Pak.J.Bot., 25(1): 21-28, 1993.

EFFECT OF AQUEOUS EXTRACTS AND LEACHATES OF ANTHERS OF *CERATONIA SILIQUA* L., ON THE INHIBITION OF LETTUCE SEED GERMINATION

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Abstract

Aqueous extracts and leachates of young, mature, and senesced anthers of *Ceratonia siliqua* were tested for their inhibition of lettuce seed germination. Only senesced anthers was found to contain substantial amounts of germination inhibitors. Isolation of the inhibitors from the anthers revealed the inhibitors to be soluble in water, methanol, ethanol, ethyl acetate and was acidic in nature. Kinetin and GA_3 were found to counteract the inhibition of the aqueous extract and leachates of the senesced anthers. Little loss in the inhibitory activity of the aqueous extract occured even after subjecting the anthers to the highest soil temperature of 80°C for 60 minutes, which indicated that the inhibitors were not proteinaceous.

Introduction

Secondary plant products, distributed throughout the plant kingdom may have several ecological roles. Some may act as allelopathic agents helping plant defence against invading insects, herbivores and other neighbouring plants (Lovett, 1982; Rice, 1984). A number of secondary metabolites with biological activity, localized in different parts of the plant are secreted from specialized structures such as trichomes (Horsley, 1977; Sterling *et al.*, 1987; Khan & Jahan, 1988, Kuti *et al.* ,1990; Heisey, 1990). Chemical analysis of leaves and leachates of *Eucalyptus globulus* has revealed a large number of water soluble compounds of known phytotoxic potential (Hillis, 1966a,b; Del Moral & Muller,1969; Molina *et al.*,1991). Pollens have also been reported to exibit allelopathy in nature (Char; 1977; Kanchan & Jayachandra, 1980). Malic & Ahluwalia (1985) have studied the growth regulator contents in immature and mature anthers of *Kigelia pinnata* where IAA, ABA and Kinin increased several times in mature as compared to young anthers. The present study was therefore, undertaken to evaluate the influence of water soluble substances from the anthers of *Ceratonia siliqua* on the inhibition of lettuce seed germination.

Materials and Methods

Male flowers were collected from a single male plant of Carob (*Ceratonia siliqua*), growing in the nursery of the Botany Department, University of Karachi, Pakistan during December 1986. Anthers of uniform size from young (YF), mature (MF) and senescing (SF) flowers were used in the present study. Fifty anthers of young (YF), mature (MF) and senescing (SF) stages were spread on a 13x2 cm strip of Whatman filter paper No.1 and slipped into a test tube. After adding 0.8 ml of water, the anthers were incubated at 20 ±1°C and discarded after 24h of leaching. Fifty seeds of lettuce (*Lactuca sativa* cv. Grand Rapids) was spread on the wet filter paper and the germination recorded after 2,3 and 4 days of incubation in the dark at 20±1°C. The open end of the tube was tightly closed with a plastic sheet. Three replicates of each treatment were used.

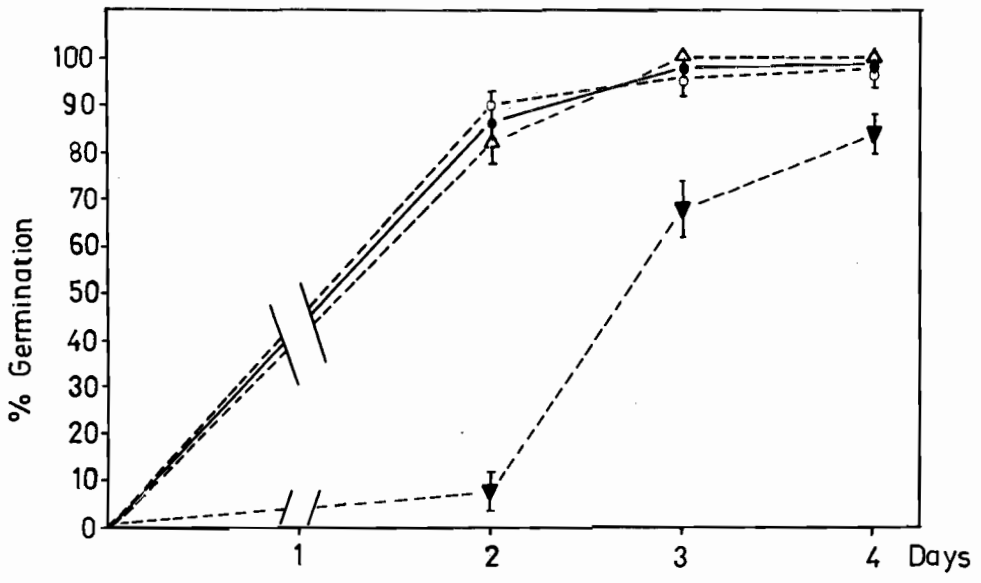

Fig.1. Germination of lettuce seeds in the presence of 50 *Ceratonia siliqua* anther leachates. Young (o), mature (Δ), and senesced (▼) anther leachates. Water control (●).

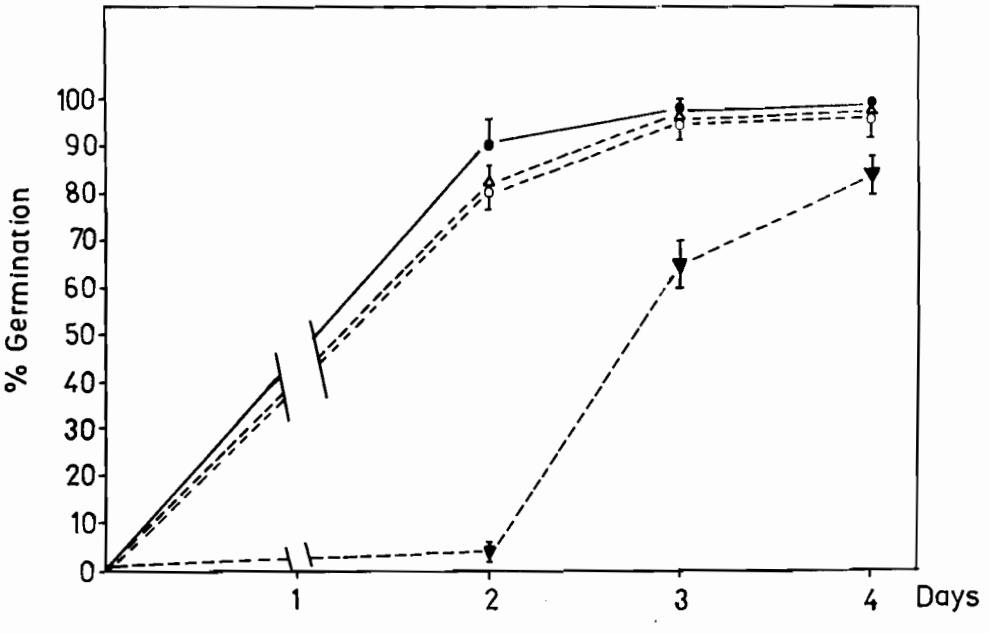

Fig.2. Effect of water soluble extract of young (o), mature (Δ), and senesced (▼) anthers of *Ceratonia. siliqua* on the germination of lettuce seeds. Water control (●).

In order to study the solubility of the germination inhibiting substance(s) from the anthers of *C. siliqua*, 200 senesced anthers were extracted with 5 ml of methanol, ethanol, ethyl acetate, chloroform and water. After 20 h of extraction at 20±1°C., 0.8 ml of all the extracts, except the water soluble one, which was used directly, were loaded on to a 13 x 2cm filter paper strip and dried using a hair drier. These strips transferred into test tubes, soaked in 0.8 ml of water in which 50 seeds of lettuce were spread. A water control was also included. The effect of growth hormones on the germination of lettuce seeds in the presence of water soluble anther leachates was also studied. Lettuce seeds were permeated for 60 min., at room temperature with 5 ml acetone solutions of Gibberellic acid (GA_3 10 mgl-1) and Kinetin (10 mgl-1) and dried as described by Khan (1977). Control seeds were similarly treated with acetone only. Fifty anthers were kept on a filter paper sheet (13x2 cm) in which 0.8 ml of water was added. After 24 h of incubation in the dark at 20±1°C, the anthers were removed, and 50 lettuce seeds with or without hormone treatment were spread after which 0.1 ml of water was dispersed. Similarly, the effect of GA_3 and Kinetin on the germination of lettuce seeds in the presence of aqueous extracts of senesced anthers (200 anthers/5 ml) of *C. siliqua* was studied.

To study the effect of temperature on the allelopathic potential of the Carob anthers, Petri dishes 6cm diam., containing 20g sandy loam were incubated for 60 min., at 25, 40, 60 and 80°C, and 50 senesced anthers placed on the surface of the soil. Three replicates were used for each treatment. After a further incubation of 60 min., the dishes were allowed to cool and soil was adjusted to water holding capacity. After two days of leaching from anthers at 20 ±1°C, 50 lettuce seeds were planted in each dish. Germination percentage was measured after 2,3 and 4 days. Total phenols of the incubated anthers was also estimated by the method of Swain & Hillis (1959) after extracting the anthers with water for 20 h at 20±1 °C (200 anthers/5ml water).

Results and Discussion

Germination of lettuce seeds after 2,3 and 4 days in the dark at 20±1°C. was reduced by leachates of 50 senesced anthers of *C. siliqua* (Fig.1). The leachates from young and mature anthers did not reduce the germination. When the anthers were kept in distilled water for 20h at 20±1°C, the anther extract showed similar effects (Fig.2).

Preplant permeation of lettuce seeds via acetone with GA_3 and Kinetin improved the germination inhibited by the leachates of the Carob anthers (Table 1). The magnitude of the hormone induced reversal effect decreased with the increase in the number of anthers from 1-7 (Table 1). When water soluble anther extract (200 anthers/5ml water) was used, Kinetin and GA_3 again substantially reversed the germination inhibition induced by the aqueous anther extract (Table 2).

The effect of different soil temperatures on the allelopathic activity of Carob anthers was not lost even after subjecting the anthers to the highest soil temperature of 80°C for 60 min. (Table 3). Similarly increase in soil temperature from 25 to 80 °C did not alter the level of total phenols of the anthers (Table 3). Since all the extracts used were aqueous, which indicated that the inhibitor(s) of seed germination were water soluble. Anthers extracted with water, methanol, ethanol, ethyl acetate and chloroform seperately indicated that the inhibitor(s) are soluble in water, methanol and ethanol (Table 4) . When the aqueous anther extract was adjusted to pH 3 and 9 and extracted with ethyl acetate, 75% of the germination inhibiting activity was found in the acidic fraction.

Table 1. Effect of number of *Ceratoria siliqua* anthers (1-7) on the germination of GA_3 and kinetin (10 mg.l^{-1}) treated lettuce seeds after 48 h of incubation at 20±1°C.

Seed Treatment	Number of Anthers														
	0			1			3			5			7		
	% Ger.	% Inh*	% Rev**	% Ger.	% Inh*	% Rev**	% Ger.	% Inh*	% Rev**	% Ger.	% Inh*	% Rev**	% Ger.	% Inh*	% Rev**
Water	91.3 ±3.75	---	---	92.5 ±4.33	---	---	76.3 ±2.39	16.43	---	53.0 ±2.38	41.95	---	41.30 ±5.15	54.76	---
GA_3 (10 mg. l^{-1})	98.8 ±1.25	---	---	93.8 ±2.39	---	---	88.8 ±5.15	2.74	83.33	75.0 ±2.04	17.85	57.44	51.25 ±7.18	43.86	19.9
Kinetin (10 mg. l^{-1}	97.5 ±1.44	---	---	91.3 ±2.39	---	---	88.8 ±3.14	2.74	83.33	72.5 ±4.33	20.59	50.91	56.25 ±2.39	38.38	29.9

$$\% \text{ Inhibition} = \frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

$$^{**}\% \text{ Reversal} = \frac{\text{(Inhibitor + Reverting agent) - Inhibitor}}{\text{Control - Inhibitor}} \times 100$$

Table 2. Effect of GA_3 and kinetin on the germination of lettuce seeds in the presence of aqueous extract of *C. siliqua* L. anthers.

Seed Treatment	24 h.			48 h.			72 h			96 h		
	% Ger.	% Inh*.	% Rev**.	% Ger.	% Inh*	% Rev**.	% Ger.	% Inh*.	% Rev**.	% Ger.	% Inh*.	% Rev**.
Water Control	70.0 ±3.46	---	---	89.33 ±2.66	---	---	94.0 ±1.15	---	---	96.66 ±0.66	---	---
Aqueous Anther extract (200 anthers/ 5 ml water)	2.66 ±0.66	96.2	---	26.0 ±5.29	70.89	---	35.0 ±2.88	62.76	---	47.3 ±9.40	51.06	---
Aqueous Anther extract + GA_3 (50 $mg.l^{-1}$)	0.66 ±0.06	99.04	---	40.0 ±5.03	55.22	22.10	82.66 ±1.33	12.06	80.78	83.33 ±2.40	13.79	73.0
Aqueous Anther extract + kinetin (50 $mg.l^{-1}$	8.66 ±0.66	87.63	8.91	62.6 ±1.67	29.85	57.88	86.66 ±1.66	7.80	87.56	89.33 ±1.76	7.58	85.15

*,** As in Table 1.

Table 3. Effect of temperatures on the seed germination inhibiting activity and total phenols of carob anthers in sandy loam soil.

Temp. (oC)	% Germination after 2 days	3 days	4 days	Total Phenols (ug Ferulic acid equivalent)
20	84.3 ±3.46	86.0 ±3.25	93.3 ±1.66	---
25	64.0 ±3.05	68.3 ±1.66	91.3 ±2.36	130 ±1.33
40	69.4 ±5.29	70.0 ±4.61	90.7 ±2.66	118 ±2.88
60	71.2 ±1.15	73.7 ±1.85	92.0 ±2.32	120 ±1.66
80	64.3 ±11.34	73.3 ±10.34	86.7 ±8.08	129 ±2.68

The present study demonstrates the allelopathic potential of the anthers of *C.siliqua*. The fact that the inhibitor(s) of seed germination was water soluble is particularly significant, since the survival of many plant species depends mainly on the presence of water soluble germination inhibitors (Went, 1948; Went & Westergaard, 1949; Koller, 1955). Senesced anthers of *Bombax ceiba* has been reported to exibit allelopathy under laboratory conditions (Khan & Jahan, 1988).

Table 4. Relative solubility of the carob anther inhibitors in different solvents on lettuce seed germination.

Solvents	% Germination after 2 days	3 days
Control (Water	64.53 ±6.23	90.34 ±3.43
Water extract	10.45 ±4.15	53.34 ±3.53
Methanolic extract	24.54 ±4.62	50.66 ±6.66
Ethanolic extract	36.66 ±4.05	68.67 ±1.65
Ethyl acetate extract	50.83 ±4.32	81.27 ±4.41
Chloroform extract	68.66 ±3.66	83.53 ±6.12

The inhibition of lettuce seed germination by anthers of *C.siliqua* was partly counteracted by 10mgl-1 GA_3 and Kinetin. An increasing number of *C.siliqua* anthers (1-7 anthers) progressively increased the inhibition of GA_3 and Kinetin induced germination of lettuce seeds. GA_3 and Kinetin at 50 mgl-1 was found to neutralize substantially the germination inhibition induced by the aqueous anther extract (Table 2).

The discharge of allelopathic substance(s) to the environment by leaching of water soluble substance(s) from the senesced dry anthers of *C.siliqua*, while still attached to the plant, in response to the action of rain or dew might influence the vegetation under this tree. The dry anthers after falling on the ground may be subjected to the high summer temperatures of the Pakistani soil and their capacity to inhibit seed germination might change. The germination inhibiting potential of the anthers was not lost even after subjecting the anthers to the highest temperature of 80°C, which indicates that the inhibitor(s) were not proteinaceous. The level of total phenols in the leachates of the anthers kept at varying soil temperatures indicated no change in its quantity. The allelochemicals released by plants after reaching the soil are subjected to the processes not fully known and may result in either antagonistic or synergistic effects (Rice, 1984). Malik Ahluwalia (1985) have analysed the ABA content in immature and mature anthers of *Kigelia pinnata* and found that ABA increased during anther maturation. The inhibitory effect of ABA on lettuce seed germination is reversed by Kinetin (Khan, 1968) as also found in the present study. This indicates the possibility that apart from phenols, ABA may also be present in the anthers of *C. siliqua*.

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(Received for Publication 21 November 1990)