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## Allelopathic potential of *Argemone mexicana* L. a tropical weed

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### Abstract

Effect of *Argemone mexicana* L. on seed germination and early seedling growth of six crop species was investigated. Aqueous extract of *A. mexicana* inhibited germination, root and shoot growth of all the test species. The species showed differential response. Germination was reduced by the aqueous shoot extract in the order: pearl-millet > mustard > wheat > carrot > corn > turnip. Root length was reduced in the order: corn > pearl-millet > wheat > mustard > turnip > carrot, whereas shoot length was reduced in the order: pearl-millet > wheat > corn > turnip > mustard > carrot. Decaying *A. mexicana* in sandy loam soil at a rate of 5, 10, 20 g / 400 g soil substantially inhibited germination and seedling growth of pearl-millet. Bioassay of the extract of *A. mexicana* revealed three phenolic inhibitors and paper chromatography revealed the presence of p-hydroxybenzoic, vanillic and salicylic acids.

### Introduction

In addition to competition for resources, some weeds interact with the crop plants through the production of chemical substances (allelochemicals) that inhibit their growth and development. Under field conditions weeds overrunning in large number might be the major cause of yield reduction in crops. The allelochemicals released from various plant parts eventually penetrate the soil and hinder the normal growth of neighbouring plants. Many workers have reported allelopathic, potential of several weeds on the crops (Schreiber, 1967; Buchholtz, 1971; Bell and Koepe, 1972; Einhellig and Rasmussen, 1973; Rasmussen and Einhellig, 1975; Ashraf and Sen, 1978; Shaukat *et al.*, 1985). Ninety weed species having allelopathic potential have been reported by Putnum and Weston (1986), while Narwal (1994) listed one hundred and twenty nine such weed species. Numerous recent studies have also concentrated on the allelopathic potential of weeds (Casado 1995; Jimenez-Osornio *et al.*, 1996; Inderjit *et al.*, 1996; Lydon *et al.*, 1997; Rajbanshi and Inubushi, 1997; Peres *et al.*, 1998; Ito *et al.*, 1998; Humaida and Warrag, 1998). The inhibitory effect of weeds may be due to a variety of allelochemicals, including phenolic acids, terpenes, terpenoids, glycosides, alkaloids and flavonoids (Whittaker and Feeny, 1971). These allelochemicals have little value outside ecological context. Many recent studies have revealed the phytotoxic effect of phenolic compounds (Shafer *et al.*, 1998; Ferreira *et al.*, 1998; Wang *et al.*, 1998). This study focuses on one such weed species that is *Argemone mexicana* which is a prickly glabrous, 30-125 cm tall, branched annual herb. It is a native of West Indies and Mexico, but naturalized in the world as a weed. Leela (1981) and Sharma and Nathawat (1987) studied the allelopathic effects of *A. mexicana* on a few crop plants and demonstrated reduction in germination and growth. However, no detailed study on the allelopathic activity of *A. mexicana* has been conducted to date. The objectives of this study were, a) to test the allelopathic activity of *Argemone mexicana*

against six crop species in vitro, b) to study the phytotoxicity of decaying *A. mexicana* in soil and c) to identify some of the allelochemicals, particularly the phenolic compounds.

### Materials and Methods

**Effect of aqueous extract of *A. mexicana* on germination and seedling growth of six test species:** *Argemone mexicana* was collected from an experimental field of Pakistan Agricultural Research Council at Karachi University Campus and the effect of its shoot material was examined on germination and seedling growth. 10 g air dried plant material was crushed and soaked in 100 ml distilled water for 24h and then filtered. The filtrate was taken as stock solution and various dilutions were made of this solution (i.e. 25%, 50%, 75%, 100%). Toxicity of the filtrate was tested against pearl-millet (*Pennisetum americanum* L. Schumann), wheat (*Triticum aestivum* L.), turnip (*Brassica napobrassica* Mill.), carrot (*Daucus carota* L.), corn (*Zea mays* L.) and mustard (*Brassica compestris* L.). Twenty seeds were taken and surface sterilized with 2 percent sodium hypochlorite for 5 minutes. They were then placed on Whatman No. 1 filter paper in 9 cm diameter sterile petri plates containing 5 ml of the test extract. Distilled water was used as control. Germination counts were made daily and the root and shoot lengths were recorded after 72 h.

**Phytotoxicity of decaying *Argemone mexicana*:** Fresh shoot fragments of *A. mexicana* were mixed with sandy loam (76.1% sand, 15.3% silt, 8.6% clay) at the rate of 5, 10, and 20 g per 400 g soil. These were placed in 8 cm diameter pots. Pots were then sprinkled with 100 ml water and left for one week for the microbial activity. Subsequently, pearl-millet was sown at a rate of 10 seeds per pot. Each treatment was replicated four times. Germination counts were made daily and root and shoot length of seedlings were measured after 96 h.

**Detection of inhibitors using wheat coleoptile bioassay:** 10

## Burhan and Shaukat: Allelopathy, germination, seedling growth, phenolic inhibitors

Table 1: Rf values (x 100) of phenolic principles in ether fraction of aqueous extracts of *Argemone mexicana* and their reactions to developing reagents.

Substance	Butanol-Acetic acid-Water	Spray	
		Ferric Chloride	Vanillin -HCl
p-hydroxybenzoic	90	Yellow	Blue
salicylic acid	95	Purple	Blue
Vanilic acid	92	Buff	Light blue

g dried shoot of *A. mexicana* was blended in 200 ml distilled water. The centrifuged homogenate adjusted to pH 3 with 0.5 N sulphuric acid, extracted thrice with peroxidase free ether and evaporated to dryness using argon gas. 2 ml absolute ethanol was added to the dry material and it was streaked on Whatman No. 1 filter paper. Duplicate 10 cm wide chromatograms were developed by using descending chromatography in iso-propanol: ammonia: water (10: 1:1, v/v/v). When the solvent had moved 30 cm, the chromatograms were removed and dried. Ten equal width strips were cut and assayed for growth regulators. 5 mm coleoptile segments of 3-days-old dark grown wheat seedlings 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 petri plates over two layers of tissue paper moistened with 4 ml of 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 shoot of *A. mexicana* was evaporated to dryness, dissolved in 2 ml ethanol and used for loading Whatman No. 1 chromatographic paper. The chromatograms were developed in butanol- acetic acid- water (50: 2: 48 v/v/v) by descending chromatography. Co-chromatography was performed using reference phenolic compounds. Phenolic principles were detected using ferric chloride and vanillin- HCl (Block *et al.*, 1958; Harborne, 1973).

**Statistical analysis:** Means and standard errors were computed using a FORTRAN 77 program called SD. Factorial analysis of variance was performed using the program FANOVA developed by us in FORTRAN 77 after arcsine transformation of the percentage germination data (Sokal and Rohlf, 1995).

## Results

**Effect of aqueous extract of *A. mexicana* on germination and seedling growth of the test species:** Seed germination of all the six test species was significantly inhibited (p at the most 0.05) by the aqueous extract of *A. mexicana* at different concentrations (Fig. 1 a-1 f). The inhibitory

effect increased with the increase in concentration in the order: pearl-millet > mustard > wheat > carrot > corn > turnip. Similar was the effect of *A. mexicana* on seedling growth of the test species that showed significant reduction in shoot and root length (p at the most 0.05) (Fig. 2a-2f). Root length was reduced in the order: corn > pearl-millet > wheat > mustard > turnip > carrot, where as shoot length was reduced in the order: pearl-millet > wheat > corn > turnip > mustard > carrot. The comparison between the reduction in root and shoot lengths showed that the root growth was reduced to a greater degree than the shoot growth.

**Phytotoxicity of decaying *Argemone mexicana*:** Seed germination was reduced remarkably in soils incorporated with 10 and 20 g decaying *A. mexicana* shoot while at 5g there was relatively lesser reduction. Both shoot and root growth were significantly suppressed in soils containing 10 and 20 g *A. mexicana* shoot (p at the most 0.05) (Fig. 3 and 4).

**Wheat coleoptile bioassay:** The bioassay of ether extract of shoot revealed significant inhibition of coleoptile Growth at Rf values of 0.7-0.8, 0.8-0.9 and 0.9-1 (Fig. 5). Growth promoters were indicated at Rf values 0.2-0.3 and 0.3-0.4.

**Chromatographic study:** When the chromatograms were sprayed with vanillin-HCl or ferric chloride they showed three spots which were identified as p-hydroxybenzoic, vanillic and salicylic acids. (Table 1).

## Discussion

This study investigates the allelopathic potential of *A. mexicana* and the nature of its phytotoxins. Aqueous leachates of shoot inhibited the germination of pearl-millet, wheat, turnip, carrot, mustard and corn. These results corroborate the findings of Leela (1981) and Sharma and Nathawat (1987) who obtained germination inhibition of some crop seeds by the aqueous extract of *A. mexicana*. Aqueous extract of a number of other allelopathic weeds are known to have inhibitory effects on crop seed germination (Shaukat *et al.*, 1985; Putnum and Weston, 1986; Narwal, 1994; Casado, 1995; Jimenez-Osornio *et al.*, 1996; Lydon *et al.*, 1997; Rajbanshi and Inubushi, 1997; Peres *et al.*, 1998; Ito *et al.*, 1998; Humaida and Warrag, 1998). The effects of phytotoxins from *A. mexicana* shoot appears to be species specific. Because all species were not equally susceptible to the extract. The species specificity May be due to physiological and morphological differences among the species. Such Species specificity of phytooxins has also been demonstrated by Naqvi and Muller (1975), Friedman *et al.* (1977), Shaukat *et al.* (1983), Kozukue *et al.* (1998) and Makarova *et al.* (1998).

Seed germination was reduced in soils incorporated with

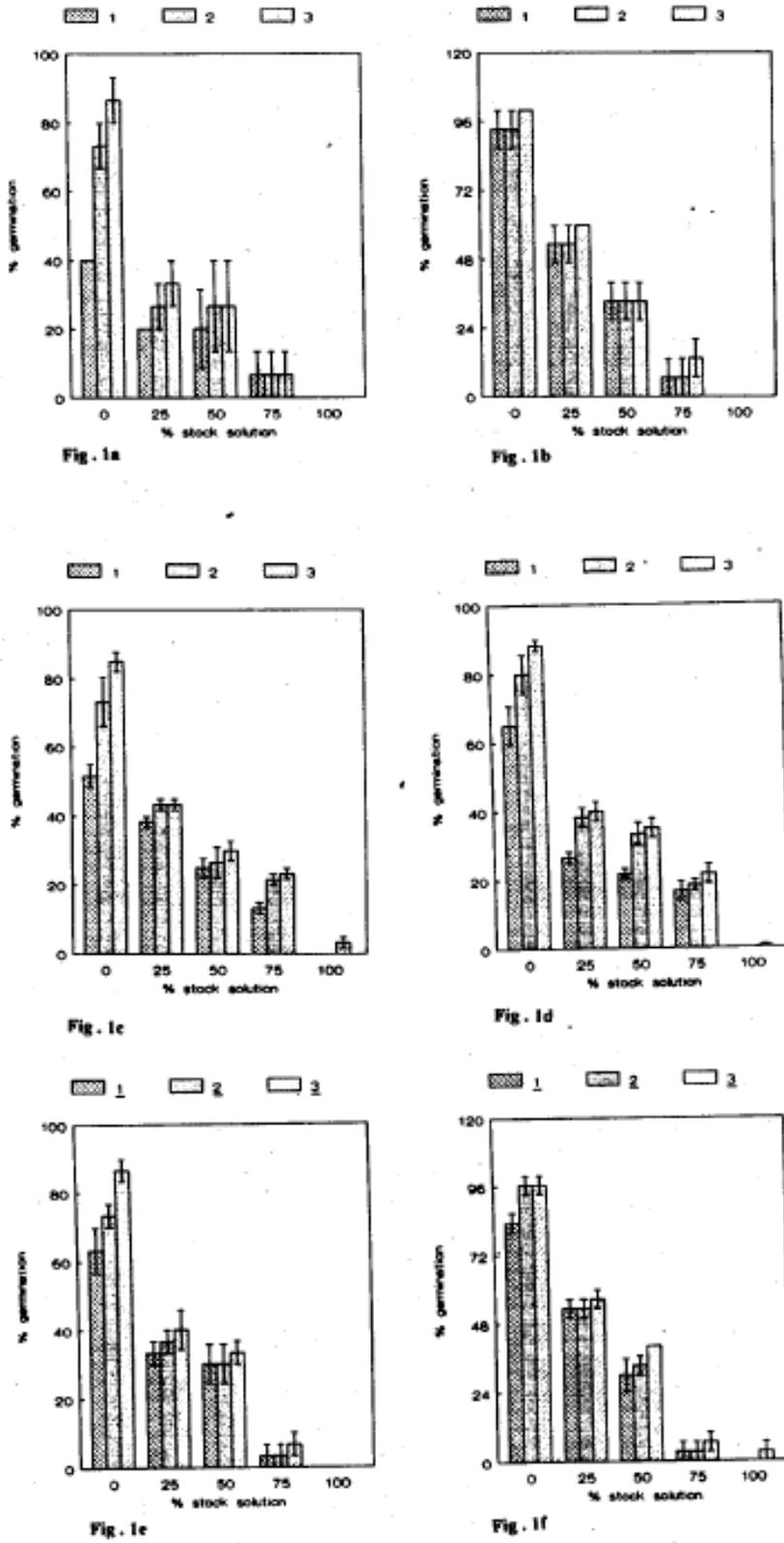


Fig. 1: Effect of shoot extract of *Argemone mexicana* on germination of test species. Pearl-millet - Wheat - Turnip - Carrot - Mustard - Corn

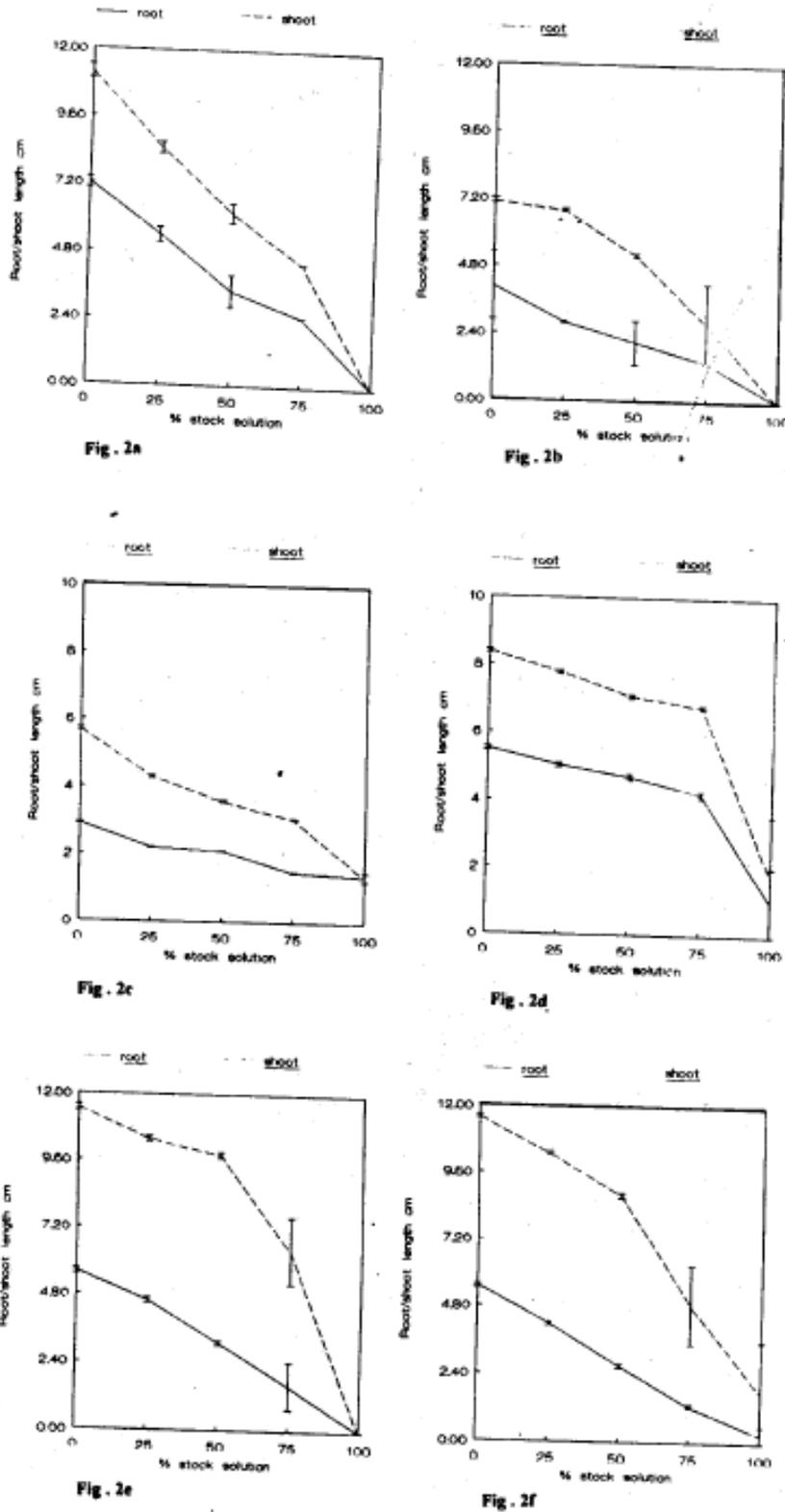


Fig. 2: Effect of shoot wxtract of *Argemone mexicana* on seedling growth of test species. Pearl-millet - Wheat - Turnip - Carrot - Mustard - Corn

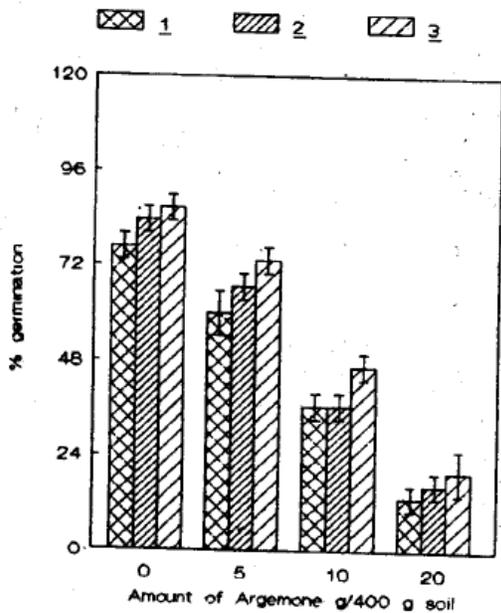


Fig. 3: Phytotoxic effect of Argemone mexicana on germination of pearl-millet.

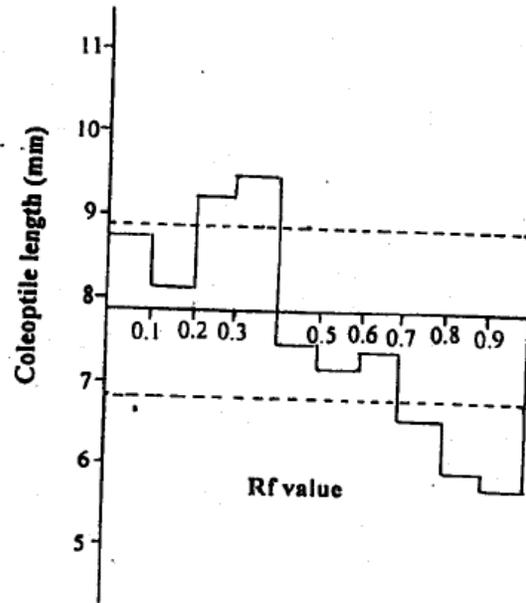


Fig.5: Histogram of ether fraction of shoot extract of Argemone mexicana. Dotted lines represent 95% confidence interval of control.

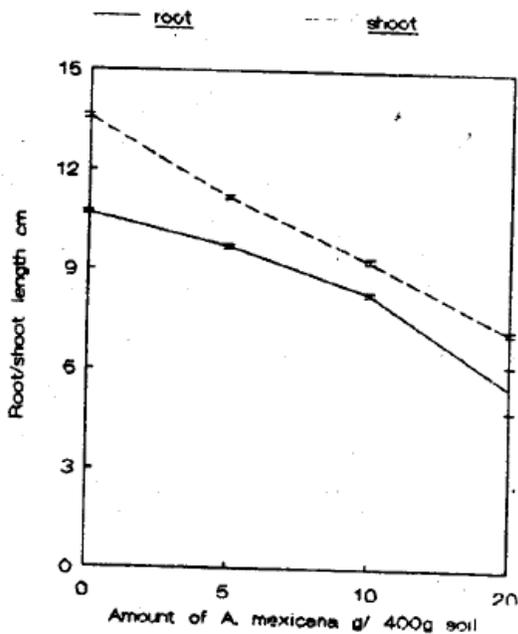


Fig. 4: Phytotoxic effect of Argemone mexicana on seedling growth of pearl-millet.

decaying *A. mexicana* shoot. Similarly, Datta and Sinha-Roy (1975) obtained inhibitory effect of decaying *Croton bonplandianum* on crop seed germination and Shaukat *et al.* (1985) found suppressive effect of decaying *Citrullus colocynthes* on germination of pearl-millet. It is possible that phytotoxins released from *A. mexicana* may accumulate in soil in biologically significant 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. mexicana* revealed three inhibitory zones. These zones were identified by paper chromatography as p-hydroxybenzoic acid, vanillic and salicylic acid. Phytotoxic chemicals other than phenolic compounds might be present, but their analysis was not attempted. The toxic nature of vanillic and p-hydroxybenzoic acids to growth at low concentrations has been reported by Janovicek *et al.* (1997). It is concluded from our results that *A. mexicana* 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 obtaining better crop yields.

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