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Allelopathic Potential of *Anagallis arvensis* L. : A Cosmopolitan Weed

Zenab Rebaz, S. Shahid Shaukat and Imran A. Siddiqui
Department of Botany, University of Karachi, Karachi-75270, Pakistan

Abstract: Effect of *Anagallis arvensis* L. on seed germination and early seedling growth of six test species was examined. Aqueous extract of *A. arvensis* inhibited germination, root and shoot growth of all the six test species. The species exhibited differential response to the extract. Germination was reduced by the shoot extract in the order: pearl millet > mustard > carrot > turnip > wheat = corn. Decaying *A. arvensis* in sandly-loam soil at 5, 10 and 20 g / kg soil substantially inhibited germination and seedling growth of pearl millet at all the dosages. Bioassay of the extract of *A. arvensis* revealed two zones of inhibition at Rf values 0.8-0.9 and 0.9-1.0. Chromatography for the phenolics revealed the presence of three phenolic acids: salicylic acid, cinnamic acid and caffeic acid.

Key words: Allelopathy, *Anagallis arvensis*, weed

Introduction

Allelopathy, an important ecological mechanism in natural and managed ecosystem (Rice, 1984), plays an important role in the intraspecific and interspecific competition. The plants may have stimulatory or inhibitory effect on germination and growth. Various scientists have reported allelopathic potential of weeds (Buchholtz 1971; Bell and Koeppe,1972; Einhellig and Rasmussen,1973; Rasmussen and Einhellig,1975; Ashraf and Sen,1978; Shaukat *et al.*,1985; Ahmed and Wardle, 1994; Burhan and Shaukat, 1999).

Chemicals released from the plants are responsible for replacing susceptible species and new invading species during succession (Al Saadawi and Rice, 1982; Rice ,1995). Putnam and Weston, (1986) have listed 90 weed spices while Narwal (1994) listed 129 weed species having allelopathic potential. Several workers have shown that allelopathy may play an important part in weed-weed interaction (Wilson and Rice 1968; Rasmussen and Rice1971; Newman and Rovira, 1975) and weed–crop interaction (Colton and Einhellig, 1980). Extensive studies regarding allelopathic potential of weeds on crops are done 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).

The secondary plant compounds including alkaloids, terpenoids, flavonoids, steroids, tannins and phenolic compounds have inhibitory effects on crops (Whittaker and Feeny 1971). Phenolic compounds often constitute the principal allelopathic agents in weeds and other allelopathic plants (Inderjit, 1998; Ferreira *et al*, 1998; Wang *et al*, 1998). Experiments were designed to determine whether allelopathic characteristics of *Anagallis arvensis* affects the germination and seedling growth of different crops. Our objectives were: 1) to evaluate the effect of aqueous extract of *A. arvensis* on six crop species *in vitro*, 2) to study the phytotoxicity of decaying *Anagallis arvensis* in soil, and 3) to identify the phenolic principles of *Anagallis arvensis*.

Materials and Methods

Effect of aqueous extract of *Anagallis arvensis* on germination and seedling growth of six test species: *Anagallis arvensis* was collected from the agricultural field of Pakistan Agricultural Research Council at Karachi University campus and the effect of its shoot extract was studied. The plant material was air-dried under shade and chopped into small pieces. Extract of *Anagallis arvensis* was prepared by soaking 10-g plant material in 100ml 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 *Pennisetum americanm* (L.) Schumann (pearl millet), *Triticum aestivum* L. (wheat), *Brassica napobrassica* Mill. (turnip), *Daucus carota* L. (carrot), *Zea mays* L. (corn), *Brassica compestris* L. (mustard).

Crop seeds were first sterilized by 0.3% calcium hypochlorite for five min and then placed on 9-cm-diam., sterile Petri dishes on Whatman No.1 filter paper. Each plate received 5ml 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 decaying *Anagallis arvensis*: Dried powdered material of *Anagallis arvensis* was mixed thoroughly with loamy sand (76.1% sand, 15.3% silt and 8.6% clay) at 5, 10 and 20g/kg of soil. Pots were watered once and soil was left for biodegradation. After one week, 10 seeds of *Pennisetium americanum* were sown in each pot. Controls and treatments were replicated thrice and pots were randomized on the greenhouse bench. Daily rate of emergence was recorded while shoot and root length were measured after four days.

Bioasssy: Ten-g air-dried shoot of *Anagallis arvensis* was blended in 200-ml distilled water. The centrifuged homogenate adjusted to pH 3 with 0.5N H_2SO_4 was extracted thrice with peroxidase-free ether and evaporated to dryness using argon gas. Two-ml of 80% ethanol was added to the dry 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 were cut and assayed for growth regulators using wheat coleoptile straight growth test of Nitsch and Nitsch (1956). Five-mm segments of 3-day-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., Petri plates over two layers of tissue paper moistened with 4-ml 0.02M citrate phosphate buffer (pH 4.8). After 48 h of growth in dark, the length of coleoptile segments was measured.

Chromatography: Ether extract of *Anagallis arvensis* was evaporated to dryness, dissolved in 2-ml of 80% ethanol and used for loading Whatman No.1 chromatographic paper. The chromatograms were developed in n-butanol-acetic acid-water (50:2:48 v/v/v) by descending chromatography using reference phenolic compounds. Phenolic principles were detected using ferric chloride-ferric cyanide reagents and UV light (long) (Block *et al.*, 1958; Harborne, 1973).

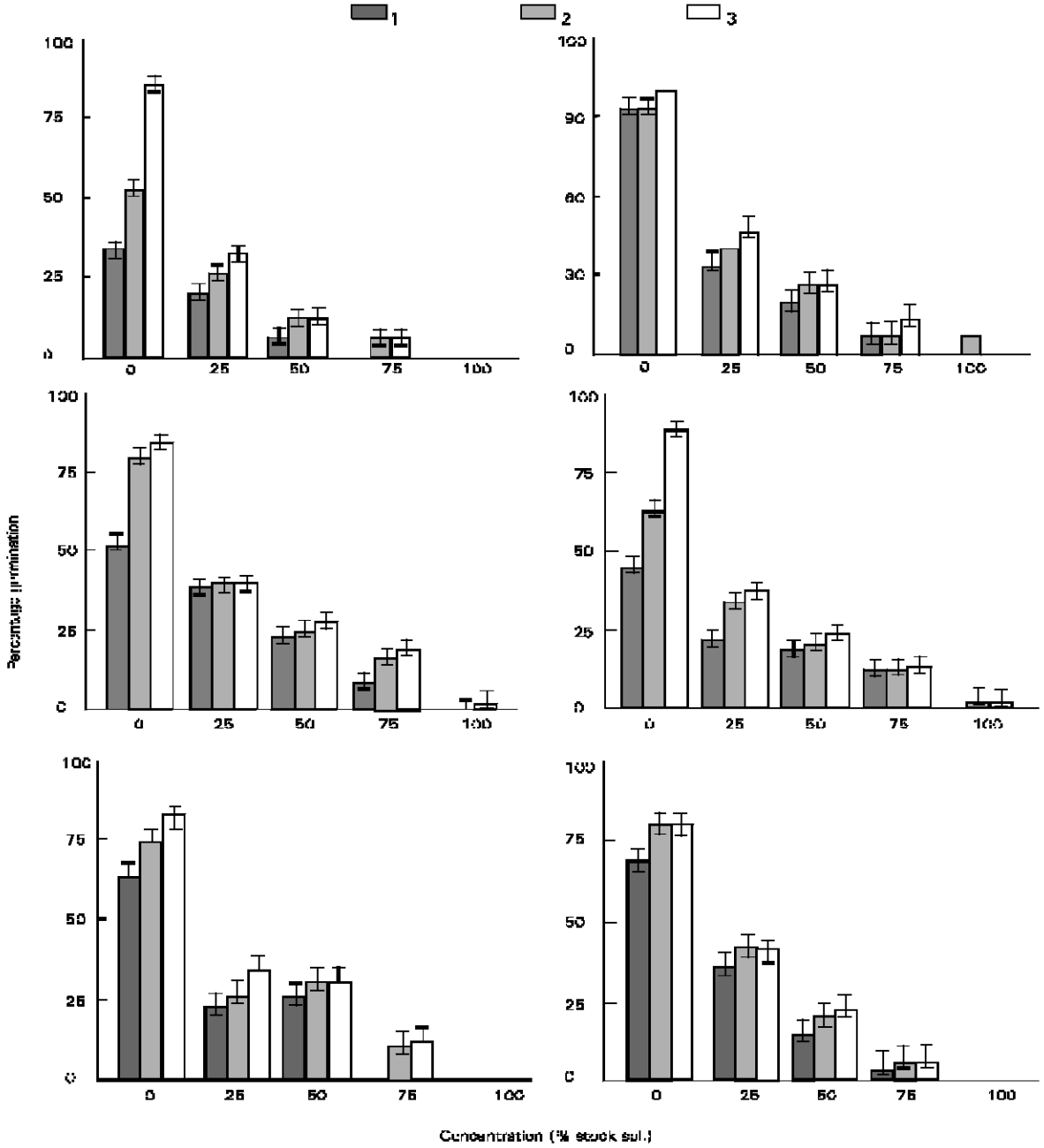

Fig. 1: Effect of shoot extract of *Anagallis arvensis* on germination of six test species, a) pearl millet, b) wheat, c) turnip, d) carrot e)mustard and f)corn

Statistical analysis: Means and standard errors were computed. Factorial analysis of variance (FANOVA) was performed, after arcsine transformation of the percentage germination data (Sokal and Rohlf, 1995). Computer programs for the analysis were developed by us in FORTRAN-77 and are available on request from the authors.

Results

Effect of aqueous extract on germination and seedling growth of the test species: Germination of all the six test species was inhibited by various concentrations of the extract (p at the most 0.05) over the controls (Fig.1a-f). The inhibitory effect increased with the increase in concentration in the order: pearl

Table 1: Effect of shoot extract of *Anagallis arvensis* on shoot and root growth (cm) of six test species

Test species	Concentrations (% stock solution)									
	0		25		50		75		100	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Millet	7.1±0.5	11.5±0.7	5.2±0.4	7.9±0.4	3.2±0.3	4.9±0.5	3.0±0.6	4.2±0.8	0±0	0±0
Wheat	4.2±0.1	7.0±0.2	3.2±0.2	6.3±0.4	2.2±0.1	5.2±0.5	0.2±0.1	3.1±0.7	0±0	0±0
Turnip	3.0±0.2	5.8±0.3	3.2±0.3	4.5±0.6	2.7±0.2	3.4±0.7	2.0±0.8	2.6±0.3	0±0	0.7±0.3
Carrot	5.4±0.4	8.2±0.3	5.1±0.2	7.5±0.6	4.7±0.3	6.8±0.4	3.1±0.2	4.2±0.4	0.2±0.2	0.3±0.2
Mustard	5.9±0.3	11.3±0.4	4.7±0.3	10.0±0.2	3.1±0.3	8.1±0.3	0.2±0.1	6.2±0.9	0.0±0.0	0.0±0.0
Corn	5.8±0.2	11.1±0.2	4.3±0.2	9.6±0.3	3.0±0.2	7.5±0.4	2.1±0.2	6.1±0.3	0.3±0.2	2.1±0.6

Table 2: Rf-values of phenolic principles in ether fraction of aqueous extract of *Anagallis arvensis* and their reaction to a developing reagent and UV light.

Compounds	Rf	Ferric chloride-ferric cyanide	UV-light
Salicylic acid	0.90	Purple	Blue
Cinnamic acid	0.93	Light blue	Blue
Caffeic acid	0.78	Dark blue	Blue

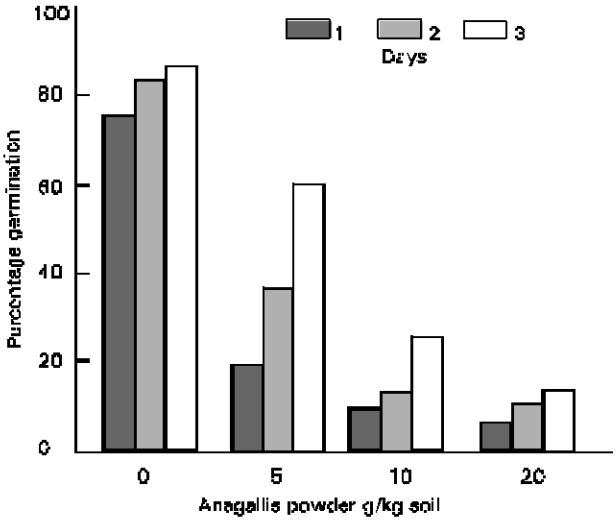

Fig. 2: Phytotoxic effect of decaying *Anagallis arvensis* shoot material on shoot and root lenght of pearl millet seedlings

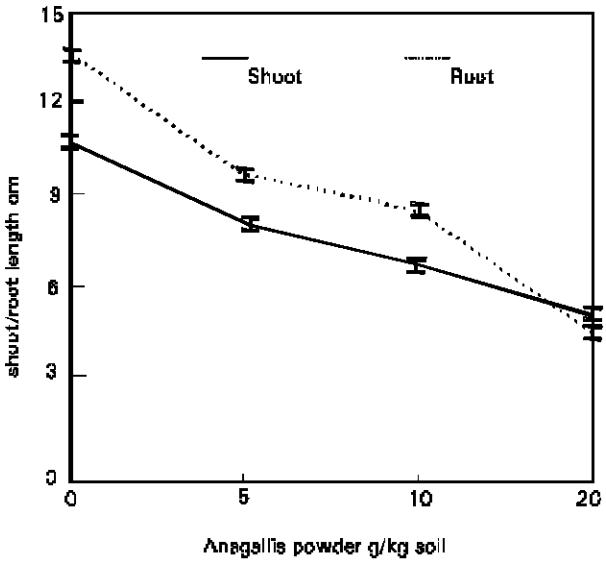

Fig. 3: Phytotoxic effect of decaying *Anagallis arvensis* shoot material on shoot and root length of pearl millet seedling

millet > mustard > carrot > turnip > wheat = corn. Similarly, seedling growth of the test species was adversely affected. Root and shoot growth of pearl millet and mustard was inhibited to a greater degree compared to the other species (Table 1). Generally, root growth was reduced to a greater extent than the shoot growth.

Phytotoxicity of decaying *Anagallis arvensis*: Germination of pearl millet was significantly ($p<0.001$) reduced in soil at all the dosages of decaying *Anagallis arvensis* (Fig. 2). 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. The effect was more pronounced at higher concentrations (Fig. 3).

Wheat coleoptile bioassy: Wheat coleoptile bioassay revealed two inhibitors at Rf-values of 0.8-0.9 and 0.9-1.0, while two significant promoters were detected at Rf-values of 0.2-0.3 and 0.3-0.4 (Fig. 4).

Chromatographic study: Chromatograms sprayed with ferric chloride-ferricyanide reagent or examined under UV light, revealed three spots which were identified as salicylic acid, cinnamic acid and caffeic acid (Table 2).

Discussion

This study provides evidence on the allelopathic potential of *Anagallis arvensis*. Seed germination and seedling growth of pearl millet, turnip, corn, wheat, carrot and mustard were inhibited by aqueous extract of *A. arvensis*. Aliotta *et al.*, (1989) found allelopathic activity in shoots of *A. arvensis* against lettuce and radish seedlings. Extracts of a number of weed species have been shown to be inhibitory to germination of crop seeds (Shaukat *et al.*, 1985; Rafique and Hafeez, 1994; Casado, 1995; Hofmann *et al.*, 1996; Demchuk and Yurchak 1996; Lydon *et al.*, 1997).

The germination of pearl millet was reduced in soil incorporated with decaying *A. arvensis*. Wilson and Rice (1968) have reported both stimulatory and inhibitory effect on

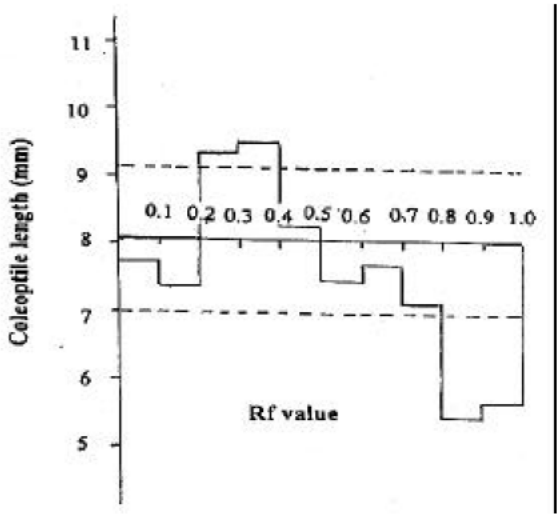

Fig. 4: Histogram of ether fraction of shoot extract of *Anagallis arvensis*. Dotted lines represent 95% confidence interval of control mean.

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 *A. arvensis* that remain active and stable for considerable duration in soil. Similarly, Shaukat *et al.* (1985) have reported adverse effects of decaying *Citrullus colocynthes* on pearl millet.

It is possible that phytotoxins released from *A. arvensis* may accumulate in soil in biologically significantly amounts and thereby play a key role as a habitat variable, exerting a causative influence on growth and development of other neighbouring plants. The wheat coleoptile bioassay of *A. arvensis* revealed two inhibitory zones which were identified by paper chromatography as salicylic acid, cinnamic acid and caffeic acid. Only the phenolic inhibitors were investigated. However, phytotoxic chemicals other than phenolic compounds might be present and could constitute important allelopathic agents. The toxic nature of the phenolic compounds have been reported by several workers (Stowe *et al.*, 1987; Blum 1996; Inderjit, 1998). It is concluded from our results that *A. arvensis* is considerably phytotoxic, and being an important weed in crop fields, it is necessary to manage its population to avoid long-term accumulation of phytotoxins and thereby protecting the crop from chemical interference.

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