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ALLELOPATHIC POTENTIAL OF SENESCED ANTHERS OF *BOMBAX CEIBA* L., INHIBITING GERMINATION AND GROWTH OF LETTUCE SEEDS

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Abstract

Aqueous extracts and leachates from senesced anthers of *Bombax ceiba* L., was tested for its effect on seed germination and seedling growth of lettuce. The inhibitors isolated from anthers were water soluble and its germination inhibiting activity increased with rise in temperature from 20 ± 1 to 26 ± 2°C. Paper chromatographic separation of acidic ethyl acetate fraction of the aqueous extract revealed the presence of three inhibiting zones, one of which was phenolic in nature. Phytotoxicity of the aqueous anther extract on the inhibition of germination and growth of lettuce seeds was overcome by Kinetin, GA_3 and red light treatments.

Introduction

Plants are known to exhibit allelopathy by releasing water soluble phytotoxins from leaves, stems, roots, rhizomes, flowers, fruits, seeds and glandular trichomes (Horsley, 1977; Sterling, 1987). Besides pollens have also been found to exibit allelopathy in nature (Char, 1977; Kachan & Jayachandra, 1980). Malik & Ahluwalia (1985) have studied the growth regulator contents in immature and mature anthers of *Kigelia pinnata* where IAA, ABA and Kinin showed an increase in anthers during maturation. Of these ABA increased several times in the mature anthers as compared to immature ones. Due to the presence of high level of ABA in mature anthers, we suspected anthers to exibit allelopathy. The present study was, therefore, undertaken to evaluate the influence of water-soluble substances from the senesced anthers of *Bombax ceiba* on the germination and growth of lettuce seeds under laboratory conditions.

Materials and Methods

Senesced flowers from a single plant of *Bombax ceiba* L., were collected in February 1987 from which anthers of uniform size were used. Water soluble extract of the anthers was prepared by suspending 15 anthers in 10 ml of water for 24 h at 15°C in the dark. Fifty seeds of lettuce (*Lactuca sativa* cv., Grand Rapids) interspersed with 15 anthers of *B. ceiba* were placed in two rows on a 13 x 2 cm strip of Whatman No. 1 filter paper and placed in a 1.5 cm diameter test tube containing 0.8 ml of distilled water (Fig. 1). In another set water-soluble anther extract was used and the tube was tightly closed with a plastic sheet (Fig.1) and kept in dark at 26 ± 2°C.There were three replicates of each treatment.

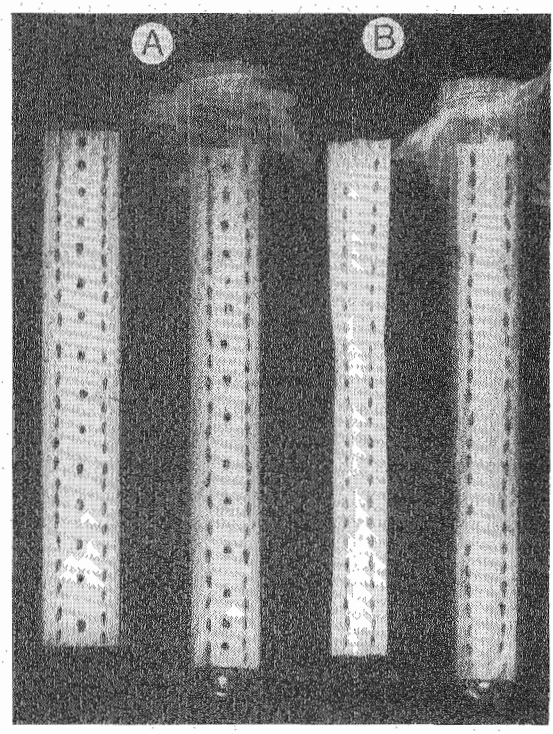

Fig. 1. Germination and growth of lettuce seeds in the presence (A) and absence (B) of *Bombax ceiba* anthers in a test tube.

To study the effect of growth hormones, lettuce seeds permeated with 5 ml acetone solution of GA_3 or Kinetin (100 and 50 mg l^{-1}) for 60 min at 20 ± 1°C and dried as described by Khan (1977) was kept in the presence of 0.8 ml water soluble anther extract (pH 6.5). Control seeds were similarly treated with acetone only. Effect of red light was studied by wrapping the test tubes with red plastic sheets and exposing the tubes for 2 h under white fluorescent light. To study the quality of different germination inhibiting substance(s), 150 anthers were mixed with 50 ml of distilled water at 15°C for 24 h in the dark and then filtered. The pH of the filterate being 6.5 was adjusted to pH 2.5 with 0.5 N HC1 and extracted 3 times using 50 ml of ethyl acetate each time. The ethyl acetate fractions were pooled, evaporated to dryness at 45°C and redissolved in 0.5 ml of ethanol. Whatman filter paper No. 1, prewashed with ethanol was loaded with the anther extract and ascending chromatography was developed in n-butanol: acetic acid: water (63:10:27 v/v). After drying the chromatogram was divided into ten equal halves and lettuce seeds germinated on these strips. These strips (Rf 0.0-1.0) were also extracted with 50% ethanol and used for the estimation of total phenols using the method of Swain & Hills (1959).

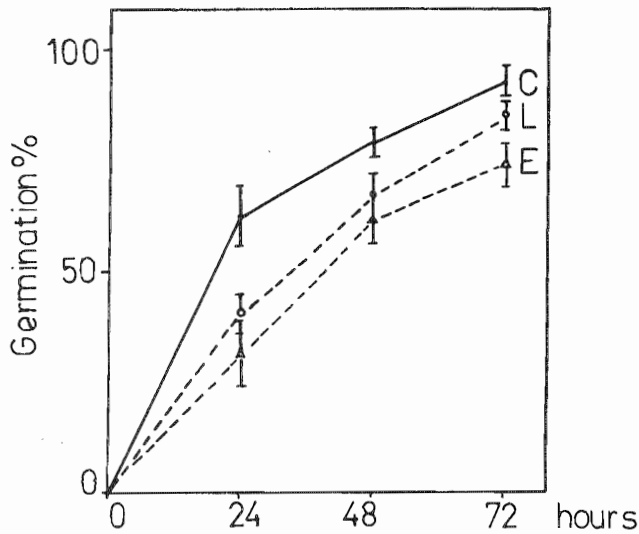

Fig. 2. Effect of leachates (L) and water-soluble extract (E) of senesced anthers of *Bombax ceiba* on the germination of lettuce seeds. C; Control. Vertical lines represent standard errors.

Results and Discussion

Anther leachates (L) and water-soluble anther extract (E) reduced the germination of lettuce seeds in the dark at 26 ± 2°C (Fig. 2). Paper chromatographic separation of acidic ethyl acetate fraction of aqueous anther extract showed the presence of 3 inhibiting zones for seed germination (Fig. 3A). Total phenols extracted from the paper revealed the presence of phenol from Rf 0.1 to 0.65 (Fig. 3B). Thus the germination inhibitor from Rf 0.25 to 0.6 appears to be phenolic in nature and the remaining two inhibitors were non phenolic. A number of phenolic growth inhibitors in the male flowers of *Cucurbita pepo* has been reported by Itokawa *et al.*, (1982).

Temperature has been shown to regulate secondary product formation in several plant species, for example, Hanson *et al.*, (1983) found increase in the content of the indole alkaloid, gramine, in barley when plants were transferred from 21/16°C to a 30/25°C environment. Similarly Sterling *et al.*, (1987) also found the exudates from velvetleaf glandular trichomes cultured at 24 and 36°C to be twice as toxic as the exudate collected from plants grown at 16°C. In the present study the effect of temperature on the allelopathic activity of aqueous anther extract showed that the inhibitory activity increased substantially with an increase in incubation temperature from 20 ± 1°C to 26 ± 2°C (Fig. 4). This may be attributed to increased uptake of allelochemics such as phenols at higher temperatures as suggested by Khan (1982). Similar observations were also made by Glass

Table 1. Effect of GA_3, Kinetin and red light on the germination of lettuce seeds in the presence of water soluble extract of *Bombax ceiba* anthers.

Treatment	24 h			48 h			72 h		
	% Ger.	% Inb.*	% Rev.**	% Ger.	% Inh.*	% Rev.**	% Ger.	% Inh.*	% Rev.**
Water Control	74.6 ± 3.2	—	—	95.3 ± 1.8	—	—	96.9 ± 6.3	—	—
Aqueous anther extracts (150 anthers/10 ml H_2O)	34.6 ± 0.9	53.6	—	62.6 ± 2.9	34.3	—	75.3 ± 3.8	22.0	—
Aqueous anther extracts + GA_3 50 mg l^{-1}	28.0 ± 4.4	62.5	—	75.3 ± 2.5	21.0	38.8	88.6 ± 5.1	8.3	62.44
Aqueous anther extracts + GA_3 100 mg l^{-1}	36.00 ± 2.1	51.7	3.5	85.3 ± 3.6	10.5	69.4	90.6 ± 3.2	6.2	71.83
Aqueous anther extracts + Kinetin, 50mg l^{-1}	41.3 ± 4.1	44.6	16.7	80.6 ± 3.5	15.4	55.0	88.0 ± 1.9	8.9	59.62
Aqueous anther extracts + Kinetin, 100 mg l^{-1}	38.0 ± 1.9	49.1	8.5	80.0 ± 1.9	16.1	53.2	93.3 ± 2.8	3.4	84.51
Aqueous anther extracts + Red Light	40.0 ± 1.9	46.4	13.5	88.0 ± 3.3	7.7	77.6	94.0 ± 2.1	2.7	87.70

* % Inhibition = Control – Treatment x 100/Control

** % Reversal = (Inhibitor + Rev. agent) – inhibitor x 100/Control.

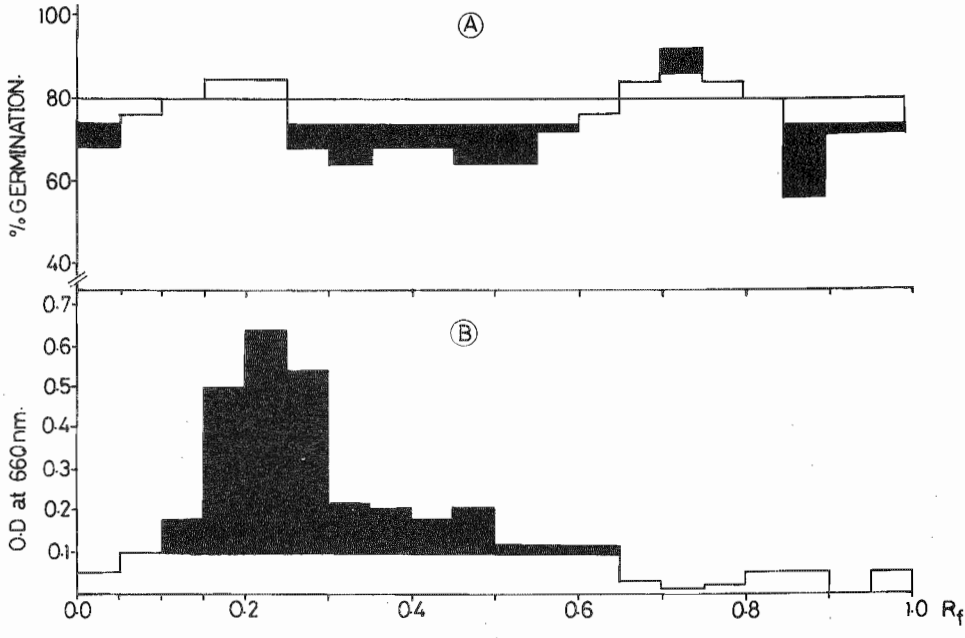

Fig. 3. Histogram of acidic fraction of water-soluble extract of the senesced anthers of *Bombax ceiba* chromatographed on Whatman paper No.1 and subjected to lettuce seed germination test (A) or to the total phenol estimation expressed as O.D. at 660 nm (B). Dark areas are significantly different (P 0.05) from the control.

& Bohm (1971) who found that the uptake of allelopathic substances such as phenolic acids by barley roots increased significantly by increasing the incubation temperature from 2°C to 28°C.

The permeation of growth hormones into lettuce seeds reduced the germination inhibiting power of the anther extract (Table 1). Gibberellic acid and Kinetin were found to reverse the germination inhibition of the inhibitor by up to 80% after 72 h of germination. Similarly, red light was also found to reverse the germination inhibition (88%) induced by the anther extract (Table 1). The inhibitory effect of abscissic acid (ABA) on lettuce seed germination is known to be reversed by Kinetin (Khan, 1968) and the effect of some growth retardants are also reported to be partly neutralized by Kinetin, GA_3 and red light as observed in the present study. This indicates the possibility of ABA as one of the inhibitory substances present in the senesced anthers of *B. ceiba*.

Reduction of root growth by 74.3% and shoot growth by 26.7% was observed in lettuce seedlings treated with water soluble anther extract (Table 2). GA_3, Kinetin and red light treatment not only neutralized the effect of anther inhibitor but also promoted the growth of the shoot substantially as compared to untreated control.

Table 2. Effect of GA_3 Kinetin and red light on the growth of lettuce seedlings after 4 days in the presence of water soluble extract of *Bombax ceiba*.

Treatment	Growth (mm) after 4 days					
	Lenght of shoot (mm)	% Inhibition*	% Reversal**	Length of Root (mm)	% Inhibition*	% Reversal**
Water control	19.7 ± 1.9	—	—	36.0 ± 2.8	—	—
Aqueous anther extracts (150 anthers/10 ml H_2O)	14.5 ± 2.3	26.7	—	9.3 ± 1.9	74.2	—
Aqueous anther extracts + GA_3 50 mg l^{-1}	19.5 ± 1.7	1.0	96.2	8.2 ± 0.5	77.2	—
Aqueous anther extracts + GA_3 100 mg l^{-1}	23.5 ± 2.8	—	173.1	8.7 ± 0.8	75.8	—
Aqueous anther extracts + Kinetin, 50 mg l^{-1}	22.5 ± 3.2	—	153.8	13.3 ± 2.5	63.0	15.0
Aqueous anther extracts + Kinetin, 100 mg l^{-1}	18.1 ± 1.9	7.7	71.1	10.6 ± 1.9	70.5	4.9
Aqueous anther extracts + Red Light	22.3 ± 2.2	—	150.0	12.6 ± 2.7	64.9	12.4

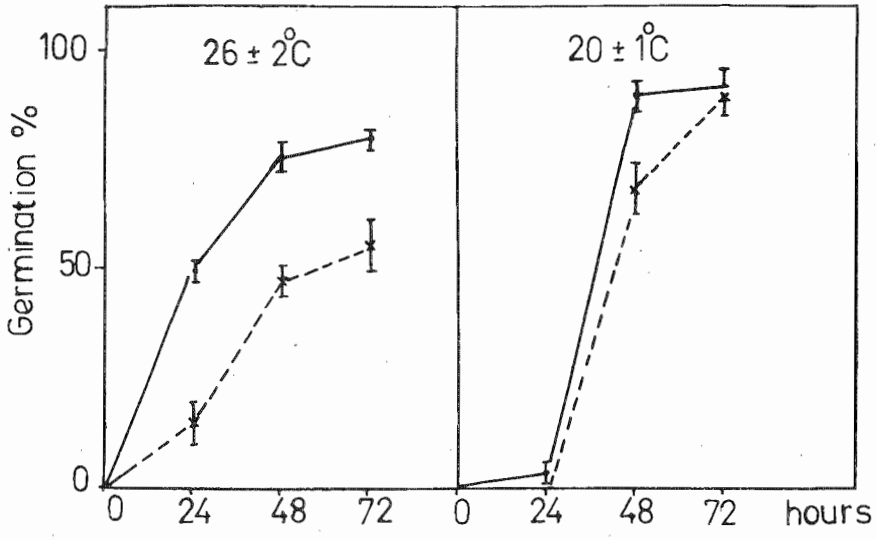

Fig. 4. Effect of temperature on the inhibition of lettuce seed germination induced by the aqueous extract of *Bombax ceiba* anthers. Vertical lines represent standard errors.

The present study would demonstrate the allelopathic potentiality of senesced anthers of *B. ceiba* in test tube bioassay. Inhibitors of seed germination and growth was found to be water soluble. This is particularly significant since the survival of many plant species depend mainly on the prsence of water soluble phytotoxins for germination and growth (Went, 1948; Went & Westergaard, 1949; Koller, 1955 a&b ; Wilson & Rice, 1968; Rice, 1984) and heavy rain is required to leach out these inhibitors before germination and growth may occur under natural environment. Although the presence of phytotoxic substances in *B. ceiba* anthers was demonstrated under laboratory conditions, their ecological role, if any, is still to be ascertained.

References

Char, M.B.S. 1977. Pollen allelopathy. *Naturewissenshaften*, 64: 489-490.

Glass, A.D.M. and B.A. Bohm. 1971. The uptake of simple phenols by barley roots. *Planta* (Berlin), 100: 93-105.

Hanson, A.D., K.M. Ditsz., G.W. Singletary and T.J. Leland. 1983. Gramine accumulation in leaves of barley grown under high temperature stress. *Plant Physiol.*, 71: 896 - 904.

Horsley, S.B. 1977. Allelopathic interference among plants. II. Physiological modes of action. *Proc. 4th North American Forest Biology Workshop*. (Eds.) H.E. Wilcox and A. Hamer. Syracuse University Press, Syracuse, N.Y. pp. 93-136.

Itokawa, H., Y. Oshida, A. Ikuta., H. Inatomi and T. Adachi. 1982. Phenolic plant growth inhibitors from the flowers of *Cucurbita pepo*. *Phytochemistry*, 21: 1935-1937.

Kanchan. S. and Jayachandra. 1980. Pollen allelopathy: A new phenomenon. *New Phytol.*, 84: 739-746.

Khan, A.A. 1977. Preconditioning, germination and performance of seeds. In: *The Physiology and Biochemistry of seed dormancy and germination*. Elevier/North Holand Biochemical Press, Amsterdam., pp. 283-316.

Khan, A.A. 1968. Inhibition of Gibberellic acid induced germination by Abscissic acid and reversal by cytokinins. *Plant Physiol.*, 43: 1463-1465.

Khan, M.I. 1982. Allelopathic potential of dry fruits of *Washingtonia filifera:* Inhibition of seed germination. *Physiol. Plant.*, 54: 323-328.

Koller, D. 1955a. Germination regulating mechanism in some desert seeds. *I. Bull. Res. Cons. Isr.*, 4: 379-381.

Koller, D. 1955b. Germination regulating mechanism in some desert seeds. II. *Zygophyllum dumosum* Boiss., *Bull. Res. Couns. Isr.*, 4: 381-387.

Malik, C.P. and K. Ahluwalia. 1985. Growth regulator contents in maturing pollen grains of *Kigelia pinnata*. In: *Recent Advances in Pollen Research* (Ed.) T.M. Varghese. Allied Publ. Pvt. Ltd., New Delhi. pp. 43-45.

Rice, E.L. 1984. *Allelopathy*. 2nd ed. Academic Press Orlando, FL.

Sterling, T.M., R.L. Houtz and A.R. Putnam. 1987. Phytotoxic exudates from Velvetleaf (*Abutilon theophrasti*) glandular trichomes. *Amer. J. Bot.*, 74: 543-550.

Swain, T. and W.E. Hillis. 1959. Phenolic constituents of *Prunus domestica*. *J. Sci. Fd. Agric.*, 10: 63-68.

Went, F.W. 1948. Ecology of desert plants. I. Observations on the germination in the Joshua Tree National-Monument, California. *Ecology*, 29: 242-253.

Went, F.W. and M. Westergaard. 1949. Ecology of desert plants. III. Development of plants in the Death Valley national Monument, California. *Ecology*, 30: 26-38.

Wilson, R.E. and E.L. Rice. 1968. Allelopathy as expressed by *Helianthus annus* and its role in old field succession. *Bull. Torrey Bot. Club*, 95: 432-448.

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