Pak.J.Bot., 30(2): 245-252, 1998.

ALLELOPATHIC POTENTIAL OF DRY FRUITS OF *WASHINGTONIA FILIFERA* (L. LINDEN) H. WENDL III. INHIBITORS OF GERMINATION AND GROWTH

M. ISHAQ KHAN

Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Germination and growth inhibiting activity of water soluble extract of *Washingtonia filifera* fruits was investigated. Paper chromatographic separation of the extract revealed the presence of two inhibiting zones of germination and growth. The fast moving beta - inhibitor was further separated by TLC into four components, one of which appears to be abscissic acid (ABA) and the rest phenolic substances. Gibberellic acid (GA_3) was found to neutralize the germination and growth inhibiting ability of the inhibitor beta-complex. Besides these, a slow moving inhibitory zone of germination and growth was due to the presence of phenolic substance.

Introduction

Secondary plant metabolites are distributed throughout the plant kingdom and some have been shown to be active allelopathic agents which help the plant defend against insects, herbivores and other plants (Lovett,1982; Rice,1984). It has been established that different parts of some species of higher plants produce chemical compounds which are toxic to the growth of other plant species (Bonner, 1950, Whittaker & Feeny, 1971). Fruits, seeds and flowers including anthers and pollens are known to exhibit allelopathy in nature (Char, 1977; Rice, 1984; Khan & Jehan, 1988). The aqueous extracts from the dry fruits of *Washingtonia filifera* has shown its allelopathic potentiality against a number of test plants under laboratory conditions (Khan, 1982; Khan, 1982 a). The present report describes the chemical and biological nature of the water-soluble inhibitors of germination and growth from the dry fruits of *Washingtonia filifera*

Materials and Methods

Mature fruits of *Washingtonia filifera* (L. Linden) H.Wendle were collected and stored for use as described earlier (Khan,1982). Fruits were surface sterilized for 5 min., with 0.1 % $HgCl_2$ solution and washed several times with sterile distilled water. Ten grams of fruits were soaked, with occasional shaking, either in 20 ml of sterile distilled water or methanol at 15° C in dark. Extract was filtered and adjusted to 300, 500 and 700 mg fruit dry weight equivalent. These extracts were applied to two sheets of Whatman filter paper No.3 in 9 cm Petri dishes and evaporated to dryness using a hair drier. Five ml of distilled water was added to all the dishes, including a water control, and 100 seeds of lettuce (*Lactuca sativa* cv. Grand Rapids) or 25 seeds of

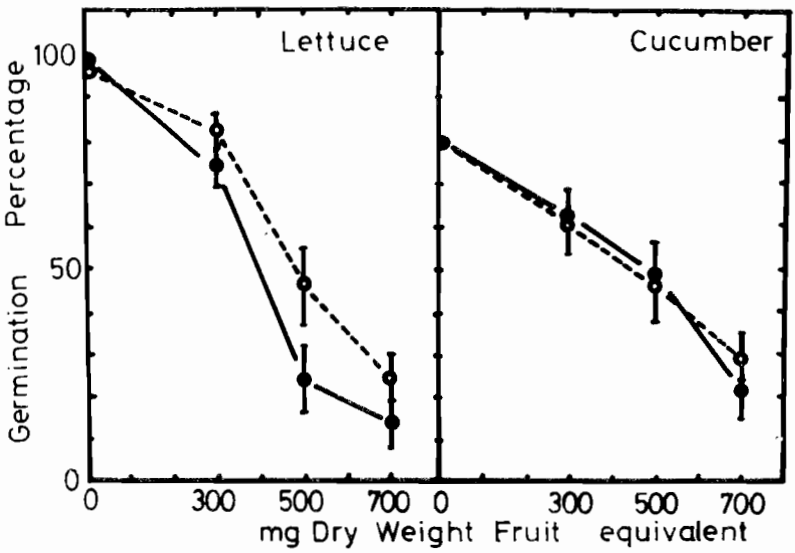

Fig.1. Effect of water-soluble extract of *Washingtonia filifera* fruits on lettuce and cucumber seed germination. Amount of water soluble (*-----*) and methanol soluble (*-----*) extract equivalent to 300, 500 and 700 mg dry weight of the fruit was used for the germination test. The vertical bars give standard error of the mean.

Cucumber (*Cucumber sativum* L. cv. Amcogreen) were spread on the paper. Germination percentage was recorded after 72 h of incubation at 25 ± 1°C in the dark.

Both paper and thin layer chromatography were used to examine the water-soluble inhibitors (400 mg F. Wt equivalent) of germination and growth. Ascending paper chromatography was carried out on Whatman paper No.4 in isopropanol: ammonia: water (10:1:1 V/V). The chromatograms were equilibrated for 2 hours and then developed for 10 - 14 h at 20 ± 1 °C. Ascending thin layer chromatography was carried out on silica gel HF_{254} in n-butanol: n-propanol: ammonia: water (2:6:1:2 V/V). Lettuce seed germination and hypocotyl growth tests were carried out after dividing the paper into 10 equal portions (Rf 0.0-1.0).

Results and Discussion

Preliminary results revealed that about 500 mg dry weight equivalent extract of the palm fruit gave 50 % inhibition of lettuce seed germination and that the inhibitors were equally soluble both in water and methanol (Fig.1).

Paper chromatographic separation of the water-soluble extract of the palm fruit revealed two distinct inhibiting zones of seed germination at Rf 0.0 to 0.2 and 0.5 to 1.0 (Fig.2). Similarly 2 growth inhibiting zones with Rf 0.0 to 0.5 and 0.7 to 1.0 were also localized in the water-soluble fraction (Fig.3). The Rf value of fast moving zone of the inhibitor was found to correspond to inhibitor beta of Bennet-Clark & Kefford (1953). Inhibitor beta is known to occur widely in higher plants. Koves (1957), Varga

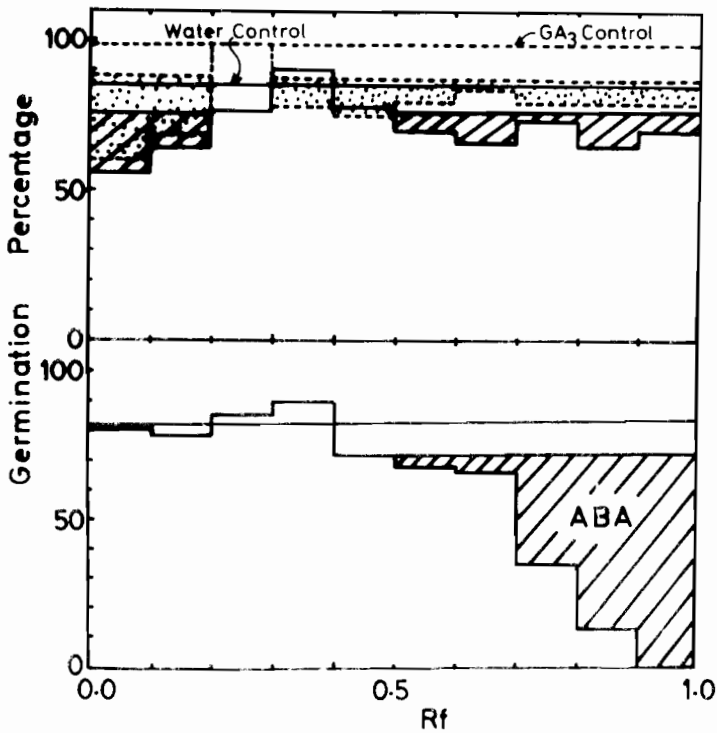

Fig.2. Histogram of water soluble extract of the fruits of *Washingtonia filifera* chromatographed on Whatman paper No.4 in isopropanol: ammonia: water (10:1:1 v /v). Paper chromatograms were divided into ten equal pieces, placed in 9 cm Petri dishes and moistened with 2 ml of either distilled water or 10-ppm Gibberellic acid solution and 100 lettuce seeds were sown. Abscisic acid was also separated on paper and subjected to lettuce seed germination test. Hatched areas are significantly different (p 0.05) from the control.

(1957) and Housley & Taylor (1958) have suggested that inhibitor beta consists of several phenolic acids while Milborow (1967) demonstrated that inhibitor beta is due to the presence of abscissic acid (ABA) only. In the present study therefore efforts were made to clarify whether the inhibitor from the fruits of *W. filifera* is solely ABA or is a mixture of several components. Paper chromatogram having Rf 0.7 to 1.0 was cut into smaller pieces and the beta inhibitor was eluted with 80 % ethanol and subjected to thin layer chromatography on silica gel HF_{254} in n-butanol : n-propanol : ammonia: water (2:6:1:2 V/V). The results showed the presence of 4 U.V. absorbing areas with one having Rf similar to that of the marker ABA (Fig.4). The other 3 U.V. absorbing spots reacted positively with Folin-phenol spray indicating their nature to be phenolic. Varga & Koves (1959) also found phenolic acids as germination and growth inhibitors in a number of dry fruits. Variation in growth response to phenolic allelopathic chemicals are known to exist within plants (Rice, 1984; Ray & Hastings, 1992) as is evident from the result presented in Fig.1.

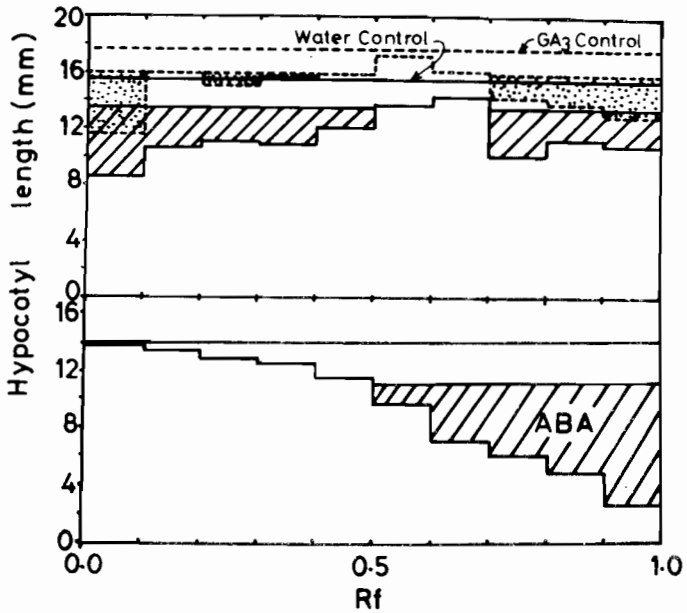

Fig.3. Histogram of water-soluble extract of the fruits of *Washingtonia filifera* chromatographed on Whatman filter paper No.4 and subjected to lettuce hypocotyle growth test. Details as mentioned in Fig.2.

ABA-induced inhibition of germination of lettuce seeds are known to be negated by gibberellic acid (GA_3) treatment at 20°C and 30°C (Robertson & Berrie, 1977). In the present study it was found that GA_3 (10 ppm) application to the fast moving chromatographic fraction of the inhibitor completely reversed the inhibitors of germination and growth (Figs.2 & 3). This further indicates that perhaps ABA is one of the components of beta inhibitor present in the fruits of *W. filifera*. Similar results were also obtained by Holst (1971) who studied the nature of the inhibitor beta from *Solanum tuberosum* where ABA and phenolic substances were found to be the major growth inhibiting component in the beta inhibitor. Besides these inhibiting substances, Holst (1971) also found many other phenolic components without having any growth inhibiting activity to be associated with the inhibitor beta complex.

The slow moving inhibiting zone from the paper chromatogram (Rf 0.0- 0.5) was also eluted with 80 % ethanol and chromatographed on Whatman No.1 filter paper employing n-butanol: acetic acid : water (6 : 1 : 2 V/V) as the ascending solvent. After drying, the paper was divided into 10 equal halves (Rf 0.0 -1.0) and extracted with 3 ml of 50 ml ethanol together with a blank. Total phenol estimation of these extracts was based on Folin-Ciocalteu method as modified by Swan & Hullis (1959). The phenol levels were expressed as O.D. units at 660 nm. The results indicated phenols to be present at higher concentration at Rf 0.0-0.1 than at 0.1 to 0.5 (Fig.5). Identification of the individual phenols from slow and fast moving inhibitory zones of the extract were not investigated.

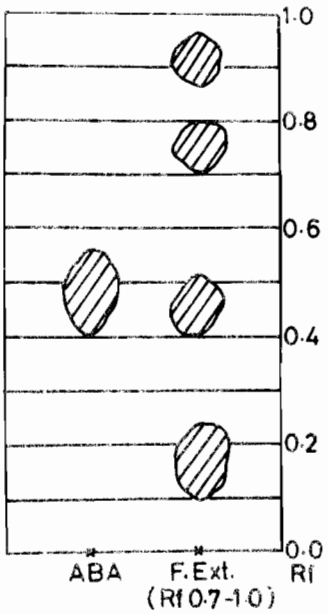

Fig.4. Inhibitor beta (Rf 0.7-1.0) from paper chromatogram separated on silica gel HF_{254} in n-butanol: n-propanol: ammonia: water (2:6:1:2 v/v). Hatched areas are U.V absorbing areas.

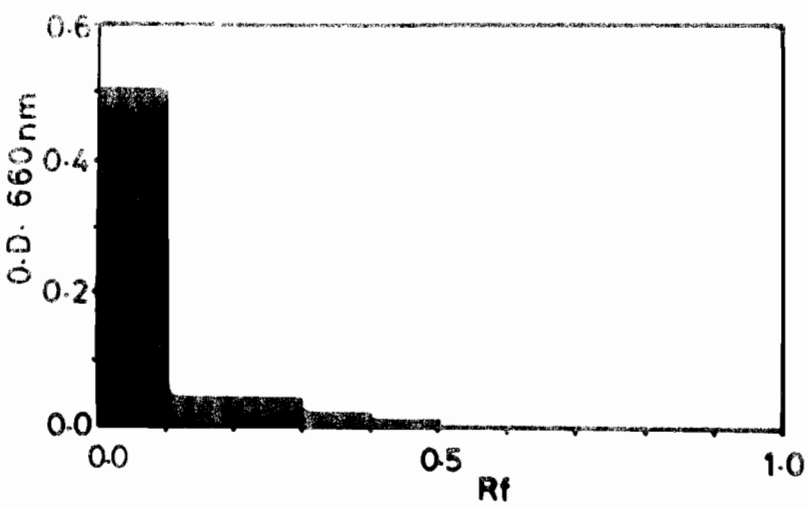

Fig.5. Chromatogram of slow moving inhibitory zone of water soluble extract of Palm fruit (Rf 0.0-0.5) subjected to ascending paper chromatography with n-butanol: acetic acid: water (6:1:2 v/v). Paper divided into ten pieces,extracted with 50% ethanol and total phenol estimated at 660 nm.

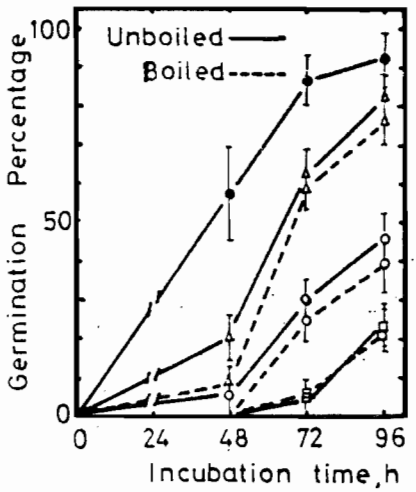

Fig.6. Effect of 10 min boiling of the water-soluble extract of *W. filifera* fruits on the germination of lettuce seeds. Amount of extract equivalent to 300 (△), 500 (○) and 700 mg (□) dry weight of the fruits. Untreated water control (●). The vertical bars give the standard error of the mean.

Different concentrations of water-soluble extract of *W.filifera* fruits were kept in a boiling water bath for 10 min and tested for its inhibitory activity. It was found that high temperature did not inactivate the germination (Fig.6) and growth (Table.1) inhibiting capacity of the extract which indicates that the inhibitors are not protienaceous in nature and are insensitive to heat. Lodhi (1975) and Lodhi & Nickell (1973) have also

Table 1. Effect of 10 minutes boiling of the water soluble extract of *Washingonia filifera* fruits on the hypocotyle and root growth of lettuce.
± = Standard error of the mean.

Extract equi. to mg D.Wt of the fruit	Hypocotyle length (mm)		Root length (mm)	
	Unboiled ext.	Boiled ext.	Unboiled ext.	Boiled ext.
0	25.0	---	112.0	---
	±0.068		±5.69	
300	19.4*	26.2^{n-s}	51.6*	55.2*
	±0.87	±1.49	±1.43	±3.33
500	14.2*	16.6*	36.6	41.0**
	±0.86	±1.66	±2.09	±6.40
700	8.6**	9.6**	26.6**	34.0**
	±0.60	±0.51	±3.25	±2.27

* $P < 0.05$, *** $p < 0.01$ (Students t-test)

reported the allelopathic inhibition of seed germination and growth of hot water extracts. Most phenolic compounds do not degrade during heating for a short duration as is evident from the results of Khizar & Khan (1977).

The present study, therefore, reveals that the fruits of *W. filifera* is strongly allelopathic atleast against cucumber and lettuce used in the present study and contains phenols and ABA-like substance as the water-soluble phytotoxins. The discharge of these allelopathic substances into the environment by leaching of substances through rain from the dry fruits of *W. filifera* while still attached to the plant is evident from the present study.

References

Benner-Clark, T.A. and W.P. Kefford. 1953. Chromatography of the growth substances in plant extracts. *Nature.*, 171: 645-647.

Bonner, J. 1950. The role of toxic substances in the interaction of higher plants. *Bot. Rev.*, 16: 51-65.

Gross, D. 1975. Growth regulating substances of plant origin. *Phytochemistry*, 14: 2105-2112.

Holst, U. 1971. Some properties of inhibitor B from *Solanum tuberosum* compared to abscissic acid . *Physiol. Plant.*, 24: 392-396.

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

Housley, S. and W.C. Taylor. 1958. Studies on plant growth hormones. VI. The nature of inhibitor B in potato. *J. Exp. Bot.*, 9:458-471.

Khan, M.I. 1982. Allelopathic potential of dry fruits of *Washingtonia filefera* (L.Linden) H. Wendl fruits. Inhibition of seed germination. *Physiol. Plant.*, 54: 323-328.

Khan, M.I. 1982a. Allelopathic potential of the dry fruits of *Washingtonia filifera* (L.Linden) H. Wendl fruits. II. Inhibition of seedling growth. *Biol. Plant.*, 24: 275-281.

Khizar, T. and M.I. Khan. 1977. Effect of temperature on the activity of growth inhibitor-A isolated from *Suaeda* leaves. *Biol. Plant.*, 19: 231-233.

Koves,E. 1957. Papierchromatographische Unterschungen der ather loslichen Keimungs-Und Wachstshemmenden Stoff der Haferspelze. *Acta Biol. Szeged*, 3: 179-187.

Lodhi, M. A. K. 1975. Allelopathic effects of hackberry in a bottom land forest community. *J. Chem. Ecol.*, 2: 171-182.

Lodhi, M. A. K. and G.L. Nickell. 1973. Effect of leaf litter of *Celtis laevigata* on growth, water contents and carbon dioxide exchange rates of three grass species. *Bull. Torrey Bot. Club.* 100: 159-165.

Milborow, B. V. 1967. The identification of (+)- abscisin II in plants and measurement of its concentrations. *Planta*, 76: 93-113.

Ray, H. and P.J. Hastings. 1992. Variation within flax (*Linum usitatissimum*) and barley (*Hordeum vulgare*) in response to allelopathic chemicals. *Theo. Appl. Genet.*, 84: 460-465.

Rice,E.L.1984. *Allelopathy*. 2nd ed. Academic Press, N. York. 22.pp.

Robertson, J. and A.M.M. Berrie. 1977. Abscissic acid and the germination of thermodormant lettuce fruits (*Lactuca sativa* cv. Grand Raphids). The fate of isotopically labelled abscissic acid. *Physiol. Plant.*, 39: 51-59.

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

Varga, M.B. 1957. Examination of growth-inhibiting substances by paper chromatography in fleshy fruits. II. Identification of the substances of growth-inhibitory zones on the chromatogram. *Acta. Biol. Szeged*, 3: 213-223.

Varga, M. B. and E. Koves. 1959. Phenolic acids as growth and germination inhibitors in dry fruits. *Nature*, 183: 401.

Wittaker, R.H. and P.P. Feeny. 1971. Allelochemics: Chemical interactions between species. *Science*, 171: 757 - 770.

(Received for publication 14 May 1997)