

STUDIES ON THE EFFECTS OF PODOPHYLLIN SUSPENSIONS IN DIFFERENT SOLVENTS ON PLANTS

I — Effects of podophyllin suspensions in acetone and alcohol
on root morphology and anatomy with reference to "Vicia-faba"

By

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Introduction

The effect of chemicals on root growth had been widely studied by many authors. Ashby (1951) studying the effect of certain acid growth regulating substances and their corresponding aldehydes on root growth found inhibition of root growth. Hussein and Hakeem (1960) studying the effects of podophyllin on *Vicia faba* and *Luffa cylindrica* found that podophyllin caused temporary inhibition followed by stimulation of root growth.

With regard to the effect of various chemicals on plant anatomy comparatively few studies were made.

Said and Naguib (1951) treating *Vicia* plants with colchicine noted definite xylem strands near the tip. Hussein and Hakeem (1960) noted several anatomical abnormalities in treated *Vicia* roots with aqueous podophyllin suspension — such as disappearance of meristematic zone, enlargement of cortical cells, pre-maturation of xylem and phloem elements and proliferation of pericyclic tissue.

As podophyllin is more soluble in alcohol and acetone than in water, Egypt. Pharmacopeia (1953), it was of interest to confirm the previously obtained results within a wider margin of concentration. Since alcohol and acetone may have their own effects on the plants, control experiments with alcohol and

acetone alone were conducted. It was also of interest to see whether alcohol and acetone act synergetically with podophyllin.

Material and Methods

Vicia faba seeds (var. Rebaya 34) soaked in water for 24 hours were treated with the experimental solutions for two, three or six hours. They were then washed with running water, and finally planted in pots containing saw dust. For each treatment 120 seeds were used. Seeds soaked in water for 24 hours were used as controls.

The treatments were carried out with aqueous alcoholic and aqueous acetone suspensions of podophyllin. (100 mg/100 ml 10% aqueous acetone or alcohol). Experiments with 10% aqueous alcohol, and 10% aqueous acetone were carried out for comparison.

The length of roots was measured every 2 days over a period of 14 days.

For anatomical studies, the roots were cut when reaching 1.5 cm., at 14 μ thickness, and stained using the technique of Sharman for meristems.

Results and Discussion

1. Effect on root germination

From Table I, the following conclusions were reached :

1. The treatment of the seeds in most of the above solutions resulted in a depression in the percentage of germination.
2. The 2-hour treatment resulted in a slight inhibition of germination.
3. No clear relation could be traced between the percentage of germination and the concentration of podophyllin.
4. No material difference between the behaviour of seeds treated with alcohol or with acetone solution was observed.

2. Effect on root growth

Inhibition of growth of the main root was a common character in all the treatments as compared with the controls (Figs. 1, 2, 3, 4, 5). The inhibiting effect of alcohol and of podophyllin in alcohol on root growth was stronger than that of acetone alone or of podophyllin in acetone, as seen from Text-Figs. 1-2). Another interesting phenomenon was that there was no relation between the time of treatment and the inhibition of growth.

a. **Effect of acetone and podophyllin in aqueous acetone.** In the two and six-hour treatments, there was significant inhibition of growth during the first 8 days of the experiments after which the inhibiting action decreased. The three-hour treatments showed slight inhibition of growth during the experimental period. In general, aqueous acetone affected more inhibition than podophyllin suspension in aqueous acetone (Fig. 1).

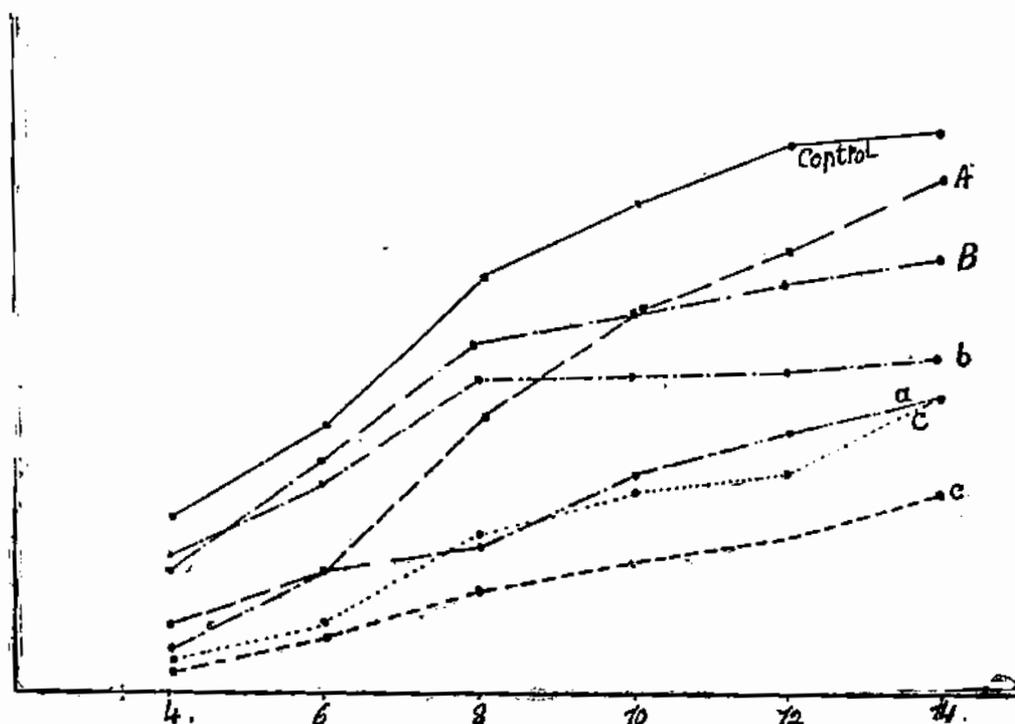


Fig. 1

Effect of podophyllin in aqueous acetone and of aqueous acetone on the root growth of *Vicia faba*.

b) **Effect of alcohol and podophyllin in alcohol.** During the first ten days the rate of inhibition of root growth was highly significant in the treatments with alcohol and podophyllin in alcohol for two and three hours. In the six hour treatments, the inhibition was strong over the time of the experiment.

It is obvious, that treatments with podophyllin in alcohol gave a stronger inhibiting effect than the same treatment with alcohol alone, especially the tow and three hour treatments (Fig. 2).

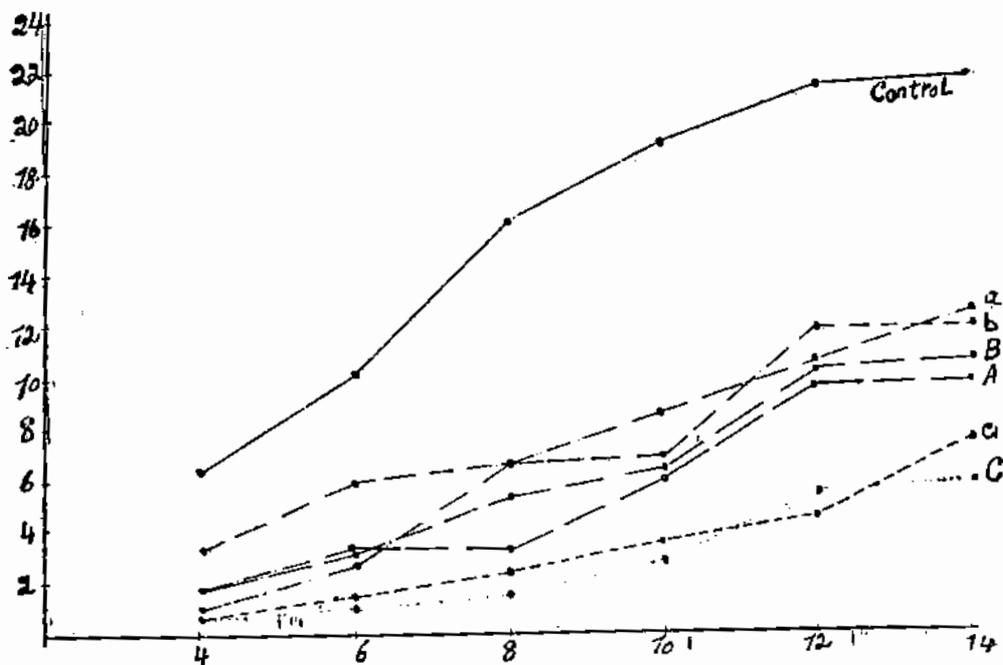


Fig. 2
Effect of podophyllin in aqueous alcohol and of aqueous alcohol on the root growth of *Vicia faba*.

Hussein and Hakeem (1960) reported that aqueous podophyllin suspension used for soaking *Vicia faba* seeds resulted in a stunted growth of the main root followed by recovery and further stimulation.

In the present work, podophyllin suspension in alcohol or acetone used for similar treatments showed in general stunted growth of the main root of *Vicia faba* seedlings in their early vegetation without any noticeable recovery. Inhibition of the

root growth of the plants treated with acetone, podophyllin in acetone, alcohol, and podophyllin in alcohol may be attributed to cessation of cell division in the apical meristem as the anatomical examination has revealed.

In addition to the inhibition of root growth, swelling of the hypocotyl, curvature of roots and division of main root tips were observed (Figs. 3, 4 & 5). These abnormalities were also resulted by aqueous podophyllin suspension (Hussein and Hakeem 1960).

In addition to the above peculiarities the following were observed in contrast to the action of aqueous podophyllin suspension.

1. Short thick roots replaced by a tuft of secondary roots which appeared to originate from the hypocotyl (Fig. 3).
2. Division of the root tips of the lateral roots. Anatomical examination of the treated main roots revealed, however, the presence of dead cells in the middle of the root tip, to which this phenomenon could be attributed (Fig. 11, 12).

In general, podophyllin in aqueous alcohol exhibited the most destructive effect on the root system. Fig. 4 shows thickened hypocotyl, whereby the main and secondary roots are severely inhibited in growth, swollen and divided at their distal ends.

B. Anatomical effects :

Anatomical studies of the untreated (Figs. 6, 8 & 10) and treated roots of *Vicia* (Figs. 7, 9, 11 & 13) showed that visual histological changes took place. The percentage of roots showing these changes increased with increase of concentration in both suspensions of podophyllin in alcohol and acetone. In case of roots treated with different concentrations of alcohol and acetone there was no clear difference as compared with the normal roots.

In the treated root tips a precortex extended over the zone of the promeristem without differentiation into a root cap. The cells of this meristematic region were all conspicuously vacuolated in contrast to the richly protoplasmic very small non-vacuolated cells of the normal tip. A clearly differentiated dermatogen was also lacking.

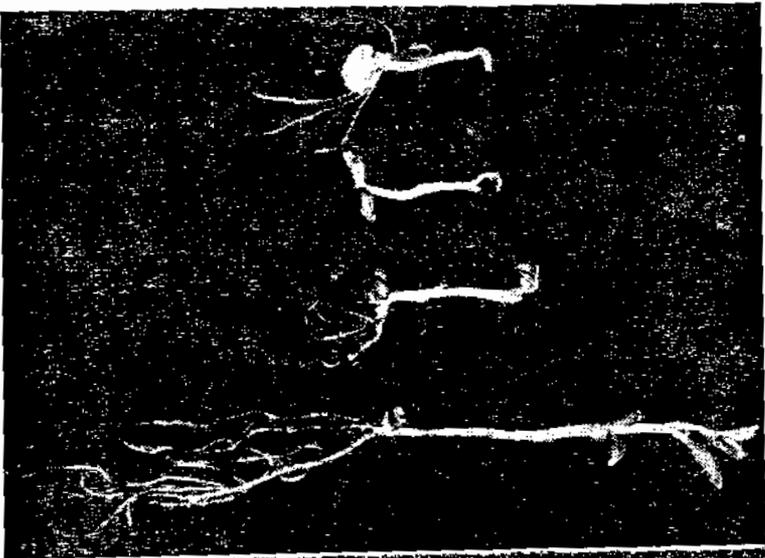


Fig. 3 : a, b, c, 2 weeks old seedlings from treated seeds showing, divided and thick roots, d, Control seedling.

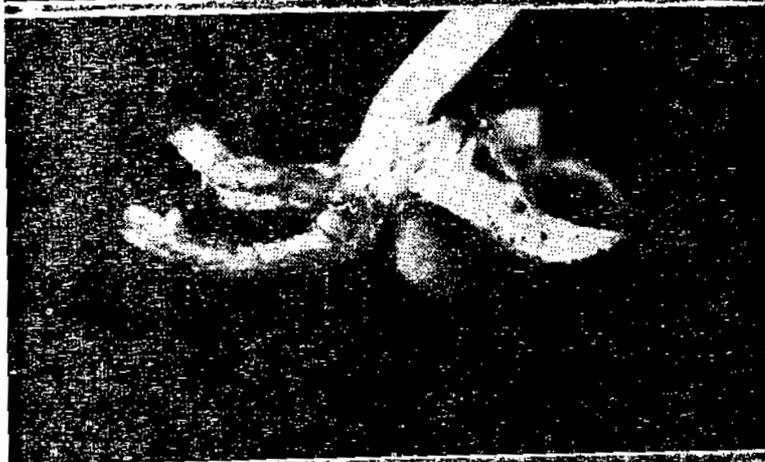


Fig. 4 : Enlarged seedling of (b) showing inhibited abnormal divided root.

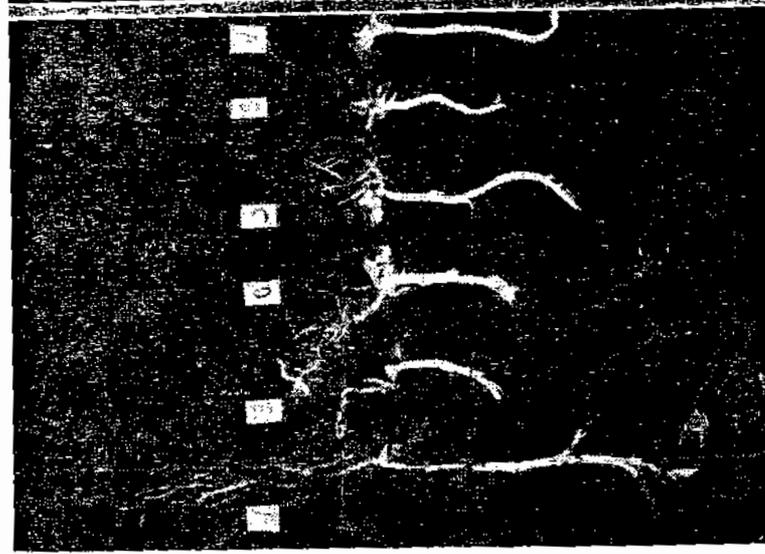


Fig. 5 : A, B, C, D, E, 2 weeks old seedlings from treated seeds showing different types of abnormalities such as inhibition & curvature. F, 2 weeks old seedling of control Plant.

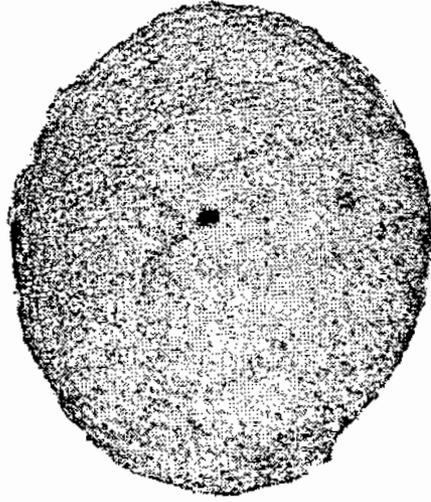


Fig. 6 : T.S. in Untreated *Vicia* root
220 u from the tip.

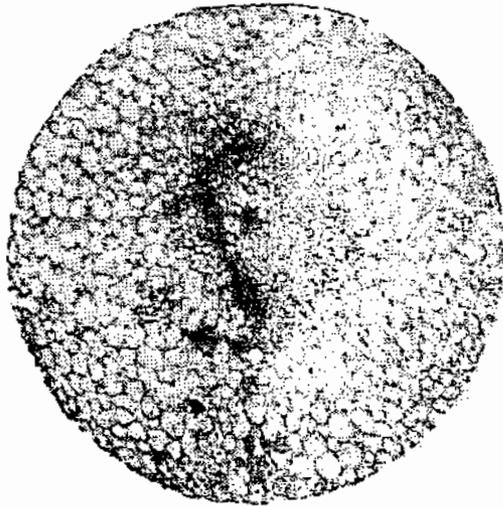


Fig. 7 : T.S. in treated *Vicia* root with
100 mg/pod/100 ml 10% aqueous acetone
for 3 hours (220 u from tip).

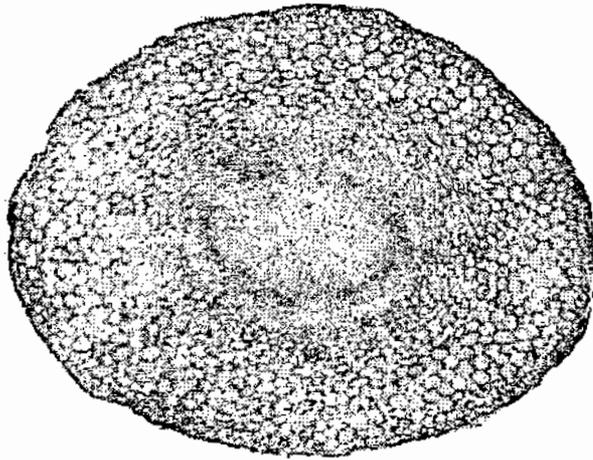


Fig. 8 : T.S. in Untreated *Vicia* root 440 u from the tip.

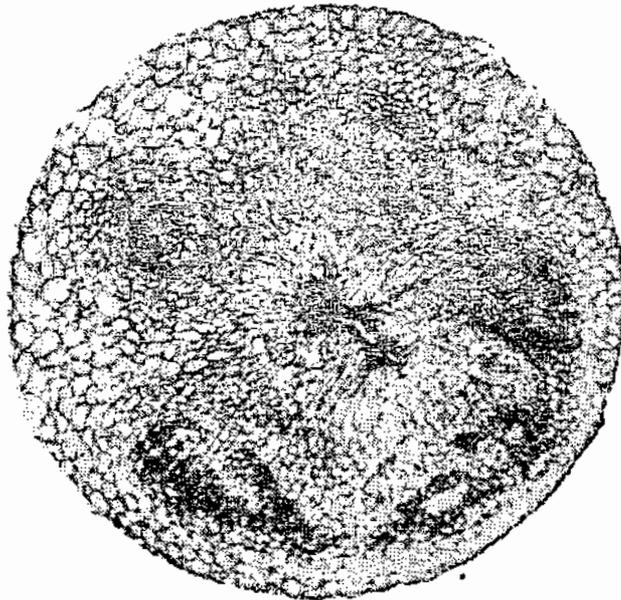


Fig. 9 : T.S. in treated *Vicia* root with 100 mg pod/100 ml 10% aqueous acetone for 3 hours (440 u from tip).



Fig. 10 : L.S. in untreated
root ($\times 10$).

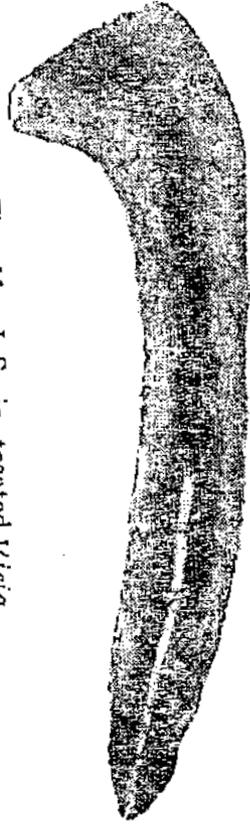


Fig. 11 : L.S. in treated *Vicia*
root with 100 mg pod/100 ml
10% aqueous alcohol for 2 hours
($\times 10$).

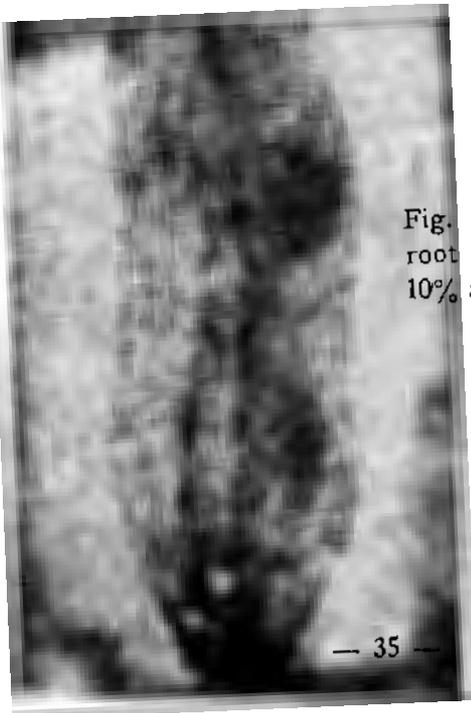


Fig. 12 : L.S. in treated *Vicia*
root with 100 mg pod/100 ml
10% aqueous acetone for 6 hours
($\times 10$).

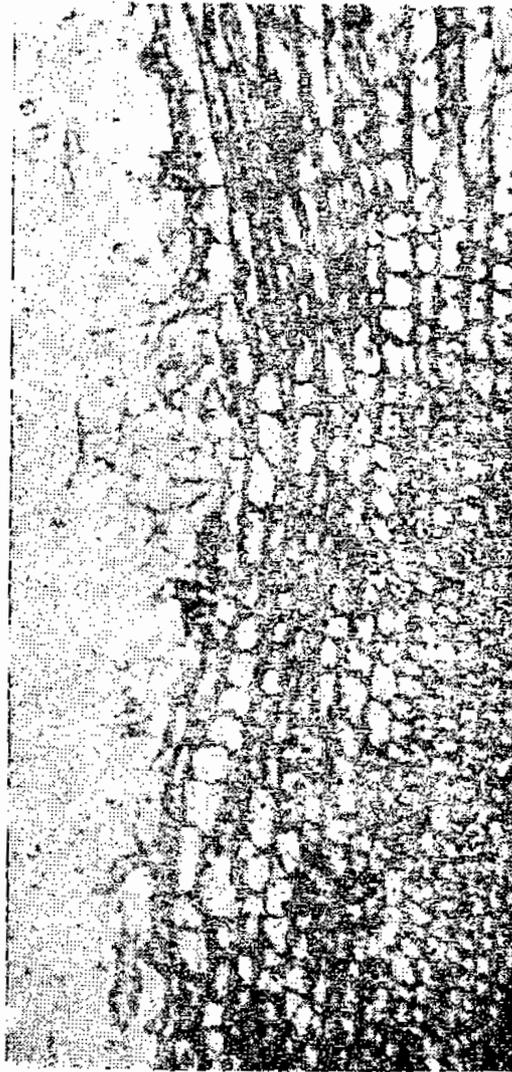


Fig. 13 : Enlarged part of Fig. 11
Showing fuzz of hairs ($\times 100$).

Abnormal enlarged cortical cells were observed with no apparent increase in the number of vertical rows of cells (Figs. 7, 9). Proliferation of the pericycle cells (Figs. 9, 12) was observed and early maturation of xylem and phloem elements was observed in treated roots (Figs. 7, 9, 11). The distance between the first xylem vessel and the tip decreased with increase of concentration and time of treatment. These prematured vessels were mostly pitted or reticulate.

Prolonged treatments (6 hours) induced degenerated or dead cells at the root tip.

These necrotic cells occurred sometimes also in longitudinal strands apparently corresponding to the strands of the protoxylem initials (Fig. 12).

Some of the roots treated with podophyllin in aqueous acetone for three hours showed stimulation of root hair formation, a thick fuzz of root hairs was observed near the root tip (Fig. 13) Ashby (1951) attributed this phenomenon to the occurrence of more epidermal cells. According to Borgstrom (1939) root hair formation is an auxin response.

These anatomical modifications of the roots after treatments with podophyllin suspension in aqueous acetone or alcohol agree to a great extent with the results of some authors using different chemicals, either polyploidising agents or growth regulating substances on different plants. They are also similar to the effect of aqueous podophyllin suspension on *Vicia* and *Luffa* roots.

Table I

Treatments	Percentage of root germination after					
	4 days			6 days		
	2h.	3 h.	6 h.	2 h.	3 h.	6 h.
1. Control (water)	100	100	100	100	100	100
2. 100 mg Pod/100 ml 10% aqueous acetone	65	100	92	75	90	82
3. 10% aqueous acetone	80	100	100	80	90	78
4. 100 mg Pod/100 ml 10% aqueous alcohol	90	85	96	75	70	60
5. 10% aqueous alcohol	80	95	80	80	70	60

Summary

1. Seeds of *Vicia faba* were treated with podophyllin suspensions in water, aqueous alcohol, and aqueous acetone, for 2, 3 and 6 hours. The effects on the growth and anatomy of roots were studied.
2. Both root growth and germination were inhibited by most of the treatments.
3. Swelling of the hypocotyl, division of the distal ends of both primary and secondary roots and curling of roots upwards were usually observed.
4. The anatomical responses of the roots were :
 - a. Partial disappearance of root cap and meristematic zone.
 - b. Hypertrophy of root cells.
 - c. Proliferation of the pericyclic cells.
 - d. Stimulation of root hair formation.
 - e. Early maturation of the vascular elements.

References

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