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Allelopathic Potential of *Launaea procumbens* (Roxb.) Rammaya and Rajgopal: A Tropical Weed

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Abstract: The effects of *Launaea procumbens* (Roxb.) Rammaya and Rajgopal on seed germination and early seedling growth of four test plant species including mustard, bulrush millet, corn and spinach was evaluated under laboratory conditions. Aqueous extract of *L. procumbens* at different concentrations (25, 50, 75 and 100% stock solution) inhibited germination of three test species in the order: spinach > mustard > corn; germination of millet was not significantly influenced. Root and shoot growth of all four species was substantially reduced by *Launaea* extract. The growth was reduced in the order: spinach > mustard > corn > millet. When different modes of extract application were tested, it was found that only the soil application of the aqueous extract had a significant retarding effect on wheat growth while shoot spray or root dip treatment had no such effect. Decaying shoot of *L. procumbens* in sandy-loam at 5, 10 and 20 g/400 g soil caused substantial inhibition of germination and seedling growth of bulrush millet (*Pennisetum americanum*) at high dosages. Bioassay of the ether extract of *L. procumbens* exhibited four zones of inhibition at Rf values 0.1-0.2, 0.7-0.8, 0.8- 0.9 and 0.9-1.0 while a promoter was detected between Rf values 0.3-04. A thin-layer chromatography for the phenolics showed the presence of seven phenolic acids including: salicylic acid, vanillic acid, syringic acid, 2-methylresorcinol, gallic acid and two unknowns.

Key words: Allelopathy, *Launaea procumbens*, phenolic compounds, germination

Introduction

The distribution of vegetation and species associations in natural, semi-natural and managed communities are often considerably influenced by allelopathic interactions (Rice, 1984). Allelopathy also plays an eminent role in the intra-specific and inter-specific competition and may determine the type of inter-specific association. The plants may exhibit inhibitory or rarely stimulatory effects on germination and growth of other plants in immediate vicinity. Allelopathic potential has been reported for a number of weed species (Einhellig and Rasmussen, 1973; Rasmussen and Einhellig, 1975; Shaukat *et al.*, 1985; Ahmed and Wardle, 1994; Burhan and Shaukat, 1999; Rebaz *et al.*, 2001; Shaukat and Siddiqui, 2002; Tajuddin *et al.*, 2002).

It has been shown that allelopathic chemicals released from the plants often play a significant role in species replacement during secondary succession (Al Saadawi and Rice, 1982; Rice, 1995) and in the horizontal patterning of plant populations (Shaukat *et al.*, 1983). Putnam and Weston (1986) listed 90 weed species while Narwal (1994) listed 129 weed species having allelopathic potential. Several workers have shown that allelopathy plays an important part in weed-weed interaction (Wilson and Rice 1968; Rasmussen and Rice, 1971; Newman and Rovira, 1975; Tajuddin *et al.,* 2002) and weed–crop interaction (Colton and Einhellig, 1980). Extensive studies regarding allelopathic potential of weeds on crops are conducted all over the world by different workers (Casado, 1995; Inderjit *et al.,* 1996; Lydon *et al.,* 1997; Rajbanshi and Inubushi, 1997; Peres *et al.,* 1998; Ito *et al.,* 1998; Al-Humaid and Warrag, 1998; Tajuddin *et al.*, 2002).

The secondary plant compounds including alkaloids, terpenoids, flavonoids, steroids, tannins and phenolic compounds usually have inhibitory effects on crops (Whittaker and Feeny, 1971; Mandava, 1985). Phenolic compounds often constitute the major allelopathic agents in weeds and other allelopathic plants (Inderjit, 1998; Ferreira *et al.,* 1998; Wang *et al.,* 1998; Burhan and Shaukat, 2000). *Launaea procumbens* (Roxb.) Rammaya and Rajgopal, a tropical ruderal and agrestal weed, grows abundantly in waste grounds, vacant lots, lawns and abandoned and cultivated fields in Sind, Baluchistan and NWFP. Since often it forms dense, almost pure stands, it is suspected that allelopathy could be involved in the suppression of the neighboring plant species. Therefore, experiments were designed to test the allelopathic potential of *L. procumbens.* The objectives of this investigation were: 1) to evaluate the effect of aqueous extract of *L. procumbens* on four crop species *in vitro,* 2) to test the effect of different modes of application of aqueous extract on plant growth, 3) to study the phytotoxicity of decaying *L. procumbens* in soil and 4) to identify the phenolic principles of. *L. procumbens.*

Materials and Methods

Effect of aqueous extract of *L. procumbens* on germination and seedling growth of four test species: *L.*

procumbens was collected from a waste ground in Malir, Karachi and its shoot extract was studied. The plant material was air-dried under shade and chopped into small pieces. Extract of *L. procumbens* was prepared by soaking 10 g plant material in 100 ml of distilled water for 24 h to obtain stock solution. Using stock solution (100%), three other concentrations were prepared i.e., 25, 50 and 75%. Effect of various concentrations was tested against *Brassica compestris* L. (mustard), *Pennisetum americanum* (L.) Schumann (bulrush millet), *Zea mays* L. (corn) and *Spinacea oleracea* L. (spinach).

Crop seeds were first surface sterilized by 0.3% calcium hypochlorite for five min and then placed on 9 cm diam., sterile petriplats on two layers of Whatman No.1 filter paper. Each plate received 5 ml of the extract. For controls, distilled water was used. Germination counts were made daily and shoot and root length of the seedlings were recorded after 72 h.

Effect of various modes of application of aqueous extract of *L. procumbens*, on growth of wheat in soil: An experiment was designed to establish whether soil application of the aqueous extract of *L. procumbens* is a prerequisite for causing the potential negative impact on the seedling growth of wheat (*Triticum aestivum* L.) or aqueous extract applied to any plant part can induce phytotoxic effects. Aqueous extract was applied on the shoot system and the root system of wheat seedlings, in addition to soil drench treatment. The experiment was carried out in 8 cm diam., plastic pots under glasshouse conditions. The sandy loam soil (pH 8.1) obtained from the experimental field of the Crop Diseases Research Institute, University of Karachi was filled in plastic pots at 350 g/pot. The soil was collected from a section of a field that had remained fellow for at least three years, therefore, the soil had low fungal populations and was relatively free of allelopathic weeds. Wheat was used as a test plant in this study because a number of weeds grow in association with this crop in almost all the agricultural fields of Pakistan.

For leaf application, the soil was excavated to a depth of 1 cm and eight wheat seeds were sown in pots. One week after emergence, the seedlings were thinned to two per pot and sprayed with 5 ml aqueous extract (75% stock solution) of *L. procumbens*. To avoid leaf washings entering the soil, the soil surface was covered with two layers of blotting paper. Control leaves were sprayed with 5 ml of sterile distilled water. For soil drench, a 5 ml aqueous extract was applied to the soil one week after seedling emergence. Soil treated with 5 ml sterile distilled water served as control. For bare-root-dip treatment, the pre-germinated wheat seedlings (seeds were previously sown in steam sterilized soil) were uprooted and roots of the seedlings dipped for 20 min. in 5 ml aqueous extract of *L. procumbens* and planted in the pots. The roots of control seedlings were dipped in 5 ml sterile distilled water. Each treatment and control was replicated four times and pots were randomized on a glasshouse bench. The plants were watered as needed. Twenty two days after seed sowing or 15 days after transplantation, the seedlings were removed from pots and their height and fresh weights determined.

Effect of decaying *L. procumbens* on germination and seedling growth of *Pennisetum americanum*: Dried powdered material of *L. procumbens* was mixed thoroughly with loamy sand (76.1% sand, 15.3% silt and 8.6% clay) at 5, 10 and 20 g/400 g of soil. Pots were watered once and soil left for biodegradation. After one week, 10 seeds of bulrush millet (*P. americanum*) were sown in each pot. Controls and treatments were replicated thrice and pots were randomized on the greenhouse bench. Final emergence percentage and shoot and root lengths were measured after four days.

Bioassay: 10 gram air dried shoot material of *L. procumbens* was blended in 200 ml distilled water. The centrifuged homogenate was adjusted to pH 3 with 0.5 N H_2SO_4, extracted thrice with peroxidase-free ether and evaporated to dryness using argon gas. 2 ml of 80% ethanol was added to the dried material and was streaked on Whatman No.1 filter paper. Duplicate 10 cm wide chromatograms were developed by descending chromatography in isopropanol:ammonia:water (10:1:1,v/v/v). When the solvent had moved 30 cm, the chromatograms were dried and 10 equal width strips cut and assayed for growth regulators using wheat coleoptile straight growth test of Nitsch and Nitsch (1956). 5 mm coleoptile segments of 3 days old dark grown wheat were excised and floated in distilled water for 1 h. Ten coleoptile segments were placed in between two strips of the same Rf value and kept in 11.5 cm diam., petriplates over two layers of tissue paper moistened with 4 ml 0.02 M citrate phosphate buffer (pH 4.8). After 48 h of growth in dark, the length of coleoptile segments was measured.

Chromatography: Ether extract of *L. procumbens* was evaporated to dryness, dissolved in 2 ml of 80% ethanol and used for loading on silica gel F_{254} thin layer chromatographic plate. The chromatogram was developed in acetic acid-chloroform 1:9 v/v by ascending chromatography using reference phenolic compounds. Phenolic compounds in the extract were detected using ferric chloride-ferric cyanide reagent and UV light (Harborne, 1973).

Statistical analysis: A factorial analysis of variance (FANOVA) was performed, after arcsine transformation of the percentage germination data (Sokal and Rohlf, 1995). As a follow up of FANOVA, a least significant difference

(LSD) test and Duncan's multiple range test were performed at $P \leq 0.05$. Computer programs for the analysis were developed in FORTRAN-77 by the senior author and are available on request.

Results

Effect of aqueous extract on germination and seedling growth of the test species: Germination of three out of four test species was inhibited by various concentrations of the extract (P at the most 0.05) over the controls (Fig. 1a-d). The inhibitory effect increased with the increase in concentration. Different species were affected to a different extent; the degree of inhibition varied in the order: spinach > mustard > corn. Germination of millet remained uninfluenced. On the other hand, seedling growth of all four-test species was adversely affected and growth reduction was greater at higher concentrations (Table 1). Root and shoot growth of spinach and mustard was inhibited to a greater degree compared to corn while millet was least affected. Generally, root growth was reduced to a greater degree than the shoot growth.

Effect of various modes of application of aqueous extract of *L. procumbens*, on growth of wheat in soil: Only soil application significantly reduced shoot height ($P < 0.05$), shoot and root weights ($P < 0.01$) of wheat plants compared to untreated controls (Table 2). Root growth was affected to a greater degree than the shoot growth. Some phytotoxic symptoms such as yellowing and chlorosis of the leaves were also observed in the treatments (data not presented). Shoot spray or bare root-dip treatments had no significant effect on any of the plant growth parameters.

Phytotoxicity of decaying *L. procumbens*: Germination of bulrush millet was significantly ($P<0.001$) reduced at high dosages (10 and 20 g/400 g soil) of decaying *L. procumbens* (Table 3). Germination percentage declined sharply with the increase in concentration. Likewise, both root and shoot growth were significantly ($P<0.001$) suppressed at all the concentrations of the decaying shoot material compared with controls. This inhibitory effect increased with the increase in concentration.

Wheat coleoptile bioassay: Wheat coleoptile bioassay revealed five inhibitors at Rf-values of 0.1-0.2, 0.2-0.3, 0.5-0.6, 0.8-0.9 and 0.9-1.0, while one significant promoter was detected at Rf-values of 0.3-0.4 (Fig. 2).

Chromatographic study: Thin layer plates when sprayed with ferric chloride-ferricyanide reagent or examined under UV light, revealed seven spots which were matched with the standard phenolic compounds and identified as salicylic acid, vanillic acid, syringic acid, 2-methylresorcinol, gallic acid and two unknown compounds (Table 4).

Table 1: Effect of aqueous shoot extract of *Launaea procumbens* on root and shoot length (cm) of mustard, spinach, corn and millet

	Concentrations (% stock solution)									
	0		25		50		75		100	
Test species	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Mustard	3.6±0.3	5.4±0.5	3.0±0.3	2.3±0.4	1.8±0.3	2.0±0.4	0.8±0.2	0.3±0.1	0±0	0±0
Spinach	3.7±0.2	4.9±0.3	2.8±0.2	2.6±0.1	1.5±0.2	1.9±0.4	0.6±0.2	0.7±0.2	0±0	0±0
Millet	4.8±0.6	7.1±0.4	4.9±0.3	6.3±0.2	4.2±0.1	4.0±0.2	2.7±0.3	2.2±0.4	1.6±0.2	0.8±0.5
Corn	4.4±0.5	7.0±0.3	3.2±0.6	4.5±0.2	3.1±0.2	2.9±0.6	0.8±0.3	1.2±0.2	0.5±0.2	0.1±0.1

Table 2: Effects of various application methods of the aqueous extract of wheat, on plant height and fresh shoot and root weight of wheat

Application method	Plant height (cm)		Shoot root weight (g)		Root weight (g)	
	Control	Treated	Control	Treated	Control	Treated
Spray on shoot	24.7	23.6	4.1	3.7	1.9	1.7
Soil drench	23.9	18.4	4.8	2.5	2.2	0.7
Root dipping	24.4	22.3	4.4	4.2	2.1	1.9
$LSD_{0.05}$ Treatment	2.1		1.0		0.4	
Application method	2.5		1.3		0.6	

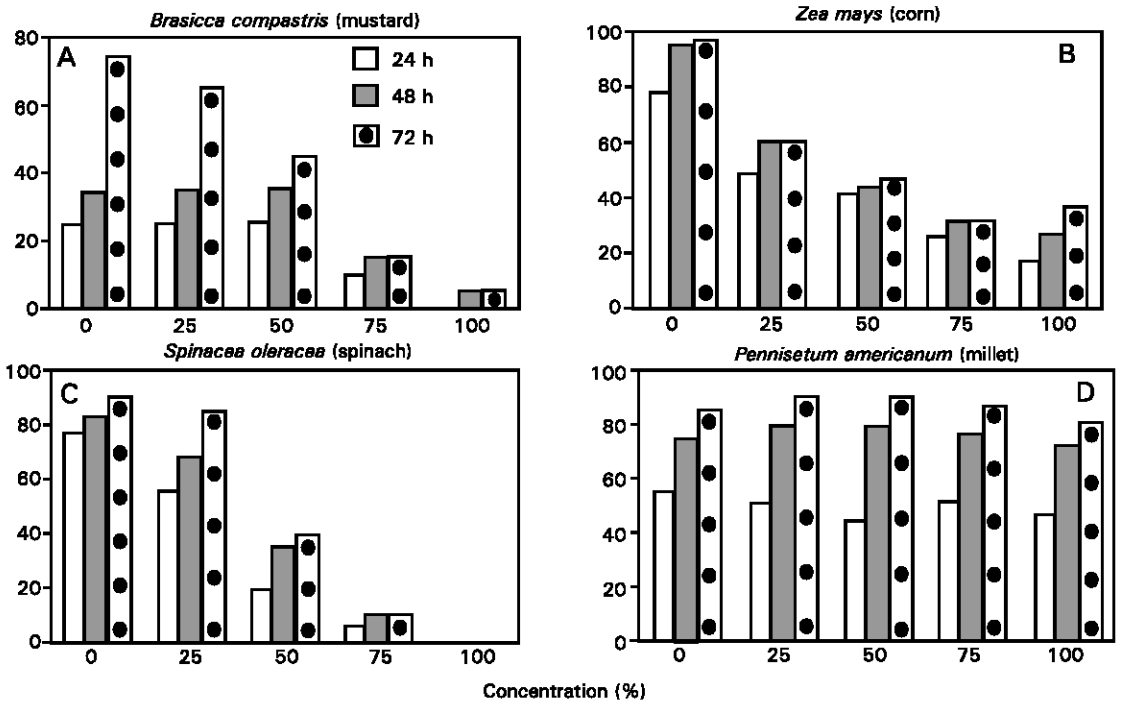

Fig. 1: Effect of various concentration of the aqueous shoot extract of *Launaea procumbens* on percentage germination of the four tested species. ($LSD_{0.05}$ Test species = 7; Concentration =9; Days = 5)

Table 3: Phytotoxic effects of decomposing *Launaea procumbens* on germination and root and shoot growth of bulrush millet (*Pennisetum americanum*)

Concentration G/400 g soil	Germination (%)	Root length (cm)	Shoot length (cm)
0	90	12.4±2.8	13.9±2.1
5	86	9.3±2.3	9.2±2.6
10	55	5.8±1.8	8.8±1.7
20	45	5.1±1.3	3.9±1.2

Table 4: Rf-values (x 100) of phenolic principles in ether fraction of aqueous extract of *Launaea procumbens* and their reaction to a developing reagent and UV light

Compounds	Rf	Ferric chloride-ferric cyanide	UV-light
Unknown I	96.34	Purple	Blue
Salicylic acid	90.85	Purple	Blue
Vanillic acid	85.32	Purple	Light blue
Syringic acid	79.87	Purple	Blue
Unknown II	77.13	Purple	Blue
2-methyl-resorcinol	44.51	Bluish	Blue
Gallic acid	4.35	Brownish	Bluish-brown

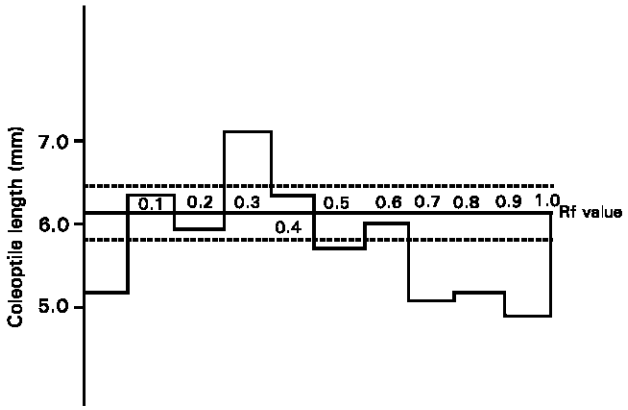

Fig. 2: Result of wheat coleoptile bioassay of the ether extract of *Launaea procumbens* showing inhibitors and promoters at different Rf values. Dotted lines represent 95% confidence interval of the control mean.

Discussion

Our results clearly indicate the allelopathic potential of *Launaea procumbens*. Seed germination and seedling growth of spinach, mustard and corn was inhibited by aqueous extract of *L. procumbens*. However, final seed

germination percentage of millet remained unchanged by the extract. Extracts of a number of weed species have been shown to be inhibitory to germination of crop seeds (Shaukat *et al*., 1985; Casado, 1995; Hofmann *et al*., 1996; Lydon *et al*., 1997; Tajuddin *et al*., 2002). Allelopathic potential of *L. procumben* has not been reported previously. The aqueous extract of *L. procumbens* produced differential inhibitory effect on germination and early seedling growth of the four test species probably because the different inhibitory compounds such as the phenolic compounds detected might elicit a differential response in different species due to morphological and physiological differences among them.

When the modes of application of the aqueous extract of *L. procumbens* were compared, it was found that only the soil application had a significant impact on the growth of wheat, while shoot spray and bare root-dip treatment had no detectable impact in this respect. Pronounced reduction in growth in the soil treatment can be explained as follows: a) soil application causes direct absorption and upward translocation of inhibitory compounds (contained in the extract) by the roots thereby causing a marked inhibition of growth and b) changes in microbial community structure and composition (Shaukat and Siddiqui, 2001; Siddiqui *et al*., 2002) following soil application which could favour certain pathogenic organisms that in turn might cause plant growth retardation. Lesser degree of absorption and presumably no basipetal movement of the extract by the leaves and the cotyledon in case of shoot application of the extract explain why the shoot spray did not affect the wheat plants significantly. Similarly, lesser uptake of the extract (containing inhibitors) in case of root dip treatment due to lesser duration of exposure to the extract seems to account for no reduction in wheat growth.

The germination of bulrush millet was reduced in soil incorporated with decaying *L. procumbens*. Wilson and Rice (1968) have reported both stimulatory and inhibitory effect on various crop species with decaying sunflower leaves. In the present study, maximum reduction was observed in soil incorporated with 20 g shoot material. This may presumably be due to the release of phytotoxins from the decaying *L. procumbens* that remain active and stable for considerable duration in soil. These results strengthen our previous findings in which the decaying weeds had inhibitory effects. Shaukat *et al*. (1985) found adverse effects on the growth of millet plants by decaying *Citrullus colocynthis* while Burhan and Shaukat (1999) reported inhibitory effects of decomposing *Argemone mexicana.*

Under natural conditions probably phytotoxins released from *L. procumbens* accumulate over the years in soil is sufficiently high concentrations and consequently play a vital role as an edaphic variate, exerting a detrimental impact on growth and development of other plants in the neighbourhood. This could be the possible mechanism of interference between *L. procumbens* and the other nearby plants. The wheat coleoptile bioassay of *L. procumbens* disclosed five inhibitory zones. These presumably represented phenolic compounds. The promoter at Rf values 0.3-04 could be a growth hormone (such as an indole compound). Thin layer chromatography showed the presence of seven phenolic compounds including salicylic acid, syringic acid, vanillic acid, 2-methyl resorcinol, gallic acid and two unknown compounds. Because they are the usual allelopathic agents, only the phenolic compounds were examined. It is noteworthy that secondary metabolites other than phenolic compounds might also constitute important allelopathic agents in *L. procumbens*. The toxic effects of the phenolic compounds on seed germination and plant growth have been previously reported (Stowe *et al*., 1987; Blum, 1996; Inderjit, 1998; Burhan and Shaukat, 2000).

The study lead us to conclude that *L. procumbens* is a considerably phytotoxic plant and being an abundant species in some of the disturbed habitats such as vacant lots in the cities and in cultivated fields and lawns, is responsible for the accumulation phytotoxins that are recurrently added over the years and eventually suppresses other species in the community and the crops in the field whose germination and growth are affected. Often in non-cultivated disturbed habitats *L. procumbens* eventually forms more or less pure populations. Thus, this species not only interferes with crop growth through its allelopathic action but also poses problem in the maintenance of biodiversity of ruderal habitats.

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