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ALLELOPATHIC POTENTIAL OF *DIGERA MURICATA*, A DESERT SUMMER ANNUAL

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Abstract

Allelopathic potential of *Digera muricata* was tested on different crops by germinating them in different concentrations of *D. muricata* extract. It was observed that germination of all test species were highly affected in the higher concentration of *D. muricata* extract. Bulrush-millet was the most affected species. Decaying plant material of *D. muricata* was also found to be highly phytotoxic. Maximum reduction in growth of bulrush-millet was observed in the decaying root material. Phenolic compounds (Quercetin, sinapic and ferluic acid), alkaloids (cystine and berbine) and a terpenoid (limonene) were detected from roots and shoots of *D. muricata*. Greater quantities of all these allelochemicals were present in the roots followed by shoots.

Introduction

Allelopathy is the direct or indirect harmful effect by one plant on another through the production of chemical compounds that escape into the environment (Rice, 1984). The effect is usually harmful but sometimes the leachate from one plant makes another more productive. Brikett *et al*., (2001), define allelopathy as "any direct or indirect harmful or beneficial effect by one plant on another via the production of chemical compounds that escape into the environment".

Phenolic compounds are considered to be the main allelopathic agents in weeds and other higher plants (Ferreira *et al*., 1998; Wang *et al*., 1998; Inderjit & Dakshini, 1996; Rebaz *et al*., 2001; Shaukat & Siddiqui, 2002). Whereas, Olofdotter *et al*., (2003), reported that phenolic compounds in rice might be one of the component in a mixture of chemicals that, when present simultaneously, were allelopathic.

Allelopathy also affects the dispersion pattern of plants in an area. Parpiev (1971), investigated the relationship between shrubs and herbaceous vegetation and found that extracts of genetically and ecologically similar species were favourable to the germination of seeds, but extracts of genetically and ecologically distinct species inhibited germination. Thus, patterning or dispersion seems to be strongly influenced by allelopathy. This is probably the main reason that introduced weeds exhibited much striking allelopathic effects. Weir *et al*., (2003), reported intraspecific and interspecific interactions mediated by a phytotoxin leachate by the roots of *Centaurea maculosa*, which is one of the most invasive weed in the western United States

Some of the common crop plants also inhibit the growth of other plants in their vicinity. This response results in natural weed control as reported by Shaukat & Siddiqui (2002), in *Lantana camara*. Neustryeva & Dobretsova (1972), reported that wheat, oat, peas and buck wheat suppress the growth and above-ground biomass of lambs quarter (*Chenopodium album*). Libel & Worsham (1983), reported inhibition of *Ipomoea lacunose* by phytotoxic contents of wheat. Saxena (2000), allowed to grow water hyacinth (*Eichhorina carssipes*) in pots containing 3 % aqueous leacate (w/v) of *Lantana camara,* a terrestrial plant. The leachate was found to be allelopathic to the growth of water hyacinth within 21 days.

The present study is designed to elucidate the effect of *D. muricata* extract on the germination and growth of different test crop species. Phytotoxicity of decaying *D. muricata* root and shoot fragments in the soil will also be studied on the most susceptible crop plant. The possible allelochemicals involved will also be determined.

Materials and Methods

Preparation of test extract of *D. muricata*: Test extract of *D. muricata* was prepared by taking 10 g air-dried crushed material, soaked in 400 ml, distilled water. The whole mixture was filtered and the filtrate was used as stock solution. From stock solution aqueous extract of 0%, 25%, 50%, 75% and 100% were prepared.

1. Effect of *D. muricata* extract on germination and growth of four crop plants: To test the toxicity of the aqueous extract of *D. muricata* on crop plants, four crop plant i.e., millet, wheat, lentil and mungbean were selected. Ten seeds of each species (soaked in water for two hrs) were kept for germination in petri plates lined with double layer of Whatman no. 2 filter paper. Three replicates of each species were used. Filter paper of Petri plate were moistened with 5 ml test extract of 0, 25, 50, 75 and 100% aqueous solution. In control distilled water was used. Petri plates were kept in laboratory at room temperature ($30\text{-}32^0$). Germination counts were made daily, whereas, plant height and dry weights were determined after 96 h of growth.

2. Phytytoxicity of decaying *D. muricata* roots and shoots: Being the most susceptible crop plant, millet was selected for further studies. For the determination of phytotoxic effect of decaying root and shoot material, 0, 5, 10 and 20 g of *D. muricata* roots and shoots were separately mixed with 400 g soil. Soil was taken in 10 cm diameter plastic pots and sprinkled with 100 ml distilled water and left for one week for the microbial activity. After one week seeds of millet were sown in each pot (10 seeds / pot). Each treatment was replicated thrice. In this

way altogether twenty four pots were taken, twelve having the decaying root material of *D. muricata* and in the remaining twelve pots decaying shoot material was taken. Germination counts were made daily. Plant dry weights were recorded when plants were one week old.

3. Chromatography: Chromatography was carried out separately in dry roots and shoots of *D. muricata*, extracted thrice with peroxidase free ether and evaporated to dryness using argon gas. After adding 1 ml 50% ethonal it was loaded on chromatographic paper as well as on TLC plates. The solvent system used for paper chromatography was n-BuOH-acetic acid-water (50: 2: 48 v / v/ v). For TLC MeOH-NH_4OH (200: 3 v/v).

Phenolic compounds were detected by spraying vanillin-HCl and ferric chloride ferricyanide reagent. Detection of alkaloids was carried out by spraying Dragondroff's, Iodoplatinate and Marelquis reagents. BuOH-aqueous citric acid (870 ml: 4.8 g citric acid in 130 ml water) was used as solvent on chromatographic paper, whereas, MeOH-NH_4OH (200: 3 v/v) was used on silica gel F_{254} plates. For terpenoids the solvent system n-BuOH-HOAc-H_2O (4: 1: 5 v /v/ v) and iso-prOH-H_2O (3: 2 v/ v) were employed. Terpinoids were detected by spraying antimony chloride and anisaldehyde H_2SO_4 .

Results

1. **Effect of *D. muricata* extract on germination and growth of four crop plants:** Germination of all the test species was significantly ($p<0.001$) inhibited by the aqueous extract of *D. muricata* as compared to controls (Fig. 1). Maximum germination was recorded in controls of all special. Final germination percentage as well as germination velocity decreased with increasing concentration of aqueous extract of *D. muricata* (Table 1). Whereas, no germination in any crop plant was observed in 100% aqueous extract of *D. muricata*.

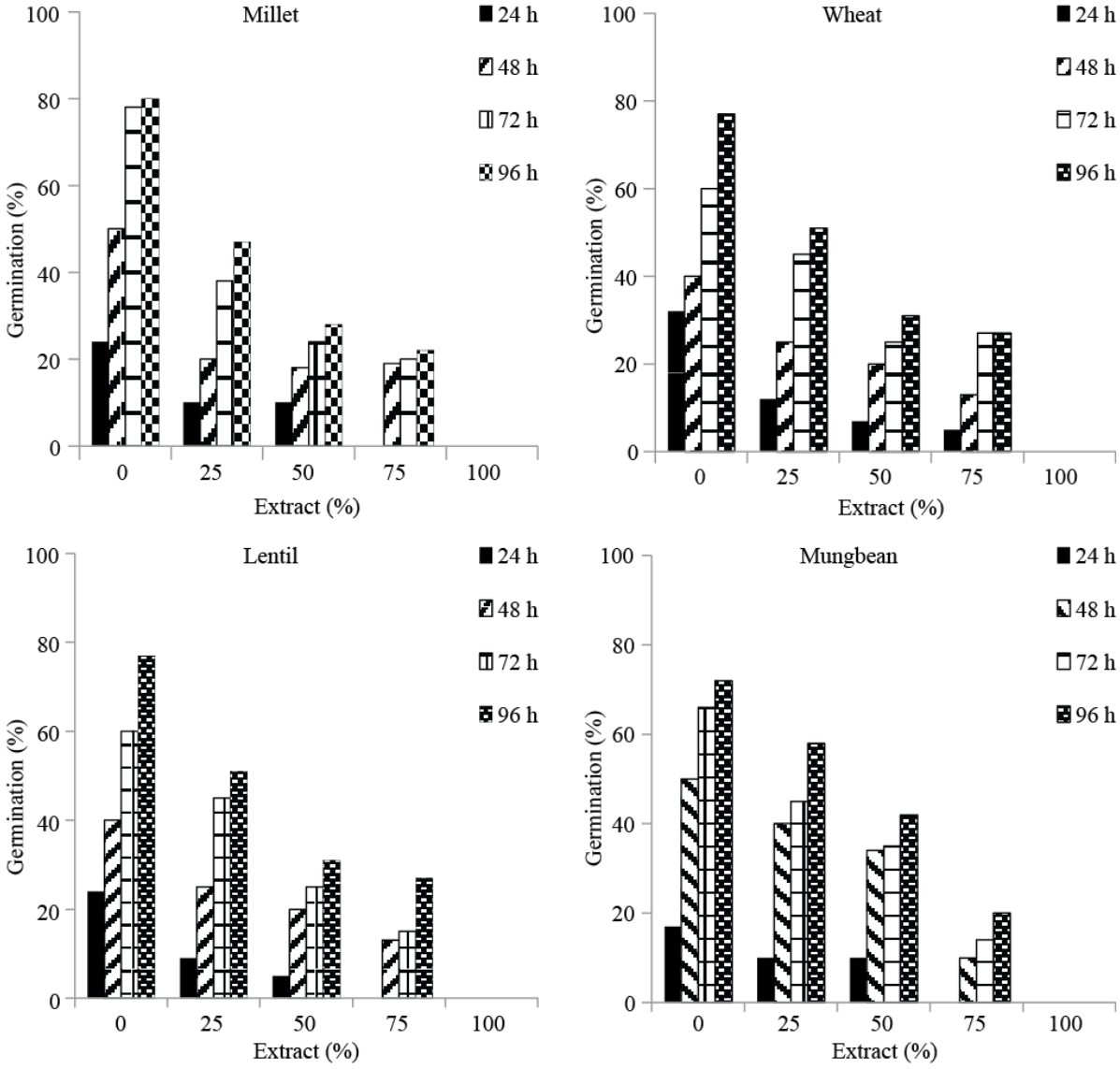

Fig. 1. Effect of *D. muricata* extract on germination of test species.

Table 1. The effect of aqueous extract of *D. muricata* on the germination velocity of the selected test species.

Species	Aqueous extract (%)	GV
Millet	0	69.54
	25	30.15
	50	21.35
	75	14.20
	100	0.00
Wheat	0	52.33
	25	22.24
	50	14.21
	75	11.33
	100	0.00
Lentil	0	50.25
	25	18.97
	50	12.71
	75	10.22
	100	0.00
Mungbean	25	44.11
	50	19.65
	75	11.78
	100	0.00

All the selected species exhibited significant differences in germination considering time (millet, $F = 399.05$, $p<0.001$; wheat, $F = 9.05$; $p<0.001$; lentil, $F = 679.25$, $p<0.001$; mungbean, $F = 877.86$, $p<0.001$) and concentration of *D. muricata* extract (millet, $F = 282.06$, $p< 0.001$; wheat, $F =20.66$; $p<0.001$; lentil, $F = 150.79$, $p<0.001$; mungbean, $F = 1969.80$ $p<0.001$) as factors. Interaction of time x concentration of aqueous extract also exhibited significant variations (millet, $F = 37.14$, $p<0.001$; lentil, $F = 150.79$; $p<0.001$ mungbean, $F = 91.50$, $p<0.001$). Whereas, two-way interaction of time x concentration of extract exhibited non-significant ($F = 0.95$, n.s.) differences in wheat. Millet was found to be the most susceptible species.

Plant size of selected test species was also suppressed by *D. muricata* extract. Among the crop plants the inhibitory effects of the extract was highest for millet and relatively lowest for mungbean (Fig. 2). In general, length of all the test species gradually decreased with the increase in the concentration of *D. muricata* extract. Significant differences in the root lengths of test species was observed (millet, $F = 471.58$, $p<0.001$; wheat, $F = 180.26$, $p<0.001$; mungbean, $F = 73.14$, $p<0.001$). Lentil exhibited non-significant ($F = 1.71$, n.s.) differences in root length. Shoot lengths of millet ($F = 50.07$, $p<0.001$), lentil ($F = 4.22$, $p<0.05$) and mung bean ($F =166.30$, $p<0.001$), also exhibited significant differences at various concentrations of *D. muricata* extract. Whereas, shoot length of wheat showed non-significant ($F = 0.97$, n.s.) differences. The inhibitory effect on germination and growth of selected crop species was in the order millet > wheat > lentil > mungbean.

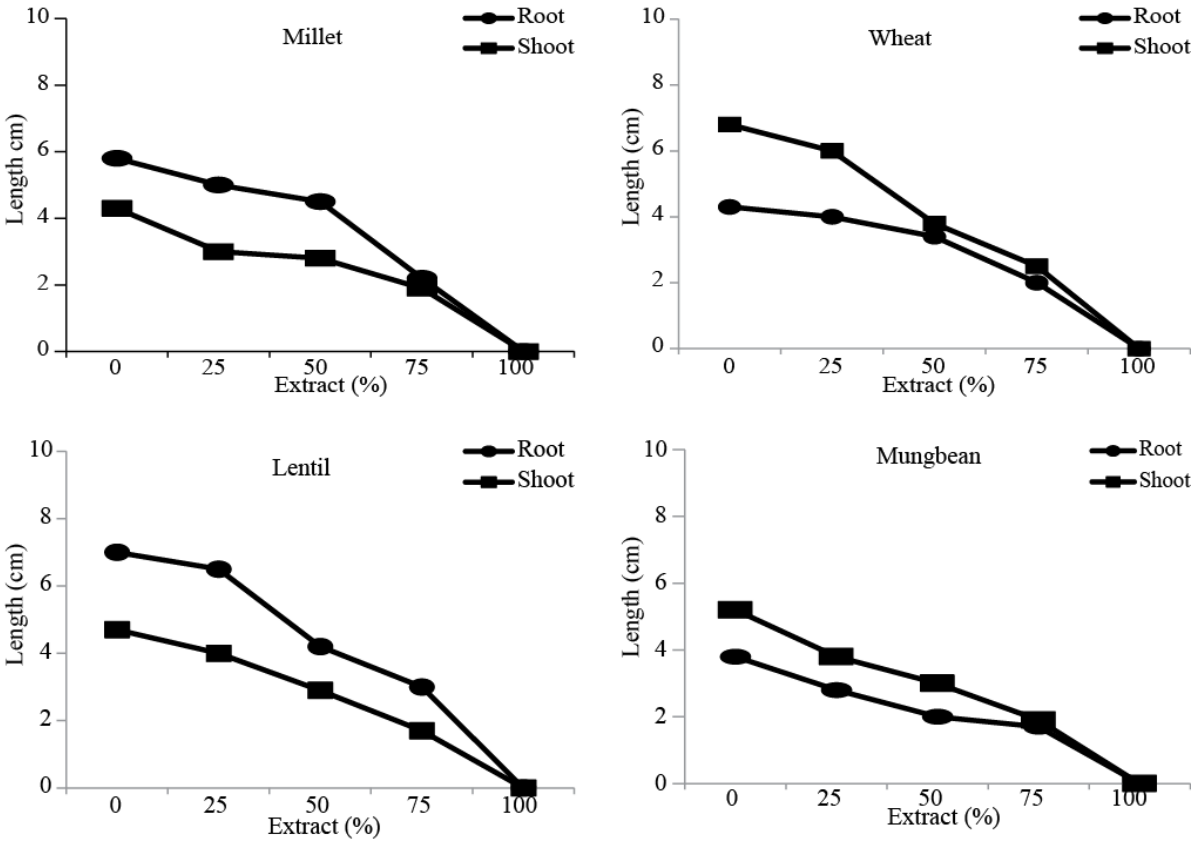

Fig. 2. Effect of *D. muricata* extract on seedling growth of test species.

2. Phytotoxicity of decaying *D. muricata* roots and shoots: Emergence of millet was recorded after 96 h of growth in 0, 5, 10, 15 and 20 g fresh shoots and roots of *D. muricata* in 400 g soil. Different concentrations in decaying shoots exhibited significant variations ($F = 44.79$, $p<0.001$). Root leachates also exhibited significant ($F = 102.75$, $p<0.001$) germination percentage at different concentration. Highest germination percentage (Fig. 3) and germination velocity (Table 2) was recorded in controls, having no plant material of *D. muricata* followed by 5, 10, 15 g per 400g soil in both root and shoot materials. Whereas, no germination was recorded in 20 g decaying root/shoot material of *D. muricata.* Reduction in emergence percentage was greater in the decaying root material of *D. muricata* than in the decaying shoots of *D. muricata*.

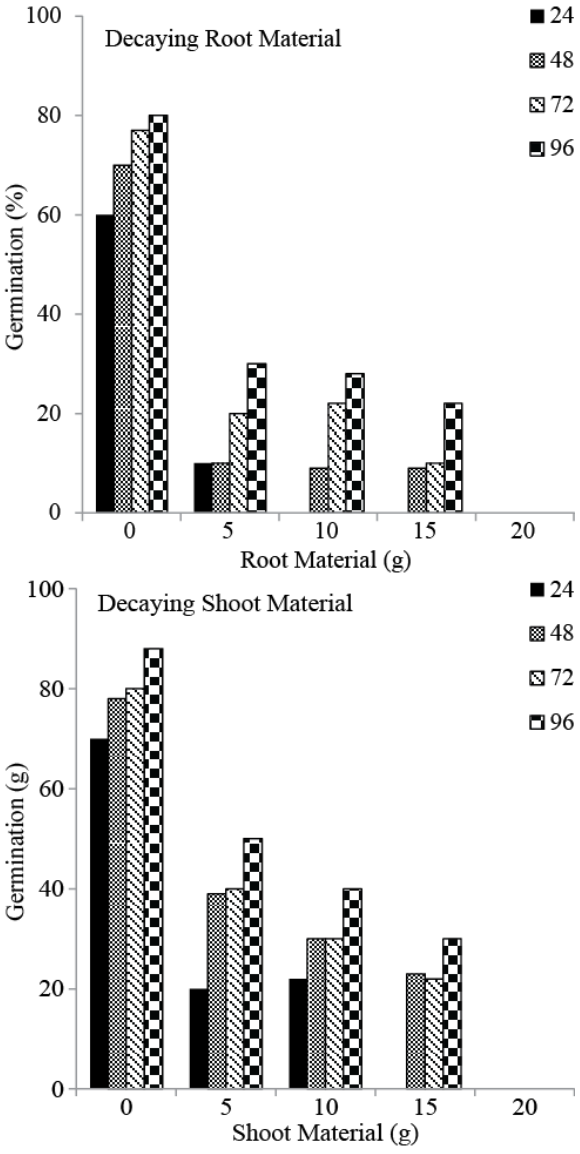

Fig. 3. Phytotoxic effects of decaying *D. muricata* fresh plant material of root and shoot on the percent germination of bulrush millet.

Table 2. Effect of decaying root or shoot material of *D. muricata* on the germination velocity of millet.

Decaying plant material (g)		Germination velocity
Root material	0	66.4
	5	41.3
	10	28.1
	15	18.2
	20	0.0
Shoot material	0	72.2
	5	60.0
	10	37.7
	15	25.4
	20	0.0

Lower dry weights were recorded in soils having 15 g decaying plant material of both root and shoot of *D. muricata* (Fig. 4). Dry weights of roots ($F = 239.44$, $p<0.001$) and shoot ($F = 249.52$, $p<0.001$) in root leachate root and shoot leachate also exhibited significant values. In general, growth of millet gradually decreased with an increase in the decaying plant material in the soil. Inhibition in growth of millet was highest in decaying roots followed by shoots. We can say that decaying root material of *D. muricata* was found to be more injurious than decaying shoot material.

3. Chromatography: Three phenolic compounds, two alkaloids and one terpenoid was extracted from *D. muricata* plant material (Table 3). Phenolic compounds, detected from *D. muricata* were quercetin acid, ferulic acid and sinapic acid, giving orangish yellow, bluish yellow/yellowish green and blue colour by spraying ferric chloride and vanillin HCl respectively. Ferulic and sinapic acids are the derivatives of cinnamic acid, whereas, quercetin is a common flavanol. Alkaloids identified by chromatography were cytosine and berberine, identified by their specific bright blue and fluorescent yellow colours respectively after sprayng the strips and TLC plates by dragondroff Iodoplatinate. Only one terpenoid i.e., limonene was found in small quantity, which is a member of monoterpenes. It gives brown colour by spraying the plates and strips with conc. H_2SO_4 Semi-quantitative data of the various phytochemicals of *D. muricata* are presented in Table 4.

Discussion

The allelochemicals released from plant parts penetrate the soil and suffers germination, growth and successful establishment of the neighbouring plants. Many members of the family Amaranthaceae are known to be allelopathic such as *Amaranthus palmeri, A. retroflexus, A. spinosa, A. tricolour, Celosia argentea* (Ashraf & Sen, 1978) and *D. arvensis* (Narwal, 1994). At our study site, *D. muricata* formed large clumps, several bare areas were found under and around *D. muricata* (visual observation), wherever it was growing singly or in a small group of a few plants. Thus, this plant is suspected to be allelopathic, exhibiting allelopathy under field conditions. With this in view, *D. muricata* was selected for the examination of it's allelopathic nature.

Table 3. Rf (x 100) values and colour produced by UV light and various reagents for the aqueous extract of *D. muricata*.

Phytochemicals	Paper	TLC	UV	Spray	Reagent
a. Phenols				Ferric chloride	Vanillin HCl
Quercetin acid	60	35	Bright yellow	Yellow	Orangish yellow
Sinapic acid	82	10	Bluish green	Yellowish green	Light blue
Ferulic acid	85	12	Bright blue	Blue	Blue
b. Alkaloids				Dragondorff's	Iodoplatinate
Cytisine	05	30	Bright blue	Blue	-
Berbine	23	10	Fluorescent yellow	-	Yellow
c. Terpenoid				Conc. H_2SO_4	
Limonene	07	15		Brown	-

Table 4. Semi-quantitative data of various phytochemicals of *D. muricata*.

Phytochemicals	Root extract	Stem extract	Leaf extract
a. Phenols			
Quercetin acid	+++	++	++
Sinapic acid	++	+	++
Ferulic acid	+++	++	++
b. Alkaloids			
Cytisine	++	_	+
Berbine	++	+	++
c. Terpenoid			
Limonene	++	_	+

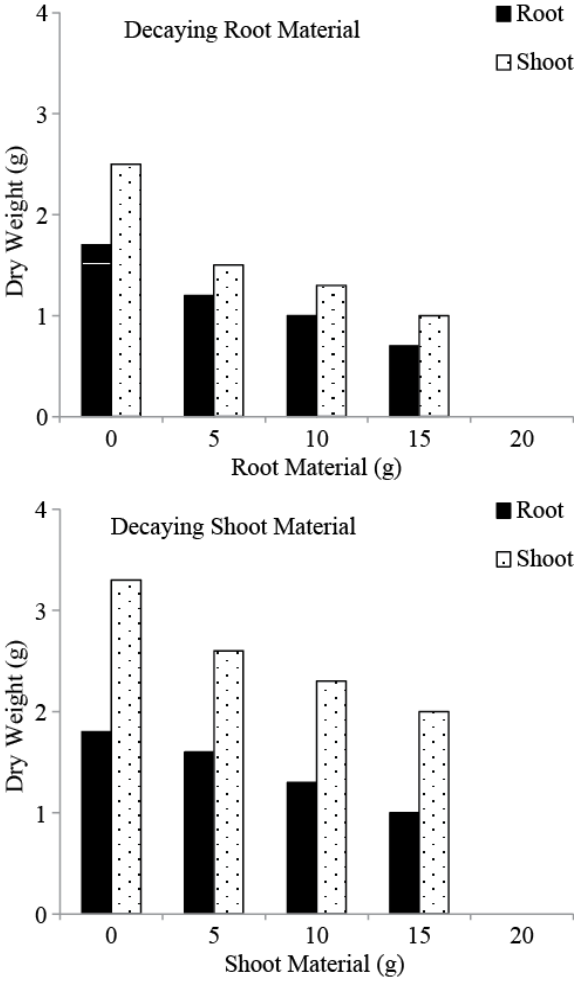

Fig. 4. Phytotoxic effects of decaying *D. muricata* fresh plant material of root and shoot on the dry weight (g) of bulrush millet.

Allelochemicals leached from *D. muricata* are found to be harmful for other plant species. Germination of four crops tested was significantly inhibited in the aqueous extract of *D. muricata*. Germination percentage of millet was highly affected whereas, mungbean was least affected. The aqueous extract of *D. muricata* produced differential inhibitory effects on germination of test species because different allelochemical compounds detected might have produced differential responses in different species due to morphological and physiological differences among them. Shaukat *et al*., (1985), also reported, the inhibition in germination of several crops, when grown in *Citrullus colocynthis* extract. Tseng *et al.,* (2003), reported suppression of germination and growth in lettuce due to allelopathic effect of *Macaranga tanarius*.

Decaying roots and shoots of *D. muricata* were found to be highly phytotoxic. Germination and seedling growth, as well as dry weights of millet were found to be positively correlated with the amount of decaying material of *D. muricata*. Maximum emergence and seedling growth was found in controls undoubtedly due to the absence of phytotoxic chemicals in the soil. Soil added with full-stength leachate of roots and shoots of *D. muricata* reduced the emergence and growth of millet, thereby providing evidence that, *D. muricata* is highly allelochemical plant. In the present study, maximum reduction of growth was observed in the soil incorporated with 15 g root material. It might be due to the release of phytotoxins from the decaying *D. muricata* that remains active and stable for considerable duration in the soil, exerting the causative influence on growth and development of other plants in the neighbourhood.

Phenolic compounds, alkaloids and a terpenoid were detected from plant material of *D. muricata*. Greater

quantities of all these allelochemicals are present in the roots followed by shoots. Phenolic compounds are present in much higher amounts than alkaloids and a terpenoid. Phenolic compounds including quercitin, sinapic and ferulic acids were identified from the extract of *D. muricata*. Toxic nature of phenolic compounds have beeen reported by several workers (Stowe *et al*., 1987; Blum, 1996; Inderjit, 1998; Rebaz *et al*., 2001). Quercetin is one of the flavanol that gives bright colour after acid hydrolysis. Flavanol is a class of flavanoid compounds that are universal in higher plants. Sinapic and ferluic acids are derivatives of hydroxycinnamic acids. Wang *et al*., (1967), reported inhibition in the growth of young sugarcane at 50 ppm concentration of hydroxycinnamic acids. Del Moral & Muller (1970), extracted phenols in the leachate from the litter of *Eucalyptus camaldulensis*. Rasmussen & Rice (1971), found hydroxycinnamic acids (p-coumaric and ferulic acids) from the shoot extracts of *Sporobolus pyramidatus*. Lehman and Blum (1999), reported inhibition of net phosphosorus uptake due to the presence of ferulic acid in the soil. Similarly, Chaves *et al*., (2001), reported negative effect of ferulic acid along with other allelochemical compounds on the germination and growth of *Rumex crispus*. Chou *et al*., (1998), isolated a number of inhibitory compounds along with ferulic acid from *Acacia confusa* and related species and showed their inhibitory effects.

Only one terpenoid was detected, which was identified as "limonene", and classified as a monoterpenoid. It is predominant terpenoid and acts as a growth inhibitor, identified from higher plants (Fischer, 1986). Many monoterpens are extremanely toxic, non-polar and safinrvolatile (Tanrisever *et al*., 1988). According to Turtola *et al*., (2003), concentration of monoterpenoids increases due to severe drought. Della Penna (1985), also detected terpenoids from *Conradina canes*. Vokou *et al*., (2003), reported adverse effect of 47 different monoterpenoids on germination and growth of *Lactuca sativa* seedlings. On the other hand, Duke *et al*., (1988), extracted terpenoids from the genus *Artemesia*, used as a potential pesticide. Alkaloids detected from the *D. muricata* were cystisine and berberine. They are known to be the strong inhibitors of seed germination and are also the main cause of growth inhibitors.

D. muricata is a desert annual, which appears only after rains at high temperatures. When plant matures and dies down, the leachates enter the soil, which with the passage of time undergo degradation and level of phenolics declines. Thus allowing the germination of other plants in the field next year after monsoon showers. The distribution of allelochemicals phytotoxic substances and the amount of these substances depend upon the number of donor plants. This study demonstrates the allelopathic potential of *D. muricata*, but it does not eliminate the possible involvement, under field conditions, of other mechanisms of interference such as resource competition, microbial nutrient immobilization and microbial competition.

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