

EXPLORING HERBICIDAL POTENTIAL OF AQUEOUS EXTRACTS OF SOME HERBACEOUS PLANTS AGAINST PARTHENIUM WEED¹

Explorando Herbicida Potencial de Aquosas Extratos de Algumas Plantas Herbáceas contra Parthenium Weed

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ABSTRACT - To assess the phytotoxic potential of *Achyranthes aspera*, *Alternanthera philoxeroides*, *Datura metel* and *Rumex dentatus* against *Parthenium hysterophorus*, 5% (w/v on dry weight basis) aqueous extracts from root, stem, leaf, flower and whole plant were tested through a Petri plate-based germination and pot-cultured seedling bioassays. *Achyranthes aspera* and *A. philoxeroides* inhibited parthenium weed germination more than extracts from other species. Whole plant, leaf and fruit extracts of *A. aspera* reduced the germination percentage (5%); leaf extract from *A. philoxeroides* caused lower germination index (0.4), higher mean germination time (14 d) and longer time to 50% germination (13.5 d) of parthenium weed. In the foliar spray bioassay, *A. aspera* reduced parthenium weed shoot growth more than the other species whereas *R. dentatus* caused more reduction in root growth. Whole plant extract from *A. aspera* caused maximum reduction in parthenium weed seedling vigor index (98%) and seedling biomass (96%). The aqueous extracts of *A. aspera* and *A. philoxeroides* contained higher concentrations of phenolics viz. gallic (16.9 mg L⁻¹), caffeic (7.4 mg L⁻¹), chromatotropic (63.8 mg L⁻¹), *p*-coumaric (10.5 mg L⁻¹), *m*-coumaric (3.1 mg L⁻¹), syringic (9.21 mg L⁻¹) and 4-hydroxy-3-methoxy benzoic (118.6 mg L⁻¹) acids compared with extracts of the other two species tested.

Keywords: allelopathy, bio-herbicides, parthenium weed, seed germination, seedling growth, weed management.

RESUMO - Para avaliar o potencial fitotóxico de *Achyranthes aspera*, *Alternanthera philoxeroides*, *Datura metel* e *Rumex dentatus* sobre *Parthenium hysterophorus*, 5% (w/v em base seca) de extratos aquosos de raiz, caule, folha, flor e toda a planta foram testados através de germinação em placa de Petri e de bioensaios de sementes cultivadas em potes. A inibição da germinação de plantas de *Parthenium* por *Achyranthes aspera* e *A. philoxeroides* foi maior que a de extratos de outras espécies. Extratos de plantas e folhas e frutos inteiros de *A. aspera* reduziram a porcentagem de germinação (5%); o extrato de folha de *A. philoxeroides* provocou menor índice de germinação (0,4), maior tempo médio de germinação (14 d) e germinação por mais tempo a 50% (13,5 d) de *Parthenium*. No bioensaio de pulverização foliar, *A. aspera* reduziu mais o crescimento de rebentos de *Parthenium* do que as outras espécies, enquanto *R. dentatus* causou maior redução no crescimento radicular. O extrato de planta inteira de *A. aspera* causou redução máxima do índice de vigor de plântulas (98%) e da biomassa das plântulas (96%) de *Parthenium*. Os extratos aquosos de *A. aspera* e *A. philoxeroides* continham as concentrações mais elevadas de compostos fenólicos, ou seja, ácidos gálico (16,9 mg L⁻¹), cafeico (7,4 mg L⁻¹), cromotrópico (63,8 mg L⁻¹), *p*-cumárico (10,5 mg L⁻¹), *m*-cumárico (3,1 mg L⁻¹), siringico (9,21 mg L⁻¹) e benzoico 4-hidroxi-3-metoxi (118,6 mg L⁻¹), em comparação com os extratos das outras duas espécies testadas.

Palavras-chave: alelopatia, bio-herbicidas, plantas de *Parthenium*, germinação das sementes, crescimento de plântulas, manejo de plantas daninhas.

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INTRODUCTION

Parthenium hysterophorus, a member of the Asteraceae family, is commonly known as carrotgrass, whitehead weed, congress grass, or parthenium weed. This weed has invaded about 30 countries in Africa, Australia and Asia, including Pakistan (Chippendale & Panetta, 1994; Shabbir & Bajwa, 2007; Nigatu et al., 2010). Parthenium is an obnoxious weed rapidly spreading across the non-cropped areas of the Khyber Pakhtunkhwa province and elsewhere in Pakistan; where it has caused serious damage to pastures, crops and biodiversity (Khan et al., 2012; Masum et al., 2013). Yield losses due to the presence of this weed in various crops range from 33 to 90% (Tamado et al., 2002; Vivek et al., 2008; Khan et al., 2013). In addition, it also causes allergy, dermatitis and asthma in human and cattle due to its poisonous nature (Suresh et al., 1994; Evans, 1997; Khan et al., 2013).

It is an alien invasive weed spreading throughout Pakistan in both irrigated and rainfed areas (Shabbir & Bajwa, 2007; Khan et al., 2012). Its presence as an aggressive colonizer in both non-cropped and cropped situations has been well documented (Javaid & Anjum, 2005; Riaz & Javaid, 2010; Khan et al., 2014). The widespread invasion of parthenium weed in natural grasslands, field crops, orchards and vegetable fields in the country has prodded weed scientists to devise strategies for its management. Although parthenium weed can be successfully controlled with herbicides (Sharma, 2003; Javaid et al., 2007; Kathiresan, 2008; Khan et al., 2012), chemical control however is not recommended due to its detrimental effects on the environment and human health. In order to minimize the usage of synthetic herbicides, plant extracts alone or in combination with herbicides should be used. These extracts contain potential allelochemicals such as phenolics, terpenoids, alkaloids, coumarins, tannins, flavonoids, steroids and quinines (Xuan et al., 2005), which are capable of selectively and non-selectively influencing plant germination and growth at various concentrations, often at very small concentrations.

Studies have been carried out using various herbaceous plants extracts for their

phytotoxic effect against parthenium weed (Anjum et al., 2006; Javaid et al., 2005; Quazi & Khan, 2010; Shafique et al., 2011). Some of these such as *Amaranthus spinosus*, *Imperata cylindrica*, *Desmostachya bipinnata*, *Dichanthium annulatum*, *Cenchrus pennisetiformis*, *Physalis minima*, *Sorghum halepense*, *Tagetes erectus* and *Xanthium strumarium* were found effective against this weed. Nevertheless, the degree and nature of phytotoxic action of aqueous extract of a plant varies by plant part (Sinha & Singh, 2004; Swain et al., 2005) and extract concentration (Safdar et al., 2013). Hence, there is a need to test other well-known allelopathic plants to explore their potential phytotoxic activity against parthenium weed. Therefore, some herbaceous plant species were evaluated for their pre and postemergence herbicidal activity against parthenium weed.

MATERIALS AND METHODS

Germination and seedling growth bioassays were conducted using four herbaceous species viz., *Achyranthes aspera*, *Alternanthera philoxeroides*, *Datura metel*, and *Rumex dentatus* in petri plates and pots. Studies were carried out at Weed Science Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan during 2011-12.

Preparation of aqueous extracts

Wild growing plants of each species were collected and kept at room temperature for drying and then oven-dried at 70 °C for 48 h. Intact plants of those species were then separated into root, stem, leaves and fruit. However, of *A. philoxeroides* into root, stem and leaves as fruit were absent in it. Some whole plants of all plant species were also kept intact. These were then cut into small (2 cm) pieces and separately soaked in distilled water at a ratio of 1: 20 w/v (herbage:water) for 24 h at room temperature (Hussain & Gadoon, 1981). Aqueous extracts (5% w/v) of different plant parts were obtained by filtering the mixture using an ASTM 40 mesh sieve and then through Sartorius Minisart syringe filters with 0.45 µm pore size.

Determination of biochemical characteristics of aqueous extracts

The pH and EC of each extract were determined with a pH meter (JENWAY 3510 pH Meter) and an EC meter (JENWAY 3510 conductivity Meter), respectively. Total soluble phenolics were determined as described by Randhir & Shetty (2005) and expressed

as gallic acid equivalents (Table 1). For identification and quantification of their suspected phytotoxins, aqueous extracts were chemically analysed using a Shimadzu HPLC system equipped with a UV detector (Model SCL-10A, Tokyo, Japan). The peaks were identified using chemical standards (Aldrich, St Louis, USA). The concentration of each isolated compound (Table 2) was determined using the following equation:

$$\text{Concentration (ppm)} = \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \text{Concentration of the standard} \times \text{Dilution factor} \quad (\text{eq. 1})$$

Table 1 - Characterization of the chemical properties of various aqueous plant extracts

Plant	Plant Part	pH	EC (dS m ⁻¹)	*Concentration of total phenolics (mg L ⁻¹)
<i>Datura metel</i> L.	Root	7.9	6.3	-
	Stem	8.6	9.8	-
	Leaf	8.2	10.9	5479
	Fruit	8.8	7.0	-
	Whole plant	8.8	8.8	-
<i>Rumex dentatus</i> L.	Root	6.8	1.0	-
	Stem	9.0	5.8	-
	Leaf	8.2	8.8	4948
	Fruit	9.1	2.1	-
	Whole plant	9.0	3.7	-
<i>Achyranthes aspera</i> L.	Root	8.3	2.7	-
	Stem	9.6	3.9	-
	Leaf	9.1	5.4	3795
	Fruit	9.4	3.8	-
	Whole plant	9.3	4.7	5779
<i>Alternanthera philoxeroides</i> Gris.	Root	9.1	4.6	-
	Stem	9.2	8.3	-
	Leaf	9.2	8.6	2618
	Whole plant	9.4	8.2	-

* Concentration of total phenolics were only determined of those aqueous extracts which showed maximum germination inhibition.

Table 2 - Chemical composition of aqueous plant extracts

Sr. No.	Phenolics	Concentration (mg L ⁻¹)			
		<i>D. metel</i>	<i>R. dentatus</i>	<i>A. aspera</i>	<i>A. philoxeroides</i>
1	Chromatotropic acid	-	-	63.8	13.8
2	Gallic acid	-	17.3	16.9	-
3	Caffeic acid	-	-	7.4	-
4	Vanillic acid	26.3	-	-	-
5	4-hydroxy-3-methoxy benzoic acid	96.7	-	24.8	118.6
6	<i>p</i> -Coumaric acid	-	-	-	10.2
7	<i>m</i> -Coumaric acid	-	-	3.1	-
8	Syringic acid	51.3	-	9.2	-
9	Ferulic acid	-	-	-	-
10	Chlorogenic acid	-	-	-	-



Biochemical characteristics of aqueous extracts of parthenium plant parts and their rhizospheric soils from various farm locations and their phenolic compositions are presented in Tables 1 and 2.

Germination studies

To evaluate preemergence herbicidal potential against parthenium, 5% aqueous extracts of root, stem, leaves, fruit and whole plant of *A. aspera*, *D. metel* and *R. dentatus* and root, stem, leaves and whole plant of *A. philoxeroides* were used in the germination bioassay. Twenty seeds of parthenium were placed in 9 cm dia. Petri plates lined with a double layer of Whatman No. 10 filter paper and moistened with 4 mL of each extract. A control treatment with 4 mL of distilled water was included. Each treatment was replicated 4 times and the plates arranged in a completely randomized design with a factorial arrangement of species X extract concentration. Petri plates were kept in an illuminated incubator maintained at 25±5 °C. Germination was recorded daily for 20 days. Germination count data were used to calculate various seed germination and vigor parameters as detailed below:

Germination percentage (GP) was calculated by the following formula:

$$GP = [N_T \times 100] / N \tag{eq. 2}$$

where N_T : proportion of germinated seeds in each treatment for the final measurement, and N: Number of seeds used in the bioassay.

The germination index (GI) was calculated by following the formula as described by Scott et al. (1984):

$$GI = N_1 / D_1 + \dots + N_L / D_L \tag{eq. 3}$$

where N_1 : number of seeds germinated in 1st count, D_1 : days to 1st count, N_L : number of seeds germinated in last count, and D_L : days to last count.

Mean germination time (MGT) was calculated by the following equation, as given by Dezfuli et al. (2008):

$$MGT = \Sigma Dn / \Sigma n \tag{eq. 4}$$

where n: Number of seeds which were germinated on day D, D: Number of days

counted from the beginning of the germination.

Time to 50% germination (T_{50}) was calculated according to the following formula modified by Farooq et al. (2005):

$$T_{50} = t_i + \{ (N/2) - n_i \} (t_j - t_i) / n_j - n_i \tag{eq. 5}$$

where N: Final number of germination, n_i , n_j : cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Seedling growth studies

Leaf extracts of *A. philoxeroides*, *D. metel* and *R. dentatus*, and whole plant extract of *A. aspera* were tested for their postemergence herbicidal potential against parthenium weed through seedling growth bioassay in pots kept in the Laboratory. Twenty seeds of parthenium weed were sown in plastic pots (10 cm dia. and 10 cm depth) filled with 650 g sandy loam soil per pot initially moistened with 200 mL of distilled water. Each treatment was replicated four times and the experimental units were arranged in a completely randomized design. Three seedlings were maintained per pot. The 4 mL aqueous extract of each plant species was sprayed over 15 days-old parthenium weed seedlings. Seedlings in control treatment were also sprayed with distilled water in a similar way. Minimum and maximum temperatures during the course of experiments were 23 °C and 41 °C, respectively. Ten days after spraying, 25-d-old seedlings were uprooted from pots and their shoot and root lengths were recorded. Shoot and root dry weights were recorded after drying them in an electrical oven at 65 °C for 48 h. Germination percentage and seedling length were used to calculate seedling vigor index (SVI) by the following formula, as described by Orchard (1977):

$$SVI = [\text{seedling length (cm)} \times \text{germination percentage}] \tag{eq. 6}$$

Data Analysis: The data collected were statistically analysed using Fisher’s analysis of variance technique in the MSTATC Software Program. Treatment means were compared on the basis of least significant difference (LSD) at the 0.05 probability level (Steel et al., 1997).



RESULTS AND DISCUSSION

Germination Assay: Table 3 presents data on various germination traits, including germination percentage (GP), germination index (GI), mean germination time (MGT), and time to 50% germination (T_{50}). Regardless of the plant tissue used, aqueous extract of

A. aspera caused maximum reduction in parthenium GP (92%) compared with the distilled water treated control (DWTC) (Table 3). However, *A. philoxeroides* delayed the germination of parthenium weed, the most (GI = 1.6, MGT = 11.8 d and T_{50} = 11.7 d). Extracts from leaves were most effective among the plant tissues used, regardless of

Table 3 - Effect of aqueous extracts of various plant parts on germination characteristics of *Parthenium hysterophorus*

Plant (P)	Plant part (PP)	GP (%)	GI	MGT	T50
Control	Distilled water	100.0 a	51.3 a	2.2 m	1.6 i
<i>Datura metel</i> L.	Root	15.0 efg	3.1 efg	7.9 def	10.0 bc
	Stem	10.0 gh	2.0 fg	8.0 def	8.5 cd
	Leaf	5.0 h	0.7 g	7.5 efg	7.0 def
	Fruit	5.0 h	1.6 fg	4.5 jk	4.0 h
	Whole plant	5.0 h	1.1 g	10.5 c	10.0 bc
<i>Rumex dentatus</i> L.	Root	21.3 e	4.5 ef	8.0 def	8.3 cde
	Stem	10.0 gh	1.2 fg	11.9 b	12.8 a
	Leaf	5.0 h	0.4 g	14.0 a	13.5 a
	Fruit	16.3 efg	3.4 efg	8.0 def	6.4 efg
	Whole plant	12.5 fgh	1.8 fg	11.0 bc	10.9 b
<i>Achyranthes aspera</i> L.	Root	10.0 gh	1.8 fg	9.0 d	8.4 cd
	Stem	5.0 h	1.1 g	7.5 efg	7.0 def
	Leaf	20.0 ef	5.4 e	6.3 ghi	6.2 fg
	Fruit	92.5 a	25.8 c	5.9 hi	5.6 fgh
	Whole plant	38.8 d	12.3 d	5.4 ij	4.8 gh
<i>Alternanthera philoxeroides</i> Gris.	Root	57.5 c	14.7 d	6.8 fgh	7.1 def
	Stem	13.8 efg	2.8 efg	8.3 de	7.3 def
	Leaf	61.3 c	24.4 c	3.7 kl	2.3 i
	Whole plant	81.3 b	38.0 b	2.8 lm	1.9 i
LSD (PXPP)		7.694	7.69	2.781	1.171
Plant means		GP (%)	GI	MGT	T50
Control		100.0 a	51.3 a	2.2 d	1.6 e
<i>Datura metel</i> L.		8.0 d ^{1/}	1.7 d	7.7 b	7.9 b
<i>Rumex dentatus</i> L.		10.8 d	1.6 d ^{2/}	11.8 a	11.7 a
<i>Achyranthes aspera</i> L.		18.0 c	4.8 c	7.3 b	6.6 c
<i>Alternanthera philoxeroides</i> Gris.		61.3 b	21.1 b	5.5 c	4.9 d
LSD (Plant)		3.44	1.24	0.52	0.77
Plant part Means		GP (%)	GI	MGT	T50
Root		51.3 a	21.8 a	5.3 c	5.3 b
Stem		37.5 c	14.2 c	7.6 a	7.3 a
Leaf		25.8 d	11.3 d	7.9 a	7.7 a
Fruit		38.3 c	16.6 b	6.1 b	5.5 b
Whole plant		45.3 b	16.6 b	7.6 a	6.9 a
LSD (Plant part)		3.44	1.24	0.52	0.78

^{1/} (92%); ^{2/} (98%). In a column, values with different letters show significant difference ($p \leq 0.05$), GP = germination percentage, GI = germination index, MGT = mean germination time, T_{50} = time to 50% germination.



species, as significantly lower GP (25.8) and GI (11.3) while higher MGT (7.9 d) and T_{50} (7.7 d) were noted with parthenium weed seeds in response to leaf extracts (Table 3). With regard to species x plant part interaction means, all plant parts of *R. dentatus* and root extract of *A. aspera*, *A. philoxeroides* and *D. metel* proved least potent inhibitors of parthenium weed germination percentage (Table 3). Germination inhibition by aqueous extracts of *A. aspera* has also been reported by Chandra et al. (2011) in broad bean (*Vicia faba*). Mandal & Mondal (2011) also found that aqueous extracts from stem, root, leaves and inflorescence of *A. philoxeroides* were found to inhibit the germination of *Ampelopteris proliferata*.

Seedling bioassay: The significantly highest shoot length (4.3 cm), root length (5.6 cm), shoot dry weight (18.4 mg) and root dry weight (2.2 mg) of parthenium weeds were recorded with a distilled water sprayed control (Table 4). On the contrary, maximum inhibition in shoot length (68%), shoot dry weight (96%), seedling biomass (96%), and seedling vigor index (98%) of parthenium weeds were observed with an extract from whole plant of *A. aspera*. Whereas parthenium weed seedlings sprayed with *R. dentatus* leaf extract showed the highest suppression in root

length (70%), root dry weight (96%) and seedling length (68%) compared with the distilled water sprayed control. These values did not differ significantly with those noted in parthenium weed seedlings in response to *A. aspera* whole plant extract. Reduction in root and shoot lengths and biomass of broad bean (*Vicia faba*) by aqueous plant extract of *A. aspera* at higher concentrations was also reported by Chandra et al. (2011), whereas retardation in seedling growth of *A. proliferata* through the application of aqueous extracts from stem, root, leaves and inflorescence of *A. philoxeroides* was also found by Mandal & Mondal (2011). Seedling growth inhibition in terms of reduced plumule and radicle lengths of wheat in pea by application of aqueous extracts of root, stem and leaves of *R. dentatus* has also been documented by Umer et al. (2010). Javaid et al. (2010), in laboratory and foliar spray bioassay studies, have proved that aqueous extracts of *D. metel* have significantly reduced germination and seedling growth of parthenium weed.

Germination and seedling growth inhibitory effects of aqueous extracts of *A. aspera* and *A. philoxeroides* on parthenium weed may be due to their higher total phenolic contents (2618 to 5779 mgL⁻¹) (Table 1), presence of gallic (16.9 mgL⁻¹),

Table 4 - Seedling growth parameters of *Parthenium hysterophorus* L. as influenced by postemergence application of aqueous extracts

Aqueous extract	Seedling growth parameters								
	SL (cm)	RL (cm)	SFW (mg)	SDW (mg)	RFW (mg)	RDW (mg)	SDL (cm)	SB (mg)	SVI
Distilled water sprayed control	4.3 a	5.6 a	160.8 a	18.4 a	28.5 a	2.2 a	9.9 a	20.6 a	986.7 a
<i>Achyranthes aspera</i> L. whole plant extract	1.35 c (68.4 %)	2.0 d (64.3 %)	13.8 c (91.4 %)	0.6 c (96.6 %)	2.7 c (90.6 %)	0.2 c (91.8 %)	3.4 c (66.1 %)	0.8 c (96.1 %)	16.8 c (98.3 %)
<i>Alternanthera philoxeroides</i> Gris. leaf extract	2.4 b (43.3 %)	3.7 b (33.8 %)	49.5 b (69.2 %)	3.7 bc (79.9 %)	10.8 b (62.3 %)	0.6 bc (72.3 %)	6.1 b (37.9 %)	4.3 bc (79.1 %)	30.6 bc (96.9 %)
<i>Datura metel</i> L. leaf extract	4.00 a (6.8 %)	2.8 c (49.5 %)	58.4 b (63.7 %)	6.4 b (65.5 %)	11.1 b (61.2 %)	0.6 b (72.7 %)	6.8 b (30.9 %)	7.00 b (66.3 %)	34.1 b (96.5 %)
<i>Rumex dentatus</i> L. leaf extract	1.4 c (66.5 %)	1.7 d (70.5 %)	19.1 c (88.1 %)	1.3 c (92.8 %)	0.8 c (97.1 %)	0.1 b (96.4 %)	3.1 c (68.8 %)	1.4 c (93.2 %)	43.5 b (95.6 %)
LSD _{0.05}	0.308	0.654	25.08	3.565	5.104	0.5202	0.769	4.005	14.00

In a column, values with different letters show significant difference ($p \leq 0.05$) by the LSD Test, Figures in parenthesis show % reduction over control in the respective column, SL = shoot length, RL = root length, SFW = shoot fresh weight, SDW = shoot dry weight, RFW = root fresh weight, RDW = root dry weight, SDL = seedling length, SB = seedling biomass, SVI = seedling vigor index.

caffeic (7.4 mg L⁻¹), chromatotropic (13.8 to 63.8 mg L⁻¹), *p*-coumaric (10.5 mg L⁻¹), *m*-coumaric (3.1 mg L⁻¹), syringic (9.2 mg L⁻¹) and 4-hydroxy-3-methoxy benzoic (24.8 to 118.6 mg L⁻¹) acids in their aqueous extracts (Table 2). Achyranthine is the major alkaloid present in all tissues of *A. aspera* whereas other allelochemicals in various plant parts are phenolics, oleonic acid, dihydroxy ketones, long chain compounds and saponin A and B (Ali, 1993; Srivastav et al., 2011).

Based on preemergence and postemergence activities against parthenium, whole plant extract of *A. aspera* and leaf extract *A. philoxeroides* are concluded as herbicidal. Therefore, these extracts should be further tested alone or in combination with existing synthetic herbicides for sustainable and eco-friendly management of parthenium weeds.

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