

# PLANT GROWTH REGULATORS FROM SALT-SENSITIVE AND TOLERANT SPECIES

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This paper reports a comparative study of the nature and level of growth regulators with particular emphasis on water-soluble growth inhibitors of salt-sensitive and salt-tolerant plants as affected by soil salinity.

## MATERIALS AND METHODS

Seeds of *Triticum aestivum* (cv. Pak-70) and *Zea mays* (cv. Akbar), sensitive to sodium chloride salinity, were planted in undrained plastic pots containing 3 kg sandy loam. The salinity was achieved by adding 125 meq/l sodium chloride prepared in half-strength Hoagland solution of pH 6.5. The pots were irrigated periodically with distilled water to field capacity. The plants were grown in a growth chamber maintained at  $22 \pm 2^\circ\text{C}$  and 10 hr light of 6000 Lux. The relative humidity ranged from 60 to 80%.

Extraction and estimation of growth inhibitors: this was done by a wheat coleoptile test. 20 g of the shoots of plants grown in saline and non-saline conditions were extracted with 200 ml. distilled water and the pH adjusted to 3.0 using 0.5N sulphuric acid. The extract was washed three times with diethyl ether and the pooled ether fraction was concentrated. Similar extracts were prepared from 10 g of fresh leaves of mature plants of *Seaeda fruticosa* Forsk. growing in saline soil having high sodium and low potassium contents. The concentrated ether extracts were streaked on 10 cm wide strips of Whatman paper No. 1 and the chromatograms developed at  $22 \pm 2^\circ\text{C}$  by descending chromatography in 10 : 1 : 1 isopropanol : ammonia : water. When the solvent front had moved 30 cm, the chromatograms were dried, cut into ten equal sections and subjected to the wheat coleoptile assay (2). Results are shown in Figures 1 - 3 (ABA = Abscissic Acid).

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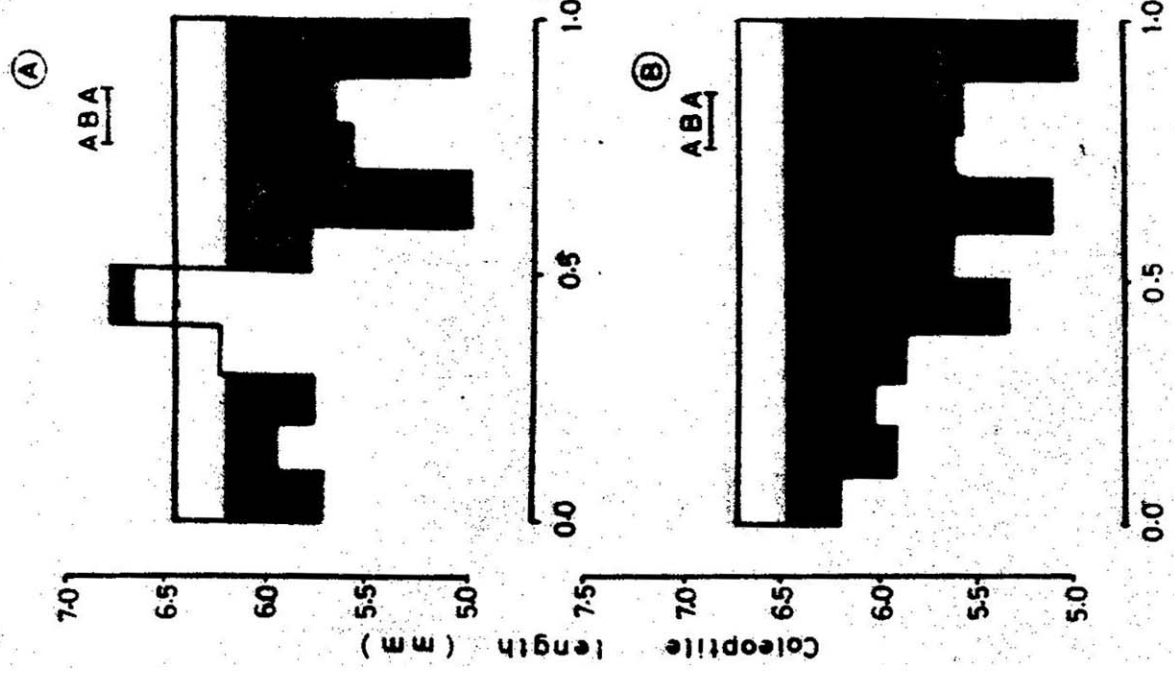


Figure 2. Wheat coleoptile assay of maize shoots grown for 40 days in non-saline (A) and saline (B) soil.

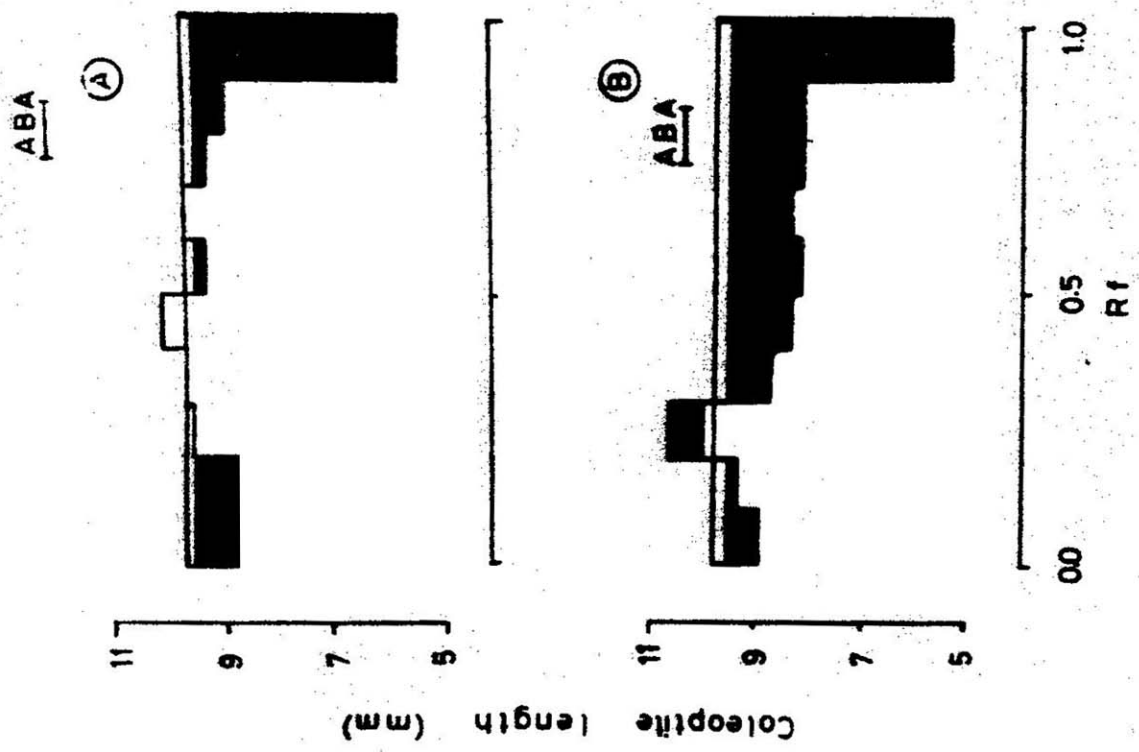


Figure 1. Wheat coleoptile assay of wheat shoots grown for 50 days in non-saline (A) and saline (B) soil.

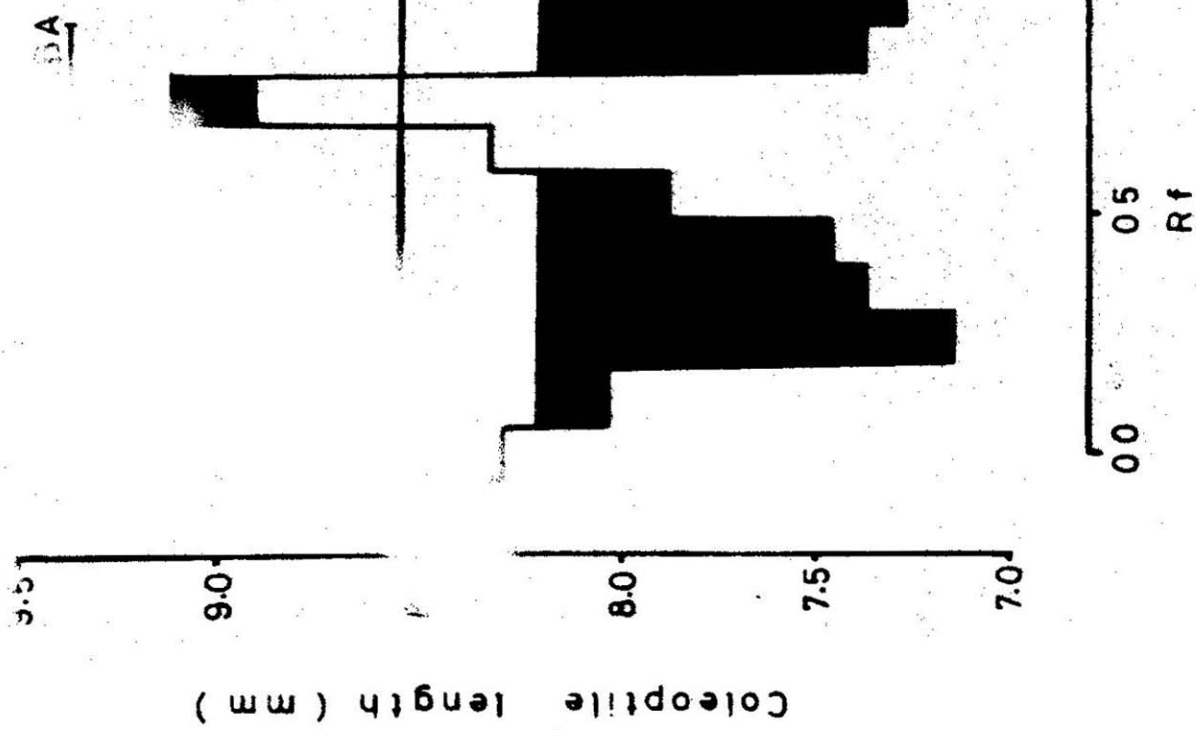


Figure 3. Wheat coleoptile assay of the acidic fraction from Suaeda leaves growing in saline soil.

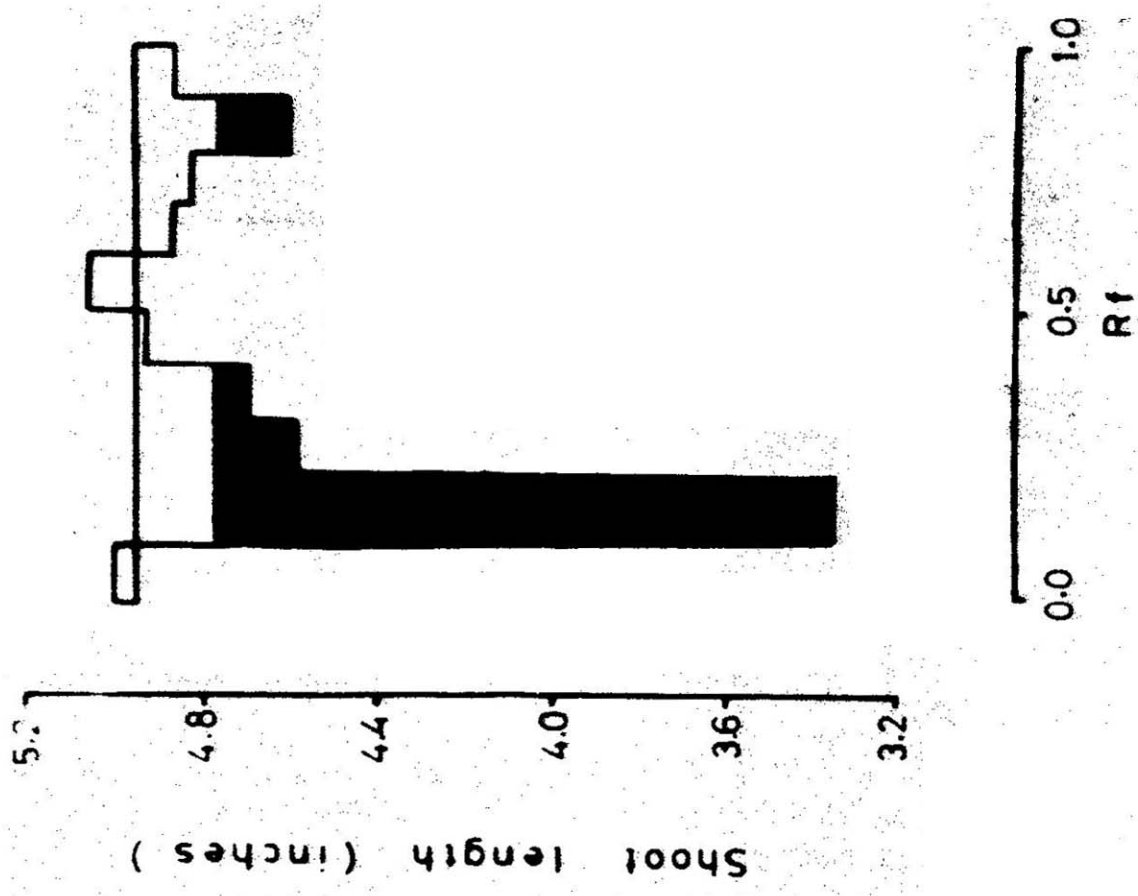


Figure 4. Effect of growth inhibitors from the Suaeda fructicosa on young wheat shoots after 10 days.

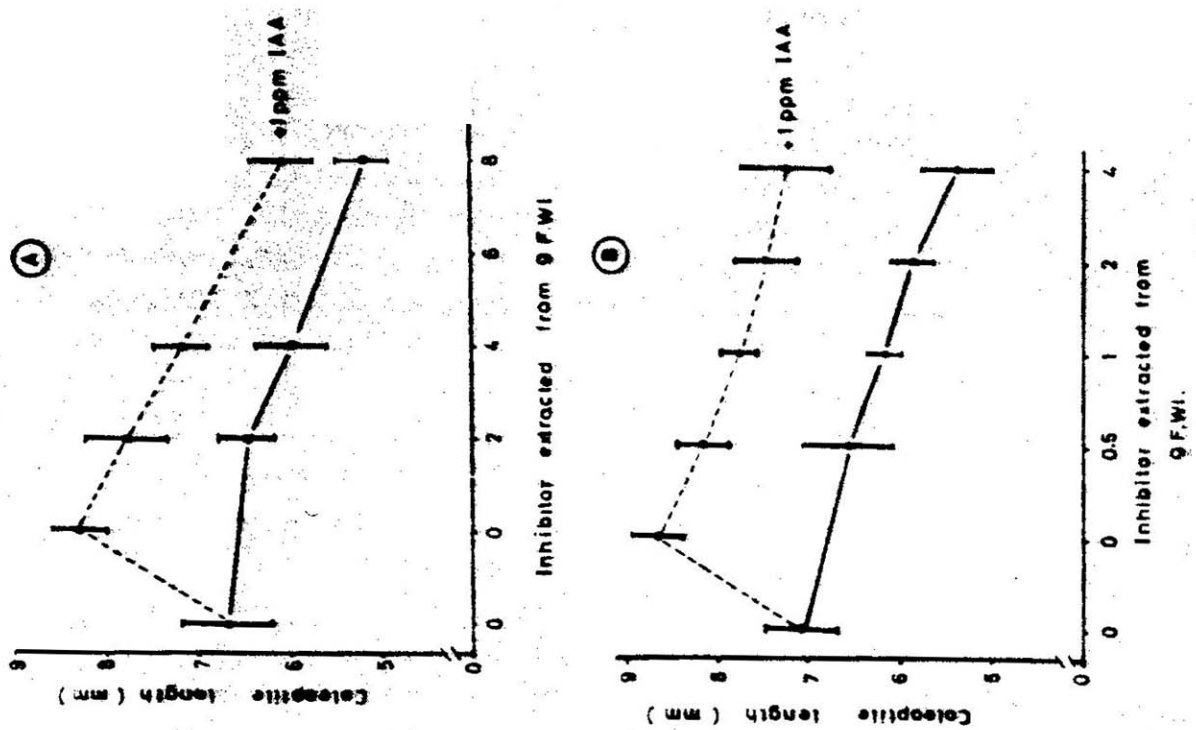


Figure 5. The effect of inhibitors from maize shoots (A) and Suaeda leaves (B) on the growth of excised wheat coleoptiles in the presence and absence of 1 ppm IAA.

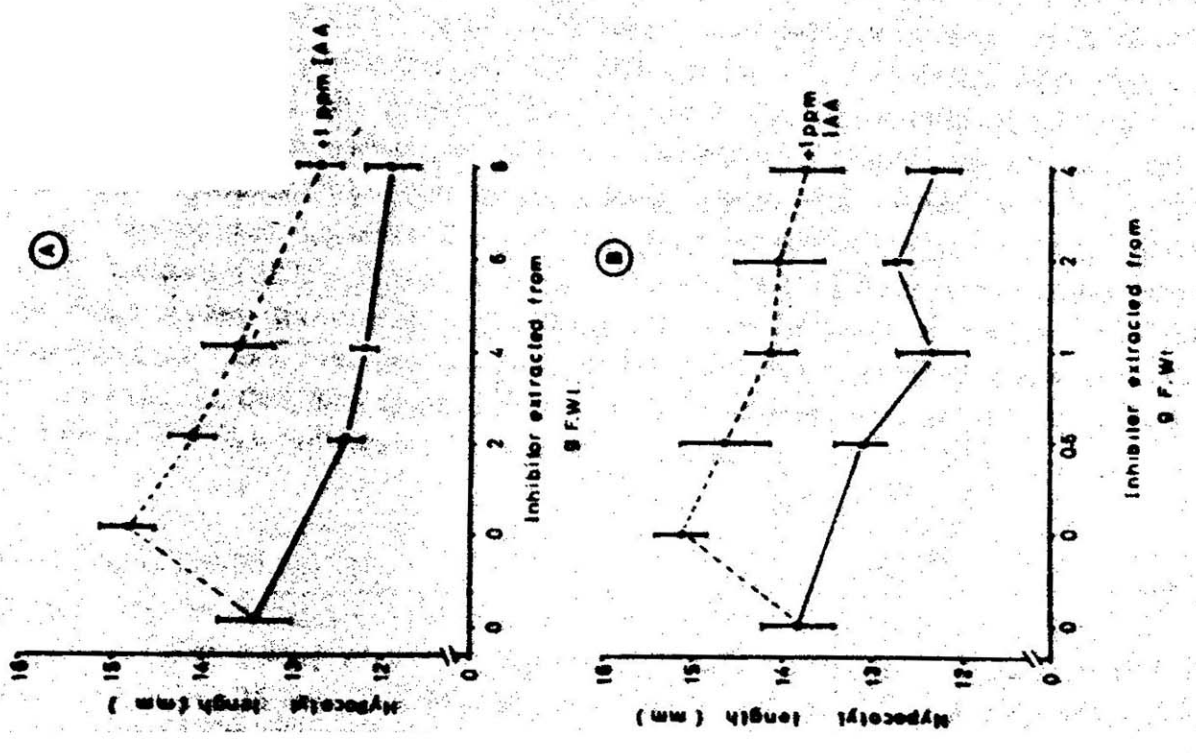


Figure 6. The effect of inhibitors from maize shoots (A) and Suaeda leaves (B) on the growth of jute hypocotyls in the presence and absence of 1 ppm IAA.

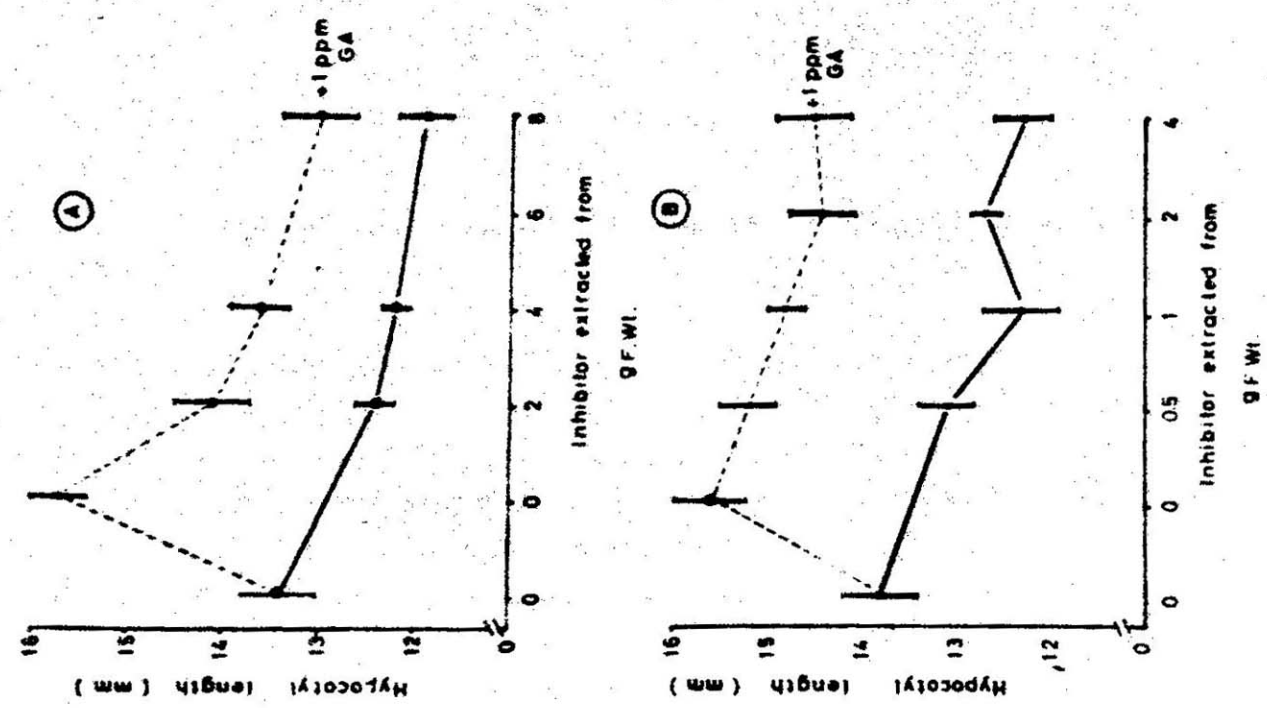


Figure 7. The effect of inhibitors from maize shoots (A) and Suaeda leaves (B) on the growth of jute hypocotyls in the presence and absence of 1 ppm GA.

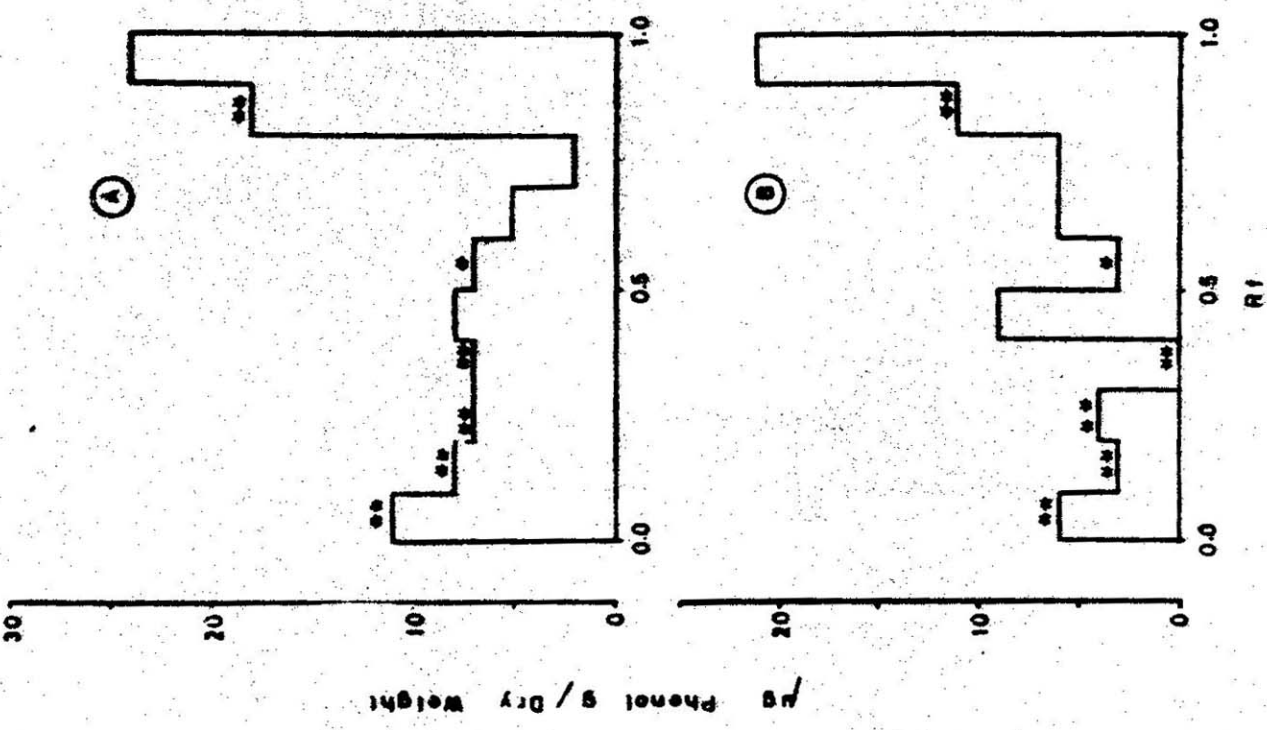


Figure 8. Total phenols from wheat shoots grown for 30 days in non-saline (A) and saline (125 meq/l sodium chloride) soil (b).

**Extraction and estimation of Phenols:** Fresh leaves equivalent to 1 g dry weight were sliced and immersed in 10 ml of 0.1N hydrochloric acid and heated for 30 minutes in a boiling water bath. The filtrate was extracted twice with 25 ml of diethyl ether and the extracts evaporated. The residue was dissolved in 0.1 ml of ethanol and used for the estimation of phenols before and after the separation by thin layer chromatography. The thin layer absorbent was Silica gel G, and the plates were developed in 4 : 1 : 2.2 n-butanol : acetic acid : water. Total phenols were estimated by the modified Folin-Ciocalteu method (3). To 0.1 ml extract solution, 5 ml of Folin reagent was added and the tubes shaken thoroughly. After 3 minutes 1 ml of saturated sodium carbonate was added and the tubes were shaken again. After 30 minutes at 25°C, the optical density at 660 nm was recorded. The results (Figures 8, 9) were calculated as the orcinol equivalent; \* denotes 95% significance and \*\*, 99% significance.

## RESULTS AND DISCUSSION

The Wheat coleoptile assay of plants grown in non-saline soil (Figure 1A) revealed the presence of growth inhibitors at Rf 0.0 - 0.2 and 0.77 - 1.0, but when the plants received 125 meq/l sodium chloride, an additional inhibiting zone at Rf 0.3 - 0.7 was observed (Figure 1B). Similarly sodium chloride treated maize shoots (Figure 2B) also showed a growth inhibiting zone at Rf 0.3 - 0.5 which was not present in the untreated plants (Figure 2A). Inhibition of growth at ABA zone in salt-treated plants were always higher than the control in both the plants. An ABA-like inhibitor (Rf 0.5 - 1.0) also occurs in the leaves of *Nicotina rusticana* grown under saline and non-saline conditions (4).

Leaves of salt-tolerant *Suaeda fruticosa* indicated marked inhibition of coleoptile growth at Rf 0.1 - 0.6 and 0.8 - 1.0 (Figure 3). The growth promoting substance was also present at Rf 0.8. Paper chromatograms of the acidic fraction of *Suaeda* leaves also showed marked inhibition of young wheat shoots at Rf 0.1 - 0.3 (Figure 4), indicating the effectiveness of the *Suaeda* inhibitors in suppressing the growth of excised coleoptiles as well as the wheat shoots.

Effect of inhibitors isolated from salt-grown maize shoots (Rf 0.0 - 1.0) and *Suaeda* leaves (Rf 0.1 - 0.6 and 0.8 - 1.0) indolylacetic acid on induced growth of wheat coleoptiles (Figure 5), jute hypocotyls (Figure 6) and gibberillic acid induced growth of jute hypocotyls (Figure 7) was studied. Both inhibitors reduced the indolylacetic acid and gibberillic acid-induced growth of coleoptiles and hypocotyls.

In order to compare the specific activities between the inhibitors and phenolics, qualitative and quantitative studies of the phenolic constituents of halophytes and non-halophytes were carried out. One or two dimensional paper and TLC chromato-

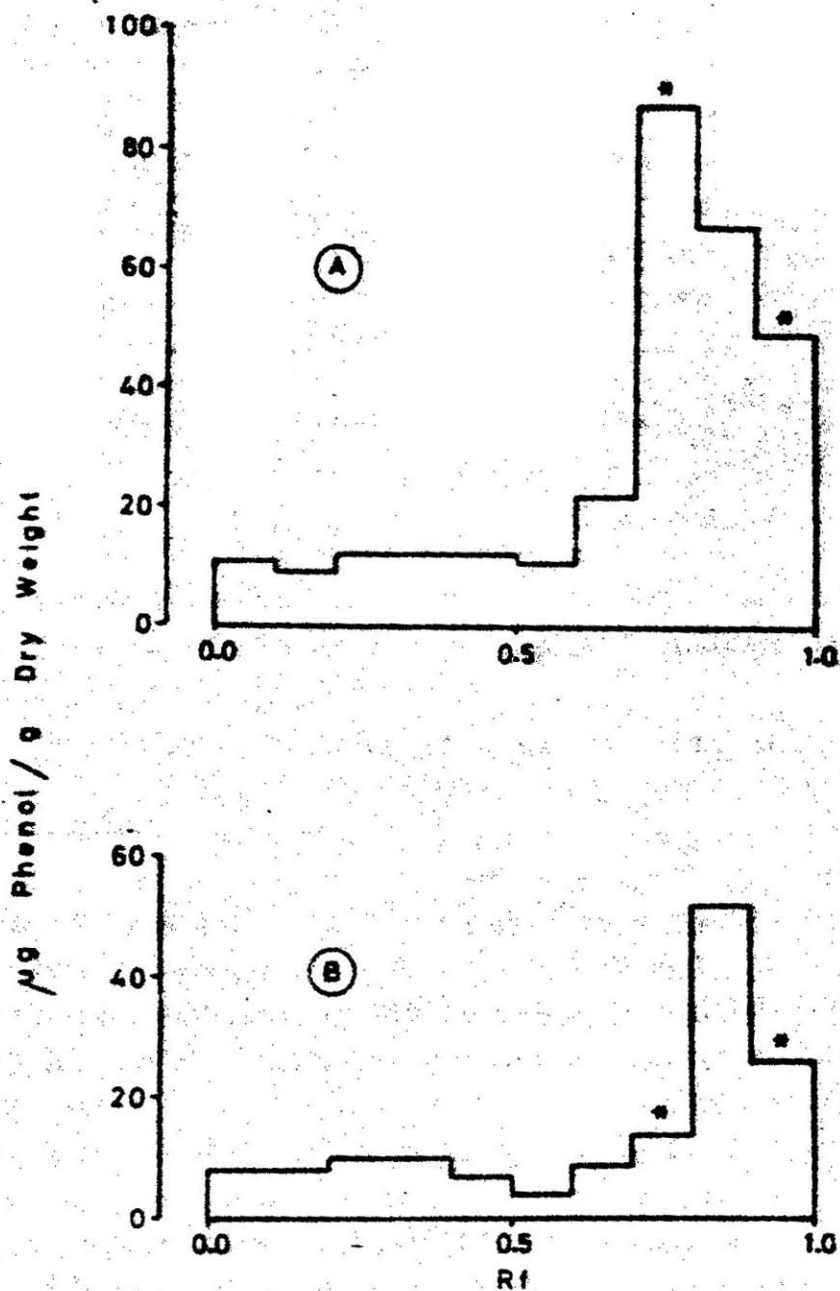


Figure 9. Total phenols from *Suaeda fruticosa* (A) and *Raphanus sativus* (B).

graphic separation revealed the presence of gallic acid, ferulic acid and salicylic acid in the inhibitory extract of *Suaeda fruticosa* and of salicylic acid in the extract of wheat. Total phenols of thin layer chromatograms loaded with the acidic fraction from extracts of wheat shoots grown for 30 days in non-saline (Figure 8A) and saline (125 meq/l sodium chloride) soil (Figure 8B) showed phenols at  $R_f$  0.0 - 0.4 and 0.6 - 0.9 in the shoots of the controls but these were significantly less in salinized plants. *Suaeda fruticosa* (Figure 9A) contained phenols at  $R_f$  0.8 and 1.0 which were significantly higher than the corresponding zones of *Raphanus sativus* phenols (Figure 9B).