old parthenium plants, respectively, due to culture filtrates of various *Drechslera* spp. The present study concludes that *Drechslera* spp. culture filtrates can be used as alternative herbicides for parthenium weed management.

**Key words:** *Drechslera* spp., fungal culture filtrates, natural herbicides, *Parthenium hysterophorus*.

*Parthenium hysterophorus* L. (Asteraceae) is one of the world's worst weeds for agriculture, the environment, and human health. It is native to the subtropics of North and South America, and it was accidentally introduced to the Indian sub-continent in the mid-1950s through wheat grains (Chandras and Vartak, 1970). It is notorious for its strong competitiveness for soil moisture and nutrients, the hazards that it poses to humans and animals, and its allelopathic effect against the associated plant species (More *et al.*, 1982; Singh *et al.*, 2005). In Pakistan, it appeared in the late 1980s and has presently invaded and become the dominant weed in most of the waste and grazing lands, mainly in the rain-fed districts of Central and Northern Punjab, Khyber Pakhtoon Khawa, and Kashmir (Javaid and Anjum, 2005; Javaid and Riaz, 2007; Riaz and Javaid, 2010).

Some synthetic chemical herbicides, such as chlorimuron ethyl, glyphosate, atrazine, ametryn, bromoxynil, and metasulfuron, are known to be very effective in controlling this weed (Mishra and Bhan, 1994; Javaid, 2007). However, for more sustainable and eco-friendly integrated weed management strategies, there is a growing trend toward alternatives to synthetic chemical herbicides that are less pesticide-dependent or based on naturally occurring compounds (Singh *et al.*,

2003). One such alternative weed management strategy is using natural herbicides from plants (Javaid *et al.*, 2010; 2011) and fungi (Javaid and Adrees, 2009; Javaid, 2010; Javaid and Ali, 2011a).

There are many examples of fungal products with herbicidal activities in the literature. Cornexistin is a nonadride phytotoxin from the coprophyllic basidiomycetous fungus, *Paecilomyces variotii*, which has good herbicidal activity against monocotyledonous and dicotyledonous weeds, but not against the maize crop (Nakajima *et al.*, 1989). AAL-toxin, a phytotoxin produced by *Alternaria alternata*, is active against many weed species (Abbas *et al.*, 1995). Similarly, a phytotoxic metabolite, trans-4 aminoproline, was isolated from *Ascochyta caulina* culture filtrates and found to be very effective in controlling *Chenopodium album* (Evidente *et al.*, 2000). *Drechslera* is a genus of fungi in which many of the species are plant pathogens. *Drechslera* species have been mostly isolated from wheat, rice, barley, and maize seeds and are known to cause leaf spot, southern leaf spot, seedling blight, foot rot, leaf blotch, stem spot, leaf streak, and blight (Neergaard, 1977; Rabbani *et al.*, 2011). Earlier studies showed that certain *Drechslera* species, such as *D. maydis*, *D. sorghicola*, *D. avenae*, and *D. siccans*, produce herbicidal metabolites (Sugawara *et al.*, 1987; Kastanias and Tokousbalides, 2000; Evidente *et al.*, 2005). However, there is a lack of studies regarding the herbicidal activity of *Drechslera* species from Pakistan. The present study was therefore conducted to explore the herbicidal potential of four *Drechslera* species from Pakistan, namely *D.*

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*australiensis*, *D. biseptata*, *D. hawaiiensis*, and *D. holmii* for parthenium weed management.

## MATERIALS AND METHODS

### Preparation of test fungi culture filtrates

Pure cultures of four species of *Drechslera*, viz. *D. australiensis* (Bugnic.) Subram. & B.L. Jain, *D. biseptata* (Sacc. & Roum.) M.J. Richardson & E.M. Fraser, *D. hawaiiensis* Bugnic. ex M.B. Ellis, and *D. holmii* (Luttr.) Subram. & P.C. Jain were procured from the Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. These cultures were maintained on malt extract agar (MEA) medium and stored at 4 °C.

Malt extract broth (2%) was autoclaved at 121 °C in 250 mL conical flasks with 100 mL medium in each flask. Flasks were inoculated with 5 mm agar discs of each test fungal species from margins of actively growing 7-d old fungal colonies. After inoculation, flasks were incubated under static conditions at 25 °C for 4 wk. The cultures were filtered after 4 wk through sterilized muslin cloth followed by Whatman filter paper N° 1 and bacterial filter papers, respectively. These filtrates were preserved at 4 °C to avoid any contamination or chemical alteration, and the culture filtrates were used within 1 wk of filtration (Javaid and Adrees, 2009).

### Laboratory bioassays

Parthenium seeds were collected in early February and the experiment was conducted in February 2009. Parthenium seeds were placed in 9 cm diameter sterilized Petri plates with sterilized filter papers moistened with 3 mL of each of the original (100%) and diluted (50%) crude fungal extracts of different *Drechslera* species. Twenty seeds were placed in each plate. The negative control treatment received 3 mL of sterilized distilled water. Since fungal metabolites were obtained with 2% malt extract broth and filtrates were also employed as 50% dilutions, two positive control treatments with 3 mL of 2% and the same quantity of 1% autoclaved malt extract broth were also included. Each treatment was replicated four times. The Petri plates were arranged in a completely randomized design in a growth room at room temperature (22 ± 2 °C) and 10-h daily light period. Harvest took place after 2 wk. Data regarding germination, root/shoot length, and fresh weight were recorded (Javaid and Adrees, 2009).

### Pot trials

A pot experiment was conducted during February-March 2009 in University of the Punjab, Lahore (31.57° N, 74.31° E), Pakistan. Plastic pots, 8 cm diameter and 12 cm deep, were filled with 350 g sandy loam soil with organic matter 0.69%, pH 7.8, N 0.035%, available P 6.3 mg kg<sup>-1</sup>, and available K 100 mg kg<sup>-1</sup>. The micronutrients B, Mn,

Fe, Cu, and Zn were 1.06, 22.8, 10.8, 1.9, and 1.3 mg kg<sup>-1</sup> soil, respectively. Ten parthenium seeds were sown in each pot. After germination, six seedlings per pot were maintained and the pots were divided into two groups to perform foliar spray on 1-wk and 2-wk old seedlings. All the pots were arranged in a completely randomized design in the open under natural environmental conditions. The mean minimum and maximum temperatures during the experimental period were 16 and 27 °C, respectively, and the mean relative humidity was 56%. Pots were irrigated with tap water when required.

Original culture filtrates of the four selected *Drechslera* species were sprayed on 1-wk and 2-wk old parthenium seedlings with a hand sprayer. Each pot was sprayed with 1 mL of the filtrates. Both groups were sprayed three times at 5-d intervals. Control treatment plants were sprayed with distilled water. Each treatment was replicated three times. After 40 d of growth, plants were carefully uprooted and washed under tap water. Roots were separated from the shoots. Data regarding shoot and root length and dry biomass were recorded (Javaid and Ali, 2011b).

### Statistical analysis

All data were subjected to ANOVA followed by Duncan's Multiple Range Test ( $P \leq 0.05$ ) to separate mean differences (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

### Laboratory bioassays

Both the original and diluted growth medium did not have a significant effect on parthenium seed germination. Original (100%) culture filtrates of all the four *Drechslera* species significantly reduced seed germination. Culture filtrates of *D. australiensis* were the most effective followed by *D. hawaiiensis*, *D. holmii*, and *D. biseptata* filtrates resulting in a 77, 47, 45, and 43% reduction in germination compared to the control, respectively. The effect of diluted (50%) *D. australiensis* and *D. biseptata* culture filtrates was also significant with a 31 and 21% reduction in germination, respectively, as compared to the control (Table 1). Similar herbicidal activity of culture filtrates of other fungal species, namely *Alternaria alternata*, *Drechslera rostrata*, *Fusarium solani*, *Trichoderma harzianum*, *T. viride*, and *T. pseudokoningii*, have also been reported against seed germination of parthenium and other weed species (Javaid and Adrees, 2009; Javaid and Ali, 2011a; 2011b).

Shoot length did not show a significant response to diluted growth medium, while original growth medium significantly reduced this growth parameter by 16% over the control. All the fungal culture filtrate treatments significantly suppressed shoot length. There was a 65 to 76% and 77 to 82% reduction in shoot length over the control due to diluted and original culture filtrates of different fungal species, respectively. The effect of either of the two concentrations of growth medium

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

Fungal species	Concentration	Germination	Shoot length	Shoot fresh weight	Root length	Root fresh weight
	— % —		mm	mg	mm	mg
Control	0	100a	9.1a	2.9a	13.4a	0.95a
Growth medium	50	94a	8.9a	2.7a	10.2b	0.85ab
	100	90ab	7.6b	2.6a	9.3b	0.82ab
<i>D. australiensis</i>	50	69cd	2.2d	0.9de	1.3c	0.41de
	100	23f	1.7d	0.6de	1.0c	0.21ef
<i>D. biseptata</i>	50	79bc	3.2c	1.5bc	2.1c	0.65bc
	100	57de	2.0d	0.9de	1.3c	0.31d-f
<i>D. hawaiiensis</i>	50	88ab	3.2c	1.9b	1.8c	0.47cd
	100	53e	1.6d	0.7de	1.1c	0.16f
<i>D. holmii</i>	50	89ab	2.5cd	1.2cd	1.9c	0.40de
	100	55e	2.1d	0.5e	1.3c	0.31d-f

Different letters in a column show a difference ( $P \leq 0.05$ ) according to Duncan's Multiple Range Test.

Note: 100% means original fungal culture filtrates.

was not significant on shoot fresh weight. However, culture filtrates of all the *Drechslera* species significantly declined shoot biomass over the control by 34 to 69% and 69 to 82% when applied in a diluted and original concentration, respectively (Table 1). These findings agree 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). Various herbicidal constituents have been identified from different *Drechslera* species. Sugawara *et al.* (1987) isolated ophiobolin I from *Drechslera maydis* and *D. sorghicola*, which exhibited herbicidal activity. Kastanias and Tokousbalides (2000) isolated pyrenophorol from a *D. avenae* pathotype that exhibited herbicidal potential against *Avena sterilis* L. Evidente *et al.* (2005) reported that drazepinone, a trisubstituted tetrahydronaphthofuroazepinone from *D. siccans*, exhibited herbicidal activity against monocot weeds. In another study, Evidente *et al.* (2006b) identified four herbicidal constituents from *D. gigantea*, viz. ophiobolin A, 6-epi-ophiobolin A, anhydro-6-epi-ophiobolin A, and ophiobolin I, which were very effective against several grass and broadleaf weeds.

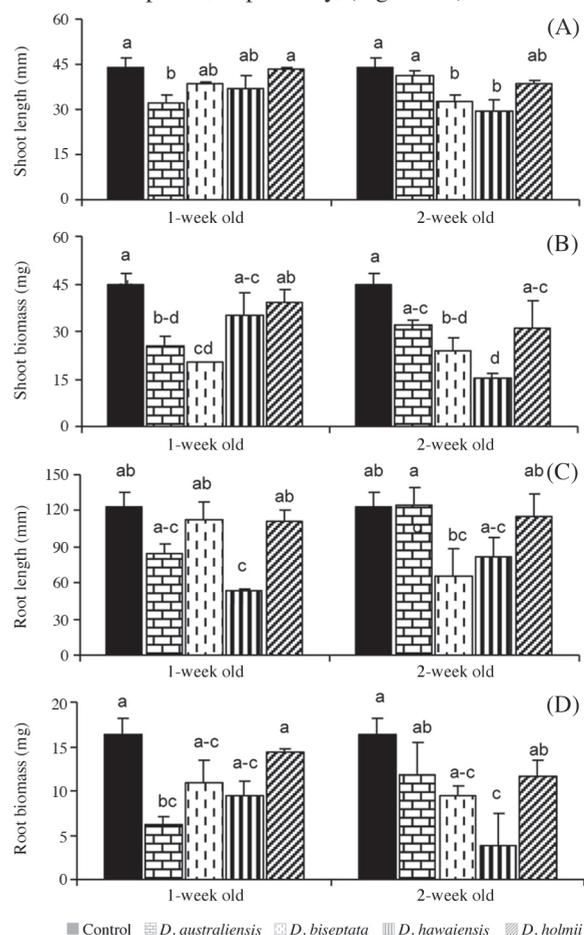
Root length was highly susceptible to various treatments. Both the original and diluted growth media significantly reduced root length by 31 and 24%, respectively. Original and diluted culture filtrates of all four *Drechslera* species significantly reduced root length by 90 to 92% and 84 to 90%, respectively, as compared to the control. Root biomass was reduced by 13 and 10% due to original and diluted growth media, but the effect was not significant. The adverse effect of all the fungal culture filtrate treatments on root biomass was significant. There was a 67 to 83% and 32 to 58% reduction in root biomass due to original and diluted culture filtrates of various *Drechslera* species, respectively, as compared to the control (Table 1).

### Pot trials

Culture filtrates of various *Drechslera* species reduced shoot length of 1-wk and 2-wk old seedlings by 2

to 26% and 6 to 33%, respectively. The effect of *D. australiensis* culture filtrates was significant in the 1-wk old plant treatment, while the effect of *D. biseptata* and *D. hawaiiensis* was significant in the 2-wk old plant treatment (Figure 1A). All foliar spray applications reduced shoot dry biomass to variable extents. In the 1-wk old plant treatment, foliar spray of *D. australiensis* and *D. biseptata* culture filtrates significantly reduced shoot biomass by 43 and 55%, respectively. The effect of culture filtrates of the other two fungal species was not significant. Two-week-old plants were more susceptible to foliar spray application than 1-wk old plants. In 2-wk old plants, different fungal culture filtrate treatments reduced shoot biomass by 27 to 65% (Figure 1B).

In general, the effect of foliar spray on root length was not very pronounced. Only *D. hawaiiensis* filtrates significantly reduced root length by 56% in 1-wk old plants. The effect of all other treatments on this root growth parameter was not significant (Figure 1C). Root dry biomass showed a more pronounced response to foliar spray application than root length. There was 13 to 62% and 27 to 76% in 1-wk and 2-wk old plants, respectively, (Figure 1D).



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

## CONCLUSIONS

The present study concludes that culture filtrates of the four tested *Drechslera* species are very effective for parthenium weed management. Further studies are required to isolate and identify the potential herbicidal constituents present in these fungal culture filtrates.

### Potencial herbicida de filtrados de cultivo de *Drechslera* spp. contra *Parthenium hysterophorus* L.

Se investigó la actividad herbicida de filtrados de cultivos de cuatro especies de *Drechslera* spp., i.e. *D. australiensis* (Bugnic.) Subram. & B.L. Jain, *D. biseptata* (Sacc. & Roum.) M.J. Richardson & E.M. Fraser, *D. hawaiiensis* Bugnic. ex M.B. Ellis, and *D. holmii* (Luttr.) Subram. & P.C. Jain, preparados en caldo de extracto de malta contra la maleza *Parthenium hysterophorus* L. en bioensayos de laboratorio y en macetas. En los bioensayos de laboratorio se estudió el efecto de filtrado de cultivo original (100%) y diluido (50%) de *Drechslera* spp. en la germinación y crecimiento de plántulas de parthenium en placas Petri de 90 mm. El filtrado de cultivo original de las cuatro especies *Drechslera* redujo significativamente germinación, longitud de brote, biomasa fresca de brote, longitud de raíz, y biomasa fresca de raíz de plántulas de parthenium en 43-77%, 77-82%, 69-82%, 90-92% y 67-83%, respectivamente, comparado con control. En ensayos en maceta, la aplicación foliar de filtrados de cultivos fúngicos originales se realizó en plántulas de parthenium de 1 y 2 semanas de edad. Filtrados de cultivo de todas las especies, excepto *D. holmii*, redujeron marcadamente el peso seco de brote de parthenium. Plantas de 2 semanas de edad fueron más susceptibles a aspersión foliar que aquellas de 1 semana. Hubo reducción de 13-55% y 28-65% en peso seco de brote de plantas de 1 y 2 semanas de edad, respectivamente, debido a filtrado de cultivo de diversas *Drechslera*. El presente estudio concluyó que los filtrados de cultivo de *Drechslera* spp. pueden usarse como herbicidas alternativos para el manejo de parthenium.

**Palabras clave:** *Drechslera* spp., filtrados de cultivo fúngico, herbicidas naturales, *Parthenium hysterophorus*.

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