

INTERRELATION BETWEEN THE COMMONLY ASSOCIATED
RICINUS COMMUNIS AND SESBANIA SESBAN

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SUMMARY

Growth substances exuded from the seeds and roots of Ricinus communis and Sesbania sesban plants which are commonly associated together were isolated by paper chromatography technique. The fractionated exudates of either Ricinus or Sesbania seeds contained one or two growth promoters of auxin type and a number of growth inhibitors. The exudates of Ricinus communis seeds contained no gibberellins, where as Sesbania sesban seeds showed only gibberellin-like substance. The seed exudates of both plants contained purine and non purine compounds having cytokinin activity, also some amino acids and sugars.

The root exudates of both plants contained auxin, growth inhibitors, gibberellins, cytokinins, amino acids and sugars.

The quantity and quality of the above mentioned substances markedly changed according to the age of these plants.

The different concentrations used of non fractionated Sesbania sesban seed or root exudates generally exhibited significantly increase in the linear growth of the roots and shoots of Ricinus communis at low concentrations. The reverse occurred at higher concentrations. The same results were obtained on using non fractionated Ricinus communis seed or root exudates.

INTRODUCTION

Ricinus communis and Sesbania sesban are commonly seen growing associated together. This observation led us to investigate if some chemical substances are released into the soil from each plant that

may affect the subsequent growth of the another. Reviews on liberation of different organic substances from some higher plants and the effects of one plant upon another include those of Borner (1960), Garb (1961), Street et al. (1964), Hurtt and Foy (1965), Rovira (1969), Tukey (1969) and Raifa (1971).

Mishustin and Naumova (1955) demonstrated that the roots of 3 or 4 years old Lucerne exuded saponins which retarded the growth of cotton but not of wheat. Street et al. (1964) detected an indole compound of auxin nature, released into the culture medium during the growth of excised tomato roots. Vancura (1964) detected B-indole acetic acid in root exudates of barley and wheat. Morris et al. (1969) have shown that radio-active IAA tended to accumulate in the developing lateral roots, while there was no evidence that IAA reached the primary root. Raifa (1971) reported the presence of a number of growth promoters of auxin type and a number of growth inhibitors in root exudates of certain Egyptian weeds. Elliot and Greenwood (1974) isolated IAA from the exudates of Zea mays roots. Mohga (1979) investigated the nature and contents of the growth substances present in the exudates of Zea mays and Corchorus olitorius seeds. She reported that the action of the root exudate upon growth of other plants depends mainly on the concentration and chemical nature of the substances present in this exudate. She also found that relatively higher concentrations of root exudates of Corchorus olitorius markedly decreased the shoot and the root length of Zea mays whereas the relatively low concentrations increased the shoot and root length.

Presence of gibberellins in either intact or excised roots was referred to by Murakami (1960, 1968), showed that certain grass roots (including rice) contain gibberellin-like activities at levels comparable to the shoot. Raifa (1971) detected many gibberellin-like substances in the root exudates of certain Egyptian weeds. Reid et al. (1972) demonstrated that tomato roots exuded certain gibberellins which decreased when the tomato roots were flooded. Frydman and Wareing (1975) demonstrated gibberellin by bioassay of root extracts of Hedera helix. Torrey (1976) reported that the evidence for the presence of gibberellins in roots comes largely from indirect evidence based on collection by diffusion and extraction combined with bioassay of materials from roots or from activity measured by bioassay from root exudates.

Concerning presence of cytokinin in plant root exudates, Vardjan and Nitsch (1961) isolated a substance having a cytokinin activity from the root extract of Cichorium. Similar substances were isolated from many plants by several investigators e.g. sunflower (Weiss and Vaadia 1965), Pisum sativum (Short and Torrey (1972), Zea mays (Feldman 1975), tomato (Carnes et al., 1975) and Coleus blumei, Impatiens Wallerana, Phaseolus vulgaris and Protea compacta (Kende 1964). Wareing & Phillips (1970) and Torrey (1976) reported that cytokinin are synthesized in the roots and can be detected in the root exudates of many plants. Koda and Okazawa (1978) reported that root tips have been considered as a major site of cytokinin synthesis in higher plants as they have observed an accumulation of cytokinins in the surrounding medium in which root tips are

cultured and Van Stadan (1976a), reported secretion of cytokinin into the culture medium from roots of corn seedlings. Radi and Maeda (1986), showed that the effect of kinetin and benzyladenin on rice excised root growth is inhibitory in the high and moderate concentration and a slight stimulation was observed with the relatively low concentrations. The inhibitory effect of both cytokinins and auxins may be due to their stimulation of ethylene production and a synergism between auxin and cytokinins was suggested by Adams and Young (1981).

Different inorganic and organic constituents were detected in seed and root exudates. Katznelson et al. (1955) stated that the dessication and subsequent rewetting of the sand in which tomatoes, soybean, barley or oats were grown resulted in the excretion of certain amino acids such as glutamic acid, aspartic acid, leucine, alanine, cysteine, glycine, lysine, phenylalanine and proline and a reducing compound of R_F value identical with glucose. Vancura (1964), analysed root exudates of barley and wheat in their initial phases of growth and identified four type of oligosaccharides in addition to two amino sugars. Fourteen amino acids were identified in root exudates of barley whereas eighteen were identified in root exudates of wheat and seven organic acids were identified in both exudates. He (1967), also reported that germinating seeds of both maize and cucumber exuded different amino acids and sugars during seeds germination at seedling stage. Youssef and Khairia (1971) analysed seed and root exudates of two cotton varieties and ten amino acids and three sugars were identified. Raifa (1971)

analysed root exudates of certain Egyptian weeds, Melilotus, Cyperus, Chenopodium, Urospermum, Amaranthus and Euphorbia and detected a number of amino acids, sugars and organic acids.

The present work was conducted to investigate the growth substances that may be present in the exudates of both, Ricinus communis and Sesbania sesban which are commonly associated together and to study the effect of seed and root exudates of each plant on germination and seedling growth of the another one.

Material and Methods:

Ricinus communis and Sesbania sesban seeds used in this investigation were collected from the Botanical Garden of the Women's College, Ain Shams University, Cairo. Grains of Hordeum vulgare Cv. Giza 120 and Sorghum (Andropogon sorghum Cv. Giza 142) and Xanthium brasiliicum Hell seeds were obtained from the Egyptian Ministry of Agriculture.

Extraction of the growth regulating substances is done in a way similar to the method adopted by El-Ghobashy (1968).

Seed and root exudates of each plants were fractionated into its major components of growth promoters and inhibitors by paper chromatography. The extract was chromatographed in descending manner using 80% isopropyl alcohol:20% distilled water as a running solvent. The chromatograms after developed, were dried in cold air, examined for fluorescence by ultraviolet chromatolite lamps, prepared for bioassay and sprayed with chemical reagent. The amount

of the exudates loaded in each case was equivalent to that obtained from 0.1 g dry weight of the plant tissue.

Each chromatogram, after air drying was cut transversely into 10 equal portions, each of which was eluted in 5 ml. distilled water in a thoroughly small Petri-dish. The dishes were kept in refrigerator overnight, and then the solution were ready for biological test.

The method used to assay promoters and inhibitors was the straight growth test of *Hordeum coleoptile*. It was applied according to the method adopted by Foda and Radwan (1962). The method used to assay gibberellins and gibberellin-like substances was the *Sorghum* first leaf test (Bently-Mowat (1966) with certain modification). The method used to assay the cytokinin was the *Xanthium* cotyledonary leaf sections (Esashi & Leopold (1969)).

The results of each test was represented graphically by a histogram of 10 columns representing the different R_F S of the chromatograms. The mean length of the control (coleoptile, first leaf and cotyledonary leaf grown in distilled water) was indicated in the histograms by a horizontal line.

The dried chromatograms were examined by an ultraviolet chromatolite lamp. They were subjected to some chemical test, where a longitudinal strip from the chromatogram was sprayed with certain reagent to test for indole compounds (Keiford, 1955 and Powell, 1959), reducing substances, amino acids (Hunt, 1959 and Smith, 1962), hydroxyl groups (Swain, 1953), gibberellins (Jones et al., 1963 and Kagawa et al. 1963), unsaturated lactones (Swain, 1953)

and cytokinin substances containing purine ring (Reguera and Ascinow, 1950).

The free amino acids of the root exudates were spotted on Whatman filter paper No. 1 Authentic amino acid mixture was simultaneously loaded with each chromatogram. The solvent system used for running was n. butanol: acetic acid: water (4:1:5 v/v/v) (Hunt, 1959, Smith 1962). The chromatograms after development were dried and sprayed with ninhydrin reagent (Block et al. 1958).

The method used for identification of the carbohydrates was similar to that of Partridge (1948). The chromatograms were sprayed with aniline hydrogen phthalate reagent.

Effect of seed or root exudates of *Ricinus communis* on the germination and the linear growth of *Sesbania sesban* and the reverse:

Five different concentrations of seed or root (15 days old) exudates of each of *Ricinus communis* or *Sesbania sesban* plants were prepared, starting with the exudates obtained from fresh seeds and roots of 10 g dry weight in 10 ml of distilled water. The different concentrations used are referred to as 1, 1/2, 1/4, 1/8 and 1/16, water being used as a control. Replicate samples from each concentration were prepared.

Ten seeds of *Ricinus communis* were sown in Petri-dishes on filter paper moisted with each of the different concentrations mentioned above of either seed or root exudates of *Sesbania sesban*. All dishes were then incubated at 25°C and the percentage of germination at the different treatments was calculated after 48 hours.

After ten days, the mean linear growth of the developed seedlings was measured.

The data were statistically analyzed according to the procedure recorded by Snedecor and Colchran (1969).

RESULTS AND DISCUSSION

It is clear from Figure 1(a) that the exudate of Ricinus communis contained only one growth promotor zone (R_F 0.1-0.2) and produced positive reactions with indole reagents and two growth inhibitor zones produced positive colour reactions with diazotized-p-nitro-aniline which may show that they are unsaturated lactones (R_F 0.2-0.3 and 0.4-0.5). On the other hand the exudate of Sesbania sesban contained at least two growth promotor zones, one of them (R_F 0.7-1.0) of indole nature and one growth inhibiting zone (R_F 0.4-0.7) may contain a phenolic lactone compound according to their chemical colour reactions.

With regard to the effect of seedling age on the root exudate, it has been shown (Fig. 2 a&b) that the root exudate of seven days old Ricinus communis seedlings was found to contain three promoting zones, two of them (R_F 0.5-0.6 and 0.7-0.9) having auxin activity of indole nature and three growth inhibitory zones (R_F 0.0-0.1, 0.1-0.3 and 0.4-0.5) the first growth inhibitor appeared likely to contain unsaturated lactone compound, while the third may contain a phenolic lactone compound. On the other hand the root exudate of seven days old Sesbania sesban seedlings contained at least three promoting zones, two of them (R_F 0.0-0.2 and 0.3-0.6) of indole

nature and one growth inhibiting zone (R_F 0.5-1.0) appear to be unsaturated lactone. Increasing the age of the two plants to 15 days, increased the level of auxins exuded from roots of both plants, while growth inhibitors disappeared completely. The root exudate of 15 days old Ricinus communis seedlings was found to contain at least four promoting zones (R_F 0.0-0.2, 0.3-0.5, 0.5-0.8 and 0.8-1.0), the first, the third and the fourth zones having auxin activity of indole nature. On the other hand the root exudate of 15 days old Sesbania sesban seedlings was found to contain five growth promoting zones, three of them (R_F 0.0-0.2, 0.4-0.6 and 0.8-1.0) of indole nature. These may bear some agreement with those of Bonner (1950), Bonner (1960), Garb (1961), Rovira (1969), Tukey (1969), Raifa (1971) and Mohga (1979). Street et al. (1964) detected an indole compound of auxin nature liberated into the cultural medium from excised tomato roots. El-Gindy (1976) detected indole auxin compounds in the root exudates of cotton and maize plants.

Concerning gibberellins content, the exudates of Ricinus communis seeds contained no gibberellins (Fig. 1b), whereas Sesbania sesban seed exudates showed only one gibberellin-like substance. On the other hand, the root exudates of both plants contained more than one gibberellin and gibberellin-like substances. The exudate of seven days old Ricinus communis seedlings (Fig. 3a), contained at least three zones having gibberellins activity (R_F 0.0-0.2, 0.4-0.5 and 0.7 - 0.8). Increasing the age (15 days old) the number of gibberellin zones detected increased to four (R_F 0.0-0.2, 0.3-0.5, 0.6-0.7 and 0.8-1.0). On the other hand, the exudate of seven

days old Sesbania sesban (Fig. 3b) contained also three zones having gibberellins activity (R_F 0.0-0.2, 0.3-0.5 and 0.6-0.7). Increasing the age to 15 days, gibberellin zones increased to four (R_F 0.0-0.2, 0.4-0.5, 0.6-0.7 and 0.9-1.0). These results may agree with those obtained by Raifa (1971) and Mohga (1979). They also reported that gibberellin content increased in the root exudate of Melilotus, Zea mays and Corchorus olitorius by increasing age.

Concerning the cytokinins content, the exudates of Ricinus seeds (Fig. 1 c), contained four promoting zones having cytokinin activity (R_F 0.0-0.2, 0.4-0.5, 0.6-0.7 and 0.9-1.0), the second zone seems likely to contain a purine compound, while the others contained non-purine substances since they produced negative colour reactions with the reagents testing for purine substances. On the other hand exudates of Sesbania sesban seeds contained three promoting zones having cytokinin activity (R_F 0.0-0.2, 0.4-0.5, 0.6-0.7 and 0.9-1.0), the second zone seems likely to contain a purine compound, while the others contained non-purine substances since they produced negative colour reactions with the reagents testing purine substances. On the other hand exudates of Sesbania sesban seeds contained three promoting zones having cytokinin activity (R_F 0.0-0.1, 0.6-0.7 and 0.8-1.0), the second zone seems to contain non-purine substance, while the others contained purine compounds. The root exudates of seven days old Ricinus communis seedlings (Fig. 4a) were found to contain two promoting zones (R_F 0.2-0.5 and 0.8-1.0), and purine compound, having cytokinin activities, while the root exudates of seven days old Sesbania sesban contained three promoting zones (Fig. 4b), having cytokinin activity (R_F 0.0-0.1,

0.2-0.4 and 0.8-1.0). The second zone seems to contain non-purine substance while the others contained Purine compounds. The root exudates of fifteen days old Ricinus communis contained two promoting zones (RF 0.0-0.2 and 0.7-1.0), having purine compounds of cytokinin activities, while exudates of 15 days old Sesbania sesban (Fig. 4b) contained four promoting zones (R_F 0.0-0.2, 0.2-0.4, 0.4-0.6 and 0.8-1.0). The second zone seems to contain non purine compounds, while the others contained purine compounds of cytokinin activities. It is clear that increasing the age, increased the cytokinins exuded and this may be due to the increase in the number of root tips. Koda and Okazawa (1978) reported that root tips have been considered as a major site of cytokinin synthesis in higher plants. They observed, an accumulation of cytokinins in the surrounding medium in which root tips are cultured. Our results also agree with those obtained by Kende (1964), Wareing & Phillips (1970), Wheeler (1971), Torrey (1976), Goodwin & Morris (1979) and Radi & Maeda (1986).

Concerning the amino acids, the exudates of 7 days old Ricinus seedling was found to contain five amino acids, namely glycine, lysine, proline, cystine and ornithine. while exudates of seven days old Sesbania seedlings, contained glutamic acid only. The exudates of fifteen days old Ricinus seedlings contained six amino acids namely glycine, cystine, threonine, lysine, proline and aspartic acid. The exudates of fifteen days old Sesbania seedlings contained four amino acids, namely glycine, lysine, alanine and glutamic acid. Many investigators also identified the amino acids present in some plants (Vancura 1964, Youssef & Khairia 1971, Raifa 1971 and Mohga 1979).

Concerning the sugar contents, the exudates of seven days old Ricinus seedlings contained glucose only, while the exudates of seven days old Sesbania seedlings contained glucose and fructose. The exudates of fifteen days old Ricinus seedlings contained three sugars, namely galactose, glucose and rhamnose, while the exudates of fifteen days old sesbania seedlings contained three sugars, namely glucose, fructose and sucrose.

It is evident that seed and root exudates of Ricinus communis and Sesbania sesban contained auxins, growth inhibitors, gibberellins, gibberellin-like substances, cytokinins, amino acids and sugars. These raise the question. whether substances exuded from each species plants which commonly grow in association influence the growth of each other. It is obvious that low concentrations of non fractionated seed or root exudates of Sesbania sesban (1/16, 1/8 and 1/4) induced significant increase in the linear growth of Ricinus communis seedlings (Table 1), while concentrations (1/2 and 1) showed non significant effect and decreased the linear growth as compared with the control (untreated plants). On the other hand the seed and root exudates of Ricinus communis induced significant increase in the linear growth of Sesbania sesban at low concentrations (1/16, 1/8, 1/4), whereas high concentration (1/2, 1) caused non-significant decrease in the linear growth of Sesbania sesban (Table 2). Mohga (1979) reported that the action of the root exudate upon growth of other plants depends mainly on the concentration and the chemical nature of the substances present in this exudate. She found that the relatively higher concentrations of root exudates of 20 days old zea mays

plants, markedly inhibit the growth of Corchorus olitorius. These inhibitory actions were obviously and gradually decreased by dilutions. Adams and Young 1981 suggested that the inhibitory effect may be due to both cytokinins and auxins which stimulate the ethylene production and a synergism between them.

In conclusion it appears that there is an apparent role of exudates of each species on the growth of the other. Both species are being naturalized and are gaining ground in several habitats. In addition to the prominent causal factor for association of species i.e. similarity of essential ecological requirements, the possible effects of interaction between the two studied species gains some evidence from the present study.

The author wishes to express her thanks to Dr. Mohamed Hamouda. Professor of Plant Ecology, Botany Department, Women's College Ain Shams University.

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Table (1): Effect of different concentrations of seed or root exudate of Sesbania sesban on shoot and root length of Ricinus communis seedlings

Relative conc. of seed exudate	Root length cm.	t	Shoot length cm.	t	Relative conc. of root exudate	Root length cm.	t	Shoot length cm.	t
Control	10.0 $\pm$ 0.16		8.2 $\pm$ 0.22		Control	10.0 $\pm$ 0.16		8.2 $\pm$ 0.22	
1/16	10.4 $\pm$ 0.16	4.0 S	9.1 $\pm$ 0.69	2.77 S	1/16	10.5 $\pm$ 0.16	5 HS	9.4 $\pm$ 0.30	7.36 HS
1/8	10.7 $\pm$ 0.36	4.01 S	9.9 $\pm$ 0.41	8.15 HS	1/8	11.2 $\pm$ 0.23	9.6 HS	10.0 $\pm$ 0.41	8.58 HS
1/4	11.0 $\pm$ 0.33	5.84 HS	10.5 $\pm$ 0.38	11.65 HS	1/4	11.8 $\pm$ 0.2	15.79 HS	11.0 $\pm$ 0.79	7.62 HS
1/2	9.8 $\pm$ 0.38	0.87 NS	7.8 $\pm$ 0.22	-2.83 NS	1/2	10.0 $\pm$ 0.16	0.0 NS	8.2 $\pm$ 0.22	0.0 NS
1	9.6 $\pm$ 0.25	-2.98 NS	7.5 $\pm$ 0.16	-5.72 NS	1	9.6 $\pm$ 0.25	-2.98 NS	8.0 $\pm$ 0.94	-4.65 NS

Each value; a mean of five replicate

Table (2): Effect of different concentrations of each of seed or root exudate of Ricinus communis on shoot and root length of Sebania sesban

Relative conc. of seed exudate	Root length cm.		Shoot length cm.		Relative conc. of root exudate	Root length cm.		Shoot length cm.	
	t		t			t		t	
Control	7.1 $\pm$ 0.25		5.0 $\pm$ 0.36		Control	7.1 $\pm$ 0.25		5.0 $\pm$ 0.36	
1/16	8.0 $\pm$ 0.38	4.45 S	6.0 $\pm$ 0.38	4.26 S	1/16	8.2 $\pm$ 0.28	6.52 HS	6.9 $\pm$ 0.2	10.3 HS
1/8	8.7 $\pm$ 0.25	10.02 HS	6.4 $\pm$ 0.23	7.28 HS	1/8	9.2 $\pm$ 0.12	18.26 HS	7.0 $\pm$ 0.38	8.53 HS
1/4	8.4 $\pm$ 0.16	9.83 HS	5.6 $\pm$ 0.14	3.46 S	1/4	8.5 $\pm$ 0.16	10.58 HS	5.7 $\pm$ 0.16	4.0 S
1/2	7.0 $\pm$ 0.41	-0.46 NS	5.3 $\pm$ 0.16	1.7 NS	1/2	7.1 $\pm$ 0.25	0.0 NS	5.2 $\pm$ 0.14	1.15 NS
1	6.5 $\pm$ 0.38	-2.95 NS	4.7 $\pm$ 0.16	-1.7 NS	1	6.9 $\pm$ 0.14	-1.56 NS	4.8 $\pm$ 0.14	-1.15 NS

Each value is a mean of five replicates

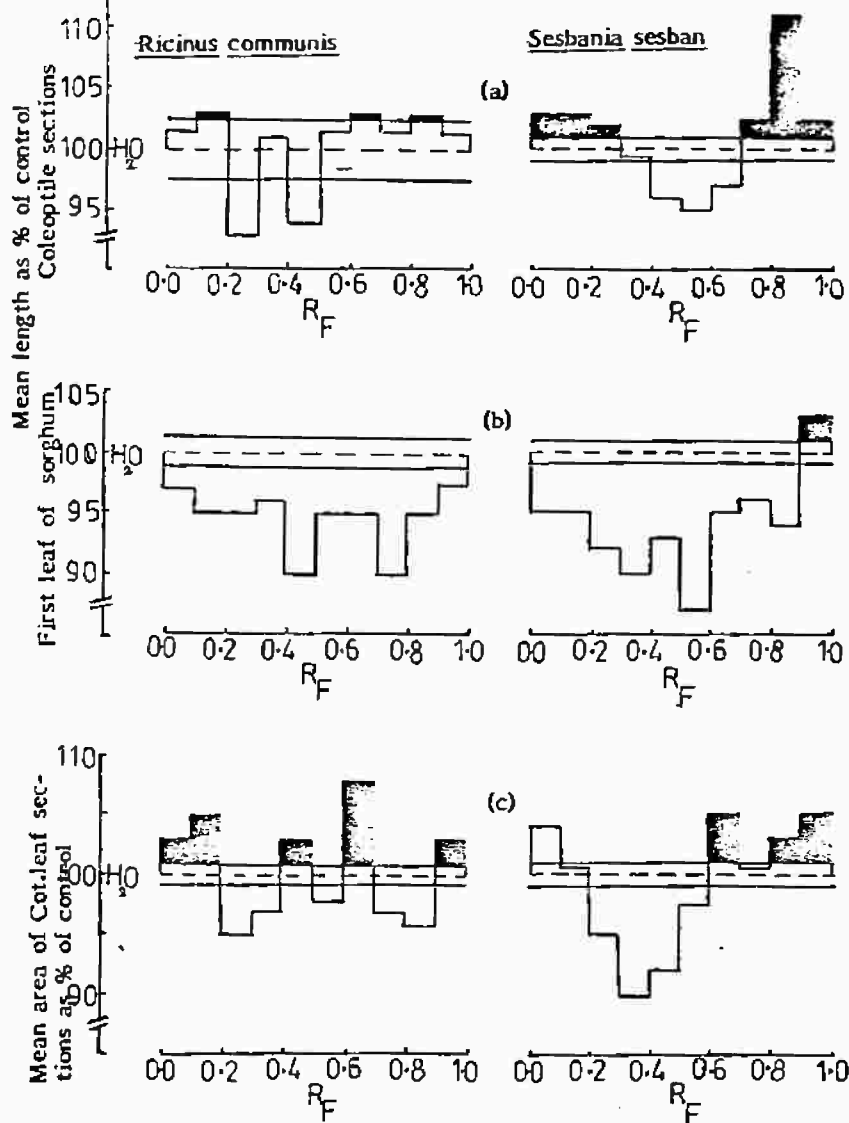


Fig. (1): Growth Regulators Exuded from Mature Seeds of *Ricinus communis* and *Sesbania sesban*.

- a) Auxins and inhibitors
- b) Gibberellins and gibberellin-like substances.
- c) Cytokinins

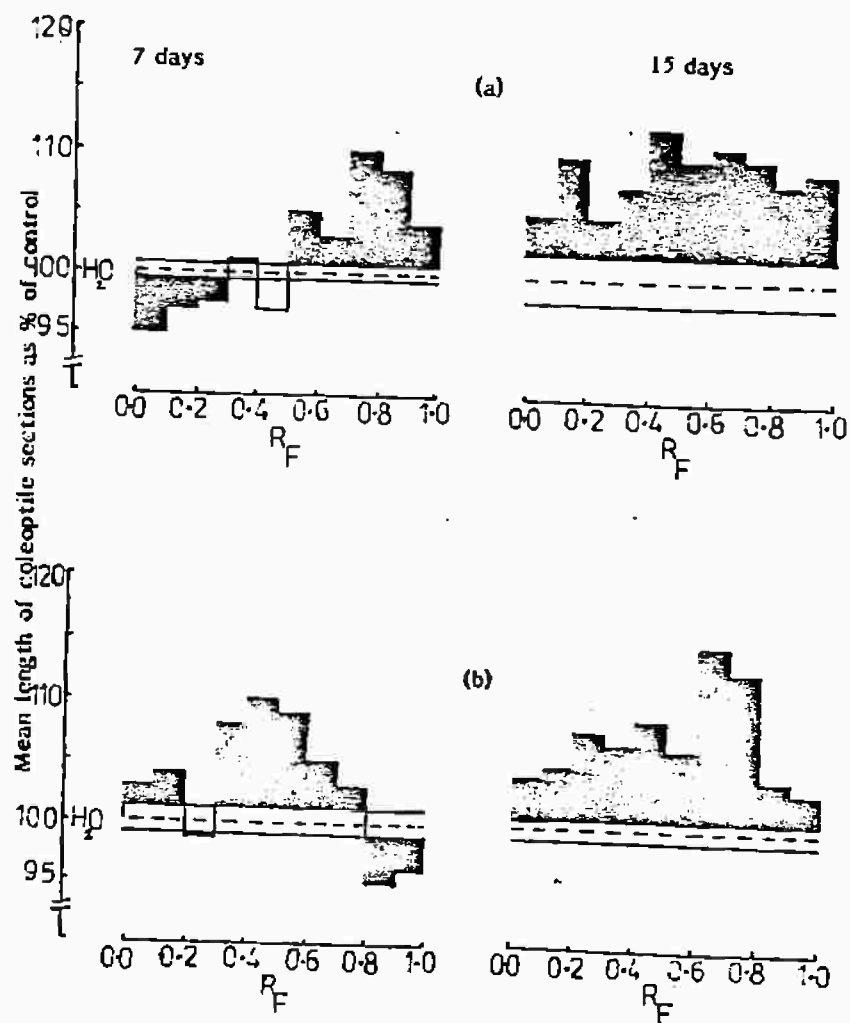


Fig. (2): Auxins and growth inhibitors exuded from:

- Ricinus communis* roots at 7 and 15 days age.
- Sesbania sesban* roots at 7 and 15 days age.

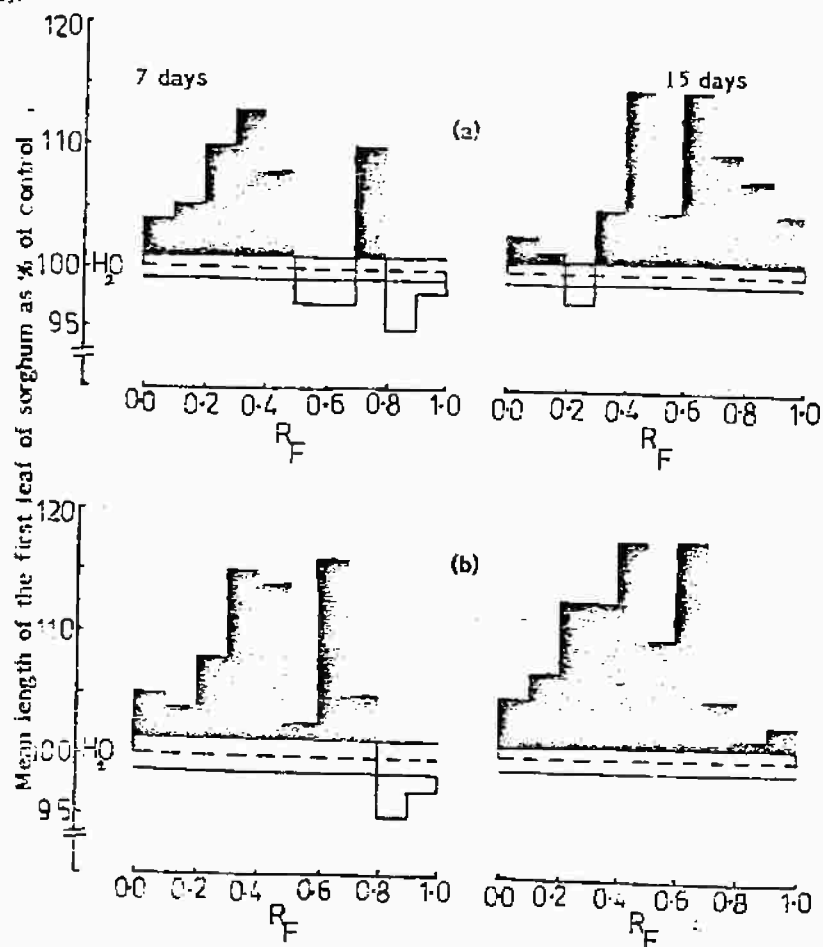


Fig. (3): Gibberellins and gibberellin-like substances exuded from:

a) *Ricinus communis* roots at 7 and 15 days age.

b) *Sesbania sesban* roots at 7 and 15 days age.

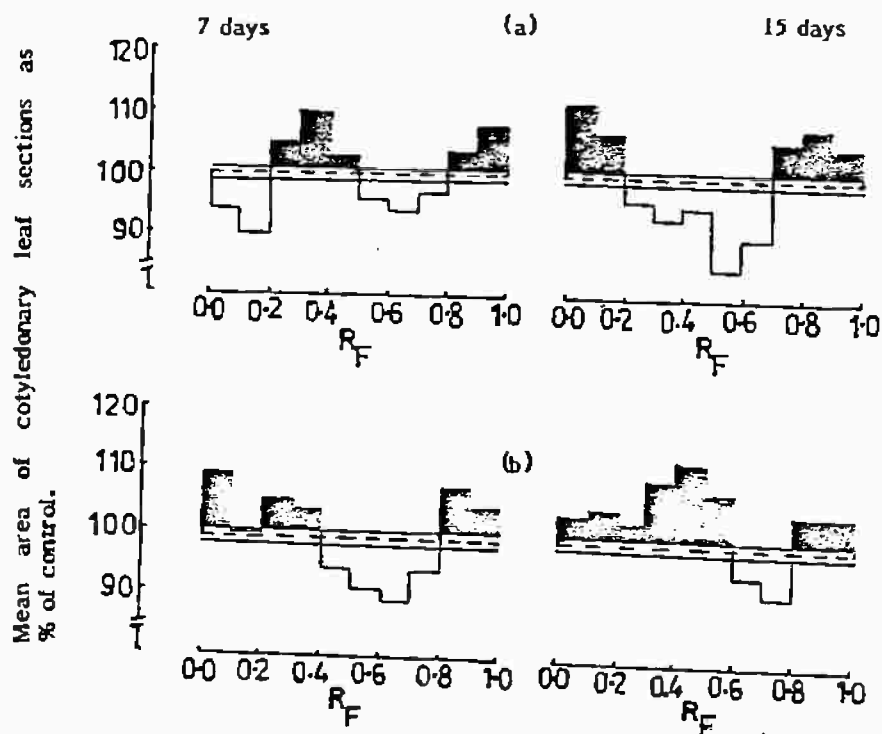


Fig. (4): Cytokinins exuded from:
a) *Ricinus communis* roots at seven and fifteen days age.
b) *Sesbania sesban* roots at seven and fifteen days age.