THE RELATION OF CHEMICAL STRUCTURE TO BIOLOGICAL ACTIVITY IN CERTAIN ORGANIC COMPOUNDS

By

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A THESIS

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Appreciation is due to Dr. C. E Wildon, Dr. H. M. Sell, Dr. L W. Mericle and Dr. L. M. Turk for their kind advices.

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Lastly, the author is deeply indebted to his brothers for their thoughtful encouragements in prosecuting the advanced studies.

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INTRODUCTION

Discovery of the effect of beta-indoleacetic acid on plant growth opened an extensive field of research for plant physiologists As a result, many investigations have been made to discover compounds which would simulate the action of indoleacetic acid The structural requirements of compounds active as auxins or anti-auxins have been investigated extensively and several theories have been formulated Recently many different new synthetic compounds have been found to be active as plant growth substances, and hence have led to a restatement of these structural requirements for growth regulators. Since the discovery of high activity of 2, 3, 6-trichlorobenzoic acid, 2, 6-dichlorobenzoic acid and 1-naphthoic acid as growth regulators, the side chain in the form originally postulated is no longer a criterion for the structural requirement The structural diversity of known growth regulators has proved to be a valuable tool for investigations into the relationships among physiological activities. The mechanism of action concerning the physiological processes which account for the chemical control of growth is not yet completely understood Therefore, research into the relationship between chemical structure and morphogenetical activity may provide a short cut to the discovery of other highly active compounds, or provide fundamental knowledge on mode of action.

The initial scope of this work was to screen out compounds which might be of some value in horticultural practices, and an attempt was then made to correlate the structures and activities of some tested compounds

Biological Activity of Organic Chemicals on Cucumber Root Growth

Review of Literature

Beta-indoleacetic acid was isolated from human urine in 1934 by Kögl (36) and his collaborators at Utrecht; it was found to be markedly active in promoting cell elongation in plants Following this discovery, Zimmerman and Hitchcock (92) showed that phenyl, alpha and beta-naphthalene, anthracene. acenaphthene, and fluorene acetic acids all possessed growth regulating activity Many other compounds, which included such indole-carboxylic acids as alpha (indole-3)-propionic acid and y-(indole-3) butyric acid have been announced by Zimmerman et al. (90) to possess growth regulating properties

Following the discovery of Irvine (32) that beta-naphthoxy-acetic acid possesses activity, Zimmerman et al. (91) examined a large number of substituted phenoxy and naphthoxy acids, many of which were found to be highly active Other types of compounds are now known to influence plant growth.

Structure and Activity

During the past few years, many organic compounds were found active in regulating the growth of plants, and attempts were made to correlate the morphogenetical activity with the chemical structure

Koepfe, Thimann and Went (35) postulated that certain features of molecular structure and configuration are required for activity These features based on the pea test, were: (a) a ring system nucleus; (b) a double bond in this

ring; (c) a side chain; (d) a carboxylic group (or a structure readily converted to a carboxyl group) on this side chain; at least one carbon atom was removed from this ring, and (e) a particular spatial relationship between the ring system and carboxyl group

As more compounds became known, these five requirements appeared to fall short Some compounds were found to meet the requirements, although they were inactive, while others did not meet their requirements even though they were active. These developments have been reviewed in a number of papers by Audus (8), Linser (42), Norman et al (52), and Thimann (70).

Attempts were then made by Veldstra (76) to condense the five requirements into two: (1) a basal ring system with high surface activity; and (2) a carboxyl group in a very definite spatial position with respect to the ring system These requirements were later formulated in greater detail by Veldstra and Booij (78), as a basal ring system (non polar part) with high interface activity and a carboxylic group (polar part), in general a group of acidic character, in a spatial position with respect to this ring system The classical example illustrating Veldstra's theory of configuration confering activity of cinnamic acid, in which the cis form fulfills Veldstra's requirement and is an auxin, whereas in the trans form the side chain cannot exist in any other plane than that of the ring and is not an auxin In another instance,

tetrahydronaphthylideneacetic acid has a cis and trans form of which this cis form is active and trans form is not In this case the activity cannot be explained by Veldstra's scheme. Thimann (70) remarked that both isomers must exist with the carboxyl in the same plane as the ring It was further discussed by Muir et al (47) the position on the benzene ring adjacent to the point of attachment of the side chain is directly involved in the growth reaction. It means if the compound is to have activity, at least one of the ortho positions needs to be free.

Optical isomers can also influence auxin activity as is evident in 2, 4-dichlorophenoxy-alpha-propionic acid which exists in dextro and levo forms. Thimann (70) found that the dextro isomer is an active auxin and levo is essentially inactive It was also confirmed from the investigations of Matell (43) and Åberg (1) in many cases where levo isomers were found as anti-auxins

Some of the important works which are connected with this investigation are discussed below.

The Ring System

Norman and Weintraub (52) explained that most active compounds possess a benzene nucleus or fused benzene nucleus having an appropriate side chain. Booij (15) comparing naphthalene-1-, indole-3, indene-3, and coumarone-3-acetic acid established that a fused nucleus has a marked

effect on activity The values for indene and coumarone were practically identical

Substituted Benzoic Acid

Zimmerman et al (91) reported first of all a mild activity for cell elongation with 2-bromo-3-nitrobenzoic acid and formative effects with 2-chloro-5-nitro- and 2, 3, 5-tri iodobenzoic acid. Bentley (13) found 2, 3, 6-trichloro-benzoic acid to be highly active in straight growth Later, the activity was also noticed by Thimann (72), Veldstra (80), and Zimmerman et al. (93). Several other substituted benzoic acids were investigated by Muir and Hansch (48), Veldstra et al (79), and Zimmerman et al. (93).

Naphthalene and Related Compounds

The introduction of a second carboxyl group in the side chain was investigated by Veldstra et al. (79). They observed no effect with (naphthalene-1-methyl) malonic acid, (naphthalene-1-methyl) bromomalonic acid, and alpha-(naphthalene-2-methyle) succinic acid in the pea test

Veldstra (77) found a high activity with 1-naphthoic acid Its 1, 2, 3, 4 tetrahydro derivatives were investigated. The high activity was noticed Mitsui et al (46) had investigated hydrogenated 1-naphthoic acid. The 1, 4-dihydro and 1, 2, 3, 4-tetrahydro derivatives were found to be strongly active

It is evident from the above results that these compounds acted in a manner similar to auxin. However, they differed widely in respect to

their specific action The activity varied with the nature of the assay used in the determination. Thimann (68) has evidently shown in his work that idene-3-acetic acid had a very small and cumaryl-2-acetic acid no activity in the Avena Curvature test, whereas considerable activity was shown both in Avena Coleoptile straight growth and pea test

Methods and Materials

Cucumber Root Test

The cucumber root test, as used by Ready et al (55), and Alamercery (5) and extensively used in this laboratory, was found to be a fairly accurate and rapid bioassay. Therefore, this simple and rapid quantitative method was deemed desirable to determine the effects upon growth of a great number of solutions of chemicals. The uniform seeds of cucumber (Cucumus sativus var. Marketer) were used for these experiments

The chemicals were received from Upjohn Chemical and Eastman Kodak Companies. The structures of some compounds used in the investigation are given in Figures 1, 2, 3, and 4. The chemicals were first dissolved in ethyl alcohol to facilitate their solubility in water

Ten seeds were uniformly distributed upon a piece of Whatman No 1 filter paper in petri dishes The filter paper was then impregnated with five mililiters of solution of a predetermined concentration of the compound to

be tested. The concentrations used were 25, 50, 100, 150, and 200 parts per million Later, the compounds which were found active were again tested at 1 and 10 ppm

All petri dishes had been previously cleaned by soaking overnight in activated charcoal suspension, then rinsing and washing with soap, and then rinsing again with tap water followed by distilled water

Three petri dishes were used for each treatment, providing three replicates The seeds were allowed to germinate for four or five days under laboratory conditions[1] Ten seedlings were selected at random from each treatment, and the length of the primary roots were measured The number of branchings on the primary root was also recorded To compare each treatment separately, all data were statistically analyzed, and the significance of differences between averages of two treatments was statistically compared by the "T" test (60)

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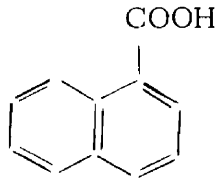
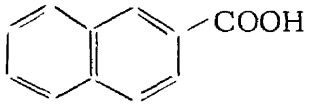
[1] In a research laboratory at the Department of Horticulture of Michigan State University, East Lansing, Michigan, July to December 1955 The temperatures ranged between 70° and 85° F The alternating photo and dark period varied with the season.

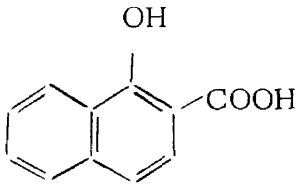
Figure No. 1



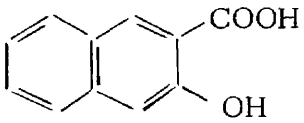
1-Naphthoic acid



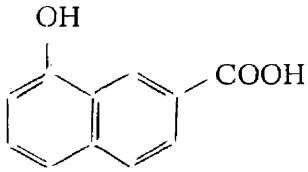
2-Naphthoic acid



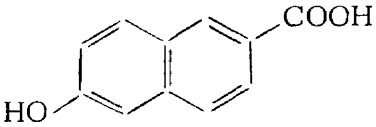
1-Hydroxy-2-naphthoic acid



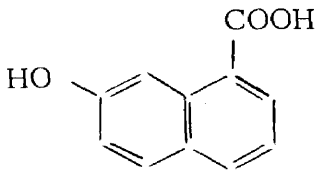
3-Hydroxy-2-naphthoic acid



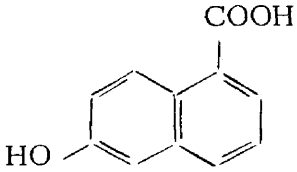
8-Hydroxy-2-naphthoic acid



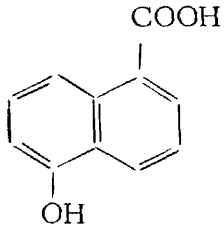
6-Hydroxy-2-naphthoic acid



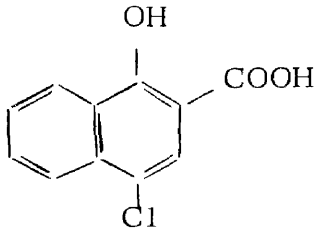
7-Hydroxy-1-naphthoic acid



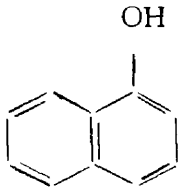
6-Hydroxy-1-naphthoic acid



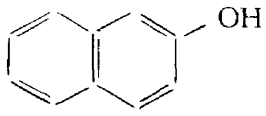
5-Hydroxy-1-naphthoic acid



1-Hydroxy-4-chloro 2-Naphthoic acid

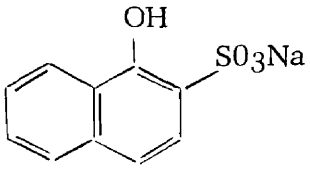


1-Naphthol

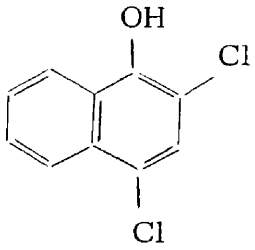


2-Naphthol

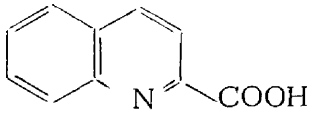
Figure No 2



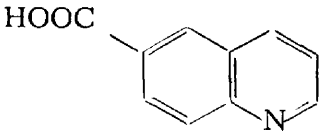
1-Hydroxy-2-naphthalene sulfonic acid (Sodium salt)



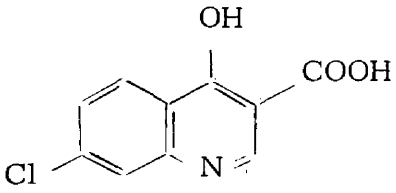
2, 4-Dichloro-1-naphthol



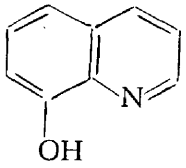
Quinaldinic acid



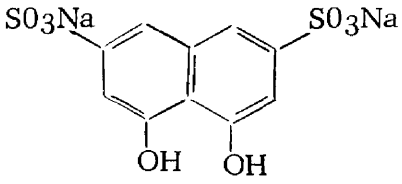
6-Quinolinecarboxylic acid



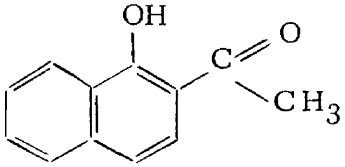
7-Chloro-4-hydroxy-3-quinolinecarboxylic acid



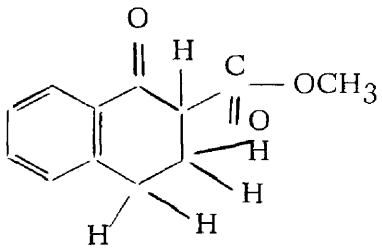
8-Quinolinol



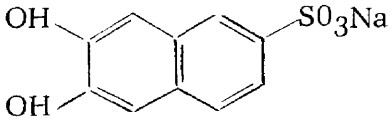
4, 5-Dihydroxy-2, 7-naphthalene disulfonic acid (Disodium salt)



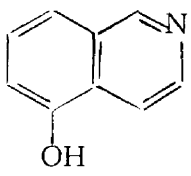
1-Hydroxy-2-acetonaphthone



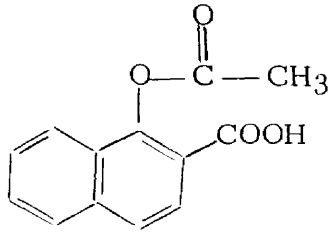
3, 4-Dihydro-1-keto-methyl-2-naphthoate



6, 6-Dihydroxy-2-naphthalene sulfonic acid (Sodium salt)

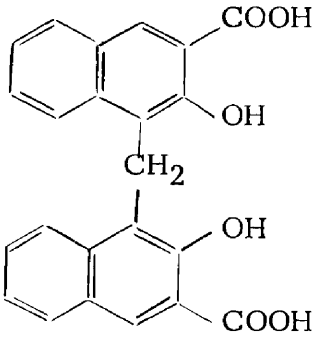


5-Isoquinolinol

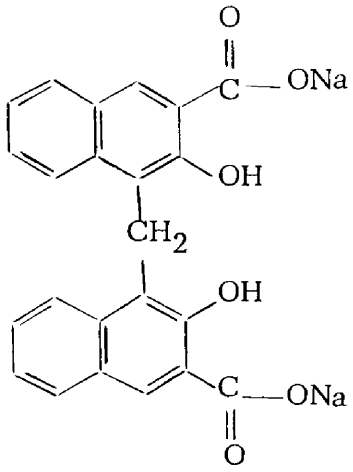


1-O-Acetyl naphthoic acid

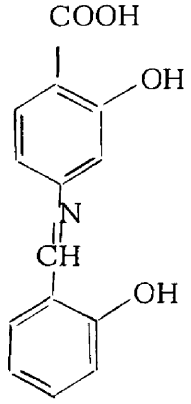
Figure No. 3



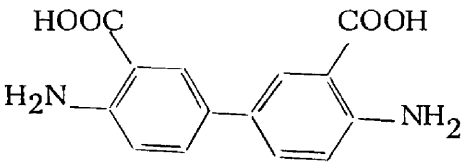
4, 4'-Methylenebis-3-hydroxy-2-naphthoic acid



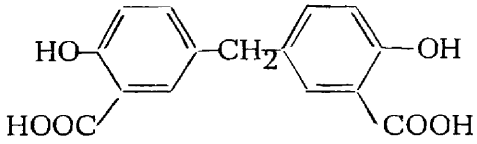
4, 4'-Methylenebis-3-hydroxy-2-naphthoic acid (Disodium salt)



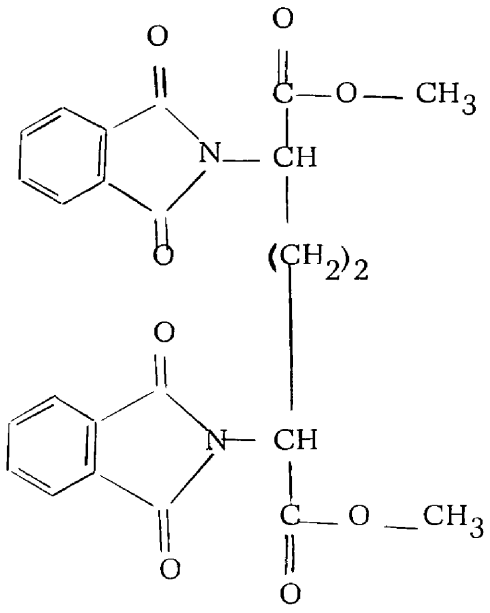
4(2-Hydroxy benzal amino) salicylic acid



4, 4'-Diamino-3, 3-dicarboxy biphenyl

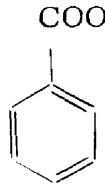


bis(3-Carboxy-4-hydroxy phenyl) methane

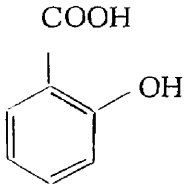


2, 5-Diphthalimido dimethyl adipate

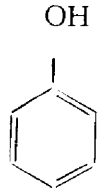
Figure No. 4



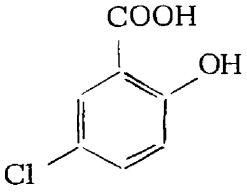
Benzoic acid



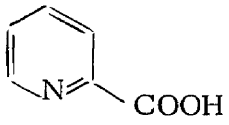
Salicylic acid



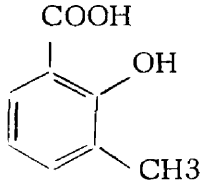
Phenol



5- Chlorosalicylic acid



2-Picolinic acid



2-Hydroxy-3-methyl-benzoic acid

Results

Significant differences in root growth was observed with various chemical treatments

Cucumber roots made significantly greater growth without injury in solutions of 25 ppm of each of the following: 1-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid (disodium salt) than in control (Table 1). However, 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid was found more effective on root elongation at 50 ppm Naphthaleneacetic acid used as a standard growth regulator had markedly inhibited the primary roots, though it significantly increased the number of root hairs

Similar results were obtained when the compounds were retested under constant temperature conditions at 80° F, with the exception of 3-hydroxy-2-naphthoic acid. The maximum growth for this compound was obtained at a concentration of 50 ppm (Table 1)

When these materials were tested at a temperature of 90° F, inhibition in activity of cucumber roots occurred with all treatments No significant differences in root growth between treatments were recorded, although slight differences were found over the control (Table 2)

As a result of these tests, two compounds (1-naphthoic acid and 6-quinolinecarboxylic acid) were discovered that closely resembled the

activity of naphthalene-acetic acid (Tables 3, 4 and 7, and Figures 5 and 10) Root elongation was significantly inhibited, even at 1 ppm and an increased number of root hairs were noticed on the primary roots This is an effect which is quite similar to the response of naphthaleneacetic acid on cucumber root growth Other compounds, 2-naphthol and 8-quinolinol at 25 ppm and 1-naphthol and 1-hydroxy-2-naphthalene sulfonic acid (sodium salt) at 50 ppm were found to significantly stimulate root elongation (Table 3) Many compounds had no significant effect (Table 3)

To compare the activity of 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid with other related compounds, two experiments were conducted and the results are presented in Table 4 Many of these related materials had pronounced biological activity, quinaldinic acid significantly inhibited root growth at 25 ppm concentration, but the root hairs were also inhibited (Figure 6) Root growth was also significantly retarded at 25 ppm of 2-naphthoic acid Significant increased root elongation was observed with treatment by 4-chloro-1-hydroxy-2-naphthoic acid at 25 ppm (Figure 8). The remainder of the compounds under this test had no significant effect on the cucumber root growth (Table 4).

Since the introduction of an hydroxyl group on the fused nucleus compound had modified the activity, it was thought desirable to test single ring compounds with hydroxyl groups or acid groups, or both, on the ring

to correlate the activity. Single ring heterocyclic compounds were also tested Growth of cucumber roots was markedly retarded in solutions of 25 ppm of 2-picolinic acid and 5-chlorosalicylic acid (Table 5) An interesting response was noticed with 2-picolinic acid (Figure 11), the number of branchings on the primary root decreased markedly by increased concentrations; also an interesting response of 2-hydroxy-3-methylbenzoic acid was recorded, the primary roots were unusually thin (Figure 12). Phenol treatments had no significant effect, however, salicylic acid markedly retarded root growth at 10 ppm and at increasing concentrations. Benzoic acid increased root growth at 1 ppm, but retarded at higher concentrations. The hydroxyl group does not have an effect unless the acid group is present, and it apparently modifies the activity of the acid group

The compounds showing the greatest activity on cucumber root were retested at 1 and 10 ppm to determine their activity in extreme dilutions (Table 7) 6-quinolenecarboxylic acid and 1-naphthoic acid retarded root growth significantly at 1 ppm, whereas 2-picolinic acid and quinaldinic acid were found to inhibit growth at 10 ppm Significant stimulation on root elongation was observed at 10 ppm with compounds 2, 4-dichloro-1-naphthol, 3-hydroxy-2-naphthoic acid, and 1-hydroxy-2-naphthoic acid

An experiment was designed to determine whether or not root stimulation with 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid could be

further enhanced by simultaneous application of a nitrogen fertilizer (Table 9) It is evident that urea and KH_2PO_4 had no significant additive effect on root elongation.

In order to explain why certain seemingly unrelated compounds stimulated root growth, it was suggested (Leeper 40) after study of the chemical structure involved, that these compounds might form a complex ion with certain chemicals and hence act as chelating agents The metal chelate compound might remove excess quantities of certain chemicals from the roots and thus permit elongation.

In order to test this theory, several chelating materials were investigated It was found that several compounds showed biological activity Resorcinol, benzotriazol, 2, 2'-biquinoline, diethyldithiocarbamic acid, 2, 4-dihydroxy-acetophenone and mercarptoacetic acid at a concentration of 25 ppm were active in stimulating root growth. This was highly significant 1-amino-4-hydroxyanthraquinone, furil dioxime and thiazole yellow also increased root growth; this, however, was significant only at the L S. D. 5% level (Tables 10 and 11) A retarding effect on root elongation was recorded when the compounds phenylglyoxal aldoxime, and 2, 2, 4, 4, 6, 6-hexanitrodiphenylamine were used (Tables 12 and 13)

TABLE 1

Effects of Various Concentrations of Organic Compounds on the Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes.

Organic Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L.S.D. 5% / L.S.D. 1%	*Average Number of Branching Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	150		0	25	50	100	150
H_2O - Control	68.5						20.7				
1-Hydroxy-2-naphthoic acid		99.6	98.2	76.1	61.1	3.9 / 5.3		30.7	28.9	25.4	18.7
3-Hydroxy-2-naphthoic acid		107.2	95.0	82.5	69.6	4.8 / 6.4		30.3	29.7	24.3	23.6
Naphthaleneacetic acid		5.0	4.0	3.0	2.0			--	--	--	--
4,4'-Methylenebis-3-hydroxy-2-naphthoic acid	80.5	89.8	112.1	99.6	95.0	5.3 / 7.0	24.8	27.5	30.6	25.6	25.6
4,4'-Methylenebis-3-hydroxy-2-naphthoic acid (Disodium salt)	67.3	76.0	65.4	48.3	33.9	4.2 / 5.6	19.5	19.1	16.8	14.6	13.6
H_2O - Control	79.7						27.7				
1-Hydroxy-2-naphthoic acid		98.5	92.4	82.9	65.3	5.2 / 6.9		34.2	29.5	27.5	19.8
3-Hydroxy-2-naphthoic acid		90.6	110.5	100.3	85.4	5.6 / 7.5		31.1	34.0	32.3	28.9
4,4'-Methylenebis-3-hydroxy-2-naphthoic acid		99.6	106.8	114.6	95.8	4.8 / 6.2		29.9	31.4	36.2	30.8
Naphthaleneacetic acid		8.0	6.0	4.0	3.0			4.0	3.0	--	--

*Average of ten seedlings.

TABLE 2

Effect of Various Concentrations of Organic Compounds on the Elongation and Branching of Tap Roots of Cucumber Seedlings Grown at 90° F for Three Days in Petri Dishes.

Organic Compounds	*Average Length of Tap Roots (mm) Concentrations of Solutions in Petri Dishes (ppm)					L S D.	
	0	25	50	100	150	5%	1%
H_2O - Control	18.3						
1-Hydroxy-2-naphthoic acid		21.3	21.2	18.3	17.0	1.4	1.8
3-Hydroxy-2-naphthoic acid		19 6	21.3	21.9	18.0	1.9	2 6
4, 4'-Methylenebis-3-hydroxy-2-naphthoic acid		22 6	20.4	20.5	18 3	1.3	1 7
4, 4'-Methylenebis-3-hydroxy-2-naphthoic acid (Disodium salt)		19.0	19.2	19 3	18.2**		
Naphthaleneacetic acid		3.0	--	--	--		

*Average of ten seedlings.

**Not significant.

TABLE 3

Effects of Various Concentrations of Organic Compounds on the Elongation and Branching of Tap Roots of Cucumber Seedlings grown under Laboratory Conditions for Four Days in Petri Dishes.

Organic Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L. S. D. 5% / L. S. D. 1%	*Average Number of Branching Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	200		0	25	50	100	200
H_2O - Control	67.3						28.7				
6-Hydroxy-1-naphthoic acid		57.5	55.6	46.6	36.4	6.5 8.6		26.7	26.5	25.3	22.2
H_2O - Control**	69.1						28.4				
1-Naphthol		82.5	84.7	61.2	39.4	8.7 11.5		32.4	32.0	25.3	15 4
2-Naphthol		77.3	61.3	38.8	29.4	8 0 10.7		31.3	28.0	22.0	13 6
2,4-Dichloro-1-naphthol		77.3	83.2	80.8	68.4	8.1 10.8		31,9	31.2	28.3	27 4
H_2O - Control	63.1						28 3				
1-Hydroxy-2-naphthalene sulfonic acid (Sodium salt)		63.9	76.2	76.6	58.3	5.8 7.8		27.7	30.5	28.8	23 6
6-Quinolinecarboxylic acid		13.0	9 5	6.8	5.4	2.6 3.5		10.9	10.0	6.7	6 3
7-Chloro-4-hydroxy-3-quinolinecarboxylic acid		56.1	55.7	51.4	44,9	6.2 8 3		24.0	25.0	23.8	21.1
8-Quinolinol		72 4	72.1	64.1	59.6	5.4 7.3		29.2	28 0	27 7	24 6

*Average of ten seedlings

**Observations were recorded after five days.

TABLE 4

Effect of Various Concentrations of Organic Compounds on Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes

Organic Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L. S. D. 5% L. S. D. 1%	*Average Number of Branching Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	200		0	25	50	100	200
H_2O - Control	62.8						20.5				
1-Naphthoic acid		14.6	10.1	8.1	6.0	3.5 4.7		9.4	8.2	5.9	--
2-Naphthoic acid		53.6	43.8	23.0	--	5.3 7.0		19 8	13.9	5.7	--
4-Chloro-1-hydroxy-2-naphthoic acid		79.9	71.1	58.0	39.2	4.9 6.6		21.8	20 1	19.1	14 0
Quinaldinic acid		42 9	24 9	13.5	4.3	4.6 6.1		14.0	6 3	--	--
4, 4'-Methylenebis-3-hydroxy-2-naphthoic acid				77.9						21.5	
H_2O - Control	75.7						26.5				
4, 4'-Methylenebis-3-hydroxy-2-naphthoic acid				89.5						27 8	
5-Hydroxy-1-naphthoic acid		68.5	53.9	39.1	18.3	5.5 7.3		25.4	23.4	20.0	13.1
7-Hydroxy-1-naphthoic acid		70.3	66.9	61.9	58.3	6.6 8.8		26 1	25.7	23 2	19 1
6-Hydroxy-2-naphthoic acid		66.7	55.5	45.4	42.6	5.6 7.5		27.4	22 5	22.0	20 7
8-Hydroxy-2-naphthoic acid		76.4	79.2	79.0	63.1	6.4 8 6		28 5	32.7	27.5	24 3

*Average of ten seedlings.

TABLE 5

Effect of Various Concentrations of Organic Compounds on Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes.

Organic Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L. S D. 5% L. S. D. 1%	*Average Number of Branching Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	200		0	25	50	100	200
H_2O - Control	76. 5						30 5				
4, 5-Dihydroxy-2, 7-naphthalene disulfonic acid (Disodium salt)		76. 6	77. 9	69. 4	64. 9	7 5 9. 9		30. 5	30. 9	30. 4	25. 5
1-Hydroxy-2-acetonaphthone		54. 0	41. 5	37. 7	24. 5	7. 2 9. 6		27. 9	25. 8	24 9	18. 0
2-Picolinic acid		36. 0	25. 2	14. 3	9. 0	6. 2 8. 2		23. 9	15 1	--	--
4, 4'-Diamino-3, 3'-carboxylic acid (biphenyl)		66. 5	58. 6	44. 6	35. 7	11. 7 15. 6		29. 1	27. 5	24. 7	21. 8
H_2O - Control	75 7						28. 1				
5-Chlorosalicylic acid		60. 0	43. 4	34. 2	23. 2	6. 0 8. 0		21. 7	17. 4	14. 0	10 0
2-Hydroxy-3-methylbenzoic acid		69. 6	58. 7	42. 6	25. 6	9. 5 12. 6		28. 5	19. 4	9. 4	6. 2
bis(3-carboxy-4-hydroxy phenyl) methane		70. 0	64. 8	64. 3	44. 0	7. 9 10. 5		28. 4	29. 6	28. 1	18. 7

*Average of ten seedlings

TABLE 6

Effect of Various Concentrations of Organic Compounds on Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes

Organic Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L. S. D. 5% / L. S. D. 1%	*Average Number of Branching Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	200		0	25	50	100	200
H_2O - Control	60 6						24. 7				
3, 4-Dihydro-1-keto-methyl-2-naphthoate		61. 3	62. 8	47 1	42. 8	5. 2 6. 9		22. 7	22. 5	21. 8	19. 5
4 (2-Hydroxy benzal amino)-salicylic acid		60. 2	51. 3	45. 0	35. 0	5. 3 7. 1		23. 4	21. 5	15. 4	11 5
2, 5-Diphthalimido dimethyl adipate		63. 4	58. 9	48. 9	42. 4	4. 7 6. 2		24. 0	22. 8	19. 0	15. 2
6, 6-Dihydroxy-2-naphthalene sulfonic acid (Sodium salt)		61. 0	55. 6	45. 6	37. 3	4 8 6. 5		25. 4	21. 2	18. 7	15. 3
1-0-Acetyl naphthoic acid		61. 1	68. 5	64. 4	46. 6	5. 5 7. 3		25. 0	26. 7	25. 1	14. 8
5-Isoquinolinol		60. 3	57. 2	43. 3	16. 9	5 7 7. 5		23. 8	23. 6	20 3	12 3

*Average of ten seedlings.

TABLE 7

Effects of Various Concentrations of Organic Compounds on Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes.

Organic Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)			L.S.D.		*Average Number of Branching Conc. Solutions in Petri Dishes (ppm)		
	0	1	10	5%	1%	0	1	10
H_2O - Control	52.6					20 5		
6-Quinolinecarboxylic acid		43.0	14.4	3.4	4.5		23.8	13.9
2-Picolinic acid		50 7	43.2	4.9	6.5		20.6	20.1
2-Hydroxy-3-methylbenzoic acid		51.9	51.8***				24 8	19 0
H_2O - Control	58 7					23.3		
1-Naphthoic acid		31.9	19.1	4.3	5.8		18 1	9.7
Quinaldinic acid		57.1	44.1	4.5	6.1		25.5	18.8
1-Naphthol		65.6	66.1**	6.0	8 1		25.8	23 9
2,4-Dichloro-1-naphthol		63.6	71.5	3.7	4.9		24 1	25.2
1-Hydroxy-2-acetonaphthone		58 9	60.3***				21 2	22 9
3-Hydroxy-2-naphthoic acid		59.0	67.6	4.1	5.6		22 6	25.2
1-Hydroxy-2-naphthoic acid		61.9	70.0	3.5	4.8		25.7	26.7

*Average of ten seedlings.

**Significant at 5% level.

***Not significant.

TABLE 8

Effects of Various Concentrations of Organic Compounds on Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes.

Organic Compounds	Concentrations of Solutions in Petri Dishes (ppm)							L. S. D.	
	0	1	10	25	50	100	200	5%	1%
H_2O - Control									
1. Average length (mm)	58.7								
2. Average No. branching	23.3								
Phenol									
1. Average length (mm)		59.3	59.8	57.8	56.5	53.0	40 9	4.2	5.6
2. Average No. branching		23.4	25.4	23.3	21.9	21.5	16.2		
Salicylic acid									
1. Average length (mm)		53.2	49.0	45.1	35.6	31.1	24.2	3 8	5.0
2. Average No. branching		20.5	21.6	21.9	19.6	15.4	14.2		
Benzoic acid									
1. Average length (mm)		63.5	55.1	50.0	37.0	31.7	26.6	3.9	5.2
2. Average No branching		24.4	22.2	20.7	20.0	17.6	16.2		

Average length and branching of tap roots of ten seedlings.

TABLE 9

Effects of Simultaneous Applications of 4, 4'-Methylenebis-3-hydroxy-2-naphthoic acid, KH_2PO_4 and Urea on Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Six Days in Petri Dishes

Concentrations of Solutions in Petri Dishes	Average Length (mm)*	Average No. Branchings
H_2O - Control	82.4	27.7
Urea 500 ppm	88.5	27.8
KH_2PO_4 100 ppm	83.4	27.1
4, 4'-Methylenebis-3-hydroxy-2-naphthoic acid 100 ppm	114.9	32.6
" " " 25 ppm + Urea 500 ppm	95.6	30 3
" " " 50 ppm + "	100.8	27.1
" " " 100 ppm + "	115.5	30 0
" " " 150 ppm + "	111.1	35.2
" " " 25 ppm + " + KH_2PO_4 100 ppm	96.4	27.5
" " " 50 ppm + " "	100.0	29 4
" " " 100 ppm + " "	118.4	30.6
" " " 150 ppm + " "	116.3	36.4
L.S.D. at 5% level	9 4	
L.S.D at 1% level	12.4	

*Average of ten seedlings

TABLE 10

Effect of Various Concentrations of Chelate Compounds on the Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes.

Chelate Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L. S. D. 5% / L. S. D. 1%	*Average Number of Branchings Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	200		0	25	50	100	200
Expt. No. 16.											
Control	34.4						11.9				
1-Amino-4-hydroxyanthra-quinone		38.3	36.8	31.1	21.9	3.8 / 5.0		13.4	12.9	7.9	5.6
Quinalizarin		28.5	28.2	27.6	23.4	2.9 / 3.9		11.5	11.0	10.3	6.8
1-Amino-2-naphthol-4-sulfonic acid		37.0	38.8	28.9	26.2	3.5 / 4.6		13.4	12.5	7.8	3.5
2, 5-Dichloro-3, 6-dihydroxy-p-quinone		34.3	28.7	26.5	21.5	3.4 / 4.5		13.4	11.1	10.7	9.6
2, 3-Dihydroxy quinoxaline		34.0	28.7	24.4	17.1	2.8 / 3.7		12.6	10.7	8.5	--
Picrolonic acid		34.7	32.5	28.8	23.8	5.7 / 7.6		13.5	13.2	11.1	6.4
1-Nitroso-2-naphthol		36.3	28.2	12.9	4.8	3.3 / 4.4		12.9	7.5	6.0	--
2, 4-Dinitrosoresorcinol		31.6	31.0	27.3	21.4	3.5 / 4.7		11.7	10.8	7.9	--
Resorcinol		43.8	42.2	30.7	18.5	4.1 / 5.5		15.9	15.0	13.0	6.7
Benzoin-a-oxime		36.1	35.9	33.2	23.9	3.6 / 4.7		14.2	15.6	11.4	8.7
Benzotriazol		41.0	33.5	30.5	15.6	3.5 / 4.7		14.7	12.2	10.6	--
2, 2'-Biquinoline		42.7	37.3	31.6	20.6	2.8 / 3.8		18.8	16.9	15.4	6.5
Diethyldithiocarbamic acid		45.0	49.2	40.0	20.6	3.1 / 4.2		16.8	16.6	13.9	6.9

*Average of ten seedlings

TABLE 11

Effects of Various Concentrations of Chelate Compounds on the Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes.

Chelate Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L. S. D. 5% / L. S. D. 1%	*Average Number of Branchings Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	200		0	25	50	100	200
Expt. No 17											
Control	33.5						10.3				
5-(p-dimethylamino-benzylidene)		28.6	28.2	25.7	20.6	2.0 / 2.7		9.1	8.4	3.9	
1, 5-Diphenylcarbohydrazide		30.4	27.5	26.2	22.2	1.9 / 2.5		10.5	7.8	7.4	--
Diphenylthiocarbazone		29.4	27.2	21.4	15.6	1.9 / 2.5		9.1	8 9	--	--
Furil dioxime		36.6	36.8	30.3	22.4	2.8 / 3.8		11.6	7.9	6.9	
Mercapto-N-2-naphthylacetamide		30.0	26.9	22.1	17.6	1.9 / 2.6		9.5	9.5	5,8	
Phenolphthalein		33.0	27.5	23.5	20.0	2.1 / 2.8		9 3	7.6	4.7	
- Phenyl-y-thiohydantoic acid		20.9	15.3	8.0	5.8	2.2 / 2.9		6.5	4 0	--	
Cupferron		29.9	26.6	14.0	4,8	1.8 / 2.4		7.7	5.8	--	--

*Average of ten seedlings.

TABLE 12

Effects of Various Concentrations of Chelate Compounds on the Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four Days in Petri Dishes.

Chelate Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L. S. D. 5% / L. S. D. 1%	*Average Number of Branchings Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	200		0	25	50	100	200
Expt. No. 18											
Control	32.0						11.9				
2.4-Dihydroxyacetophenone		40.1	36.4	31.2	20.9	2.6 3.4		13.3	11.8	11.3	
Dimethylglyoxime		32.6	30.6	27.0	17.8	2.2 2.9		11.5	10.9	7.1	--
Hexamethylenetetramine		31.1	30.6	28.4	21.6	2.2 2.9		11.3	10.4	9.4	2.8
8-Hydroxy-7-iodo-5-quinoline sulfonic acid		33.5	34.7	30.8	23.8	3.1 4.1		14.3	10.6	9.6	4.6
Mercaptoacetic acid		36.2	35.8	32.9	27.7	2.5 3.3		11.8	10.8	6.7	3.2
Expt. No. 19											
Control	34.1						13.7				
Phenylglyoxal aldoxime		24.0	14.1	11.8	6.7	2.7 3.6		11.7	10.6	8.1	
5-Sulfosalicylic acid		35.7	36.7	28.7	23.2	3.0 4.0		11.3	11.7	8.0	--
4-(p-nitrophenylazo) resorcinol		36.2	31.8	22.6	15.2	3.4 4.5		14.1	11.1	8.8	5.3
Thiazole yellow		37.8	31.1	22.9	15.9	2.8 3.8		13.6	9.8	7.7	--

*Average of ten seedlings.

TABLE 13

Effects of Various Concentrations of Chelate Compounds on the Elongation and Branching of Tap Roots of Cucumber Seedlings Grown Under Laboratory Conditions for Four and a Half Days in Petri Dishes.

Chelate Compounds	*Average Length of Tap Roots (mm) Conc. Solutions in Petri Dishes (ppm)					L. S. D. 5% / L. S. D. 1%	*Average Number of Branchings Conc. Solutions in Petri Dishes (ppm)				
	0	25	50	100	200		0	25	50	100	200
Expt. No. 20											
Control	56.1						20.6				
2, 3-Butanedione oxime-thiosemicarbazone		46.8	44.7	42.9	34.6	4.5 / 5.9		19.5	17.7	15.1	12.7
p, p-methylenebis (N, N-dimethylaniline)		51.6	51.5	41.0	26.0	4.1 / 5.4		18.7	18.3	15.4	9.9
Iso-butyraldoxime		50.9	48.8	46.6	24.2	5.5 / 7.3		19.4	18.5	17.1	10.2
Diphenylglyoxime		56.3	49.9	43.4	32.9	4.2 / 5.6		20.6	18.1	15.9	14.3
Guanylurea sulfate		49.0	45.0	38.8	25.1	3.6 / 4.8		15.9	15.2	14.5	8.7
Dithioloxalic acid (dipotassium salt)		54.9	47.0	44.2	41.1	3.8 / 5.0		19.0	17.4	16.4	14.4
2, 2, 4, 4, 6, 6-hexanitrodiphenyl-amine		33.3	27.4	19.2	5.3	4.6 / 6.1		14.3	13.7	11.2	--
N, N-diphenylbenzidine		50.6	48.5	43.5	33.3	4.4 / 5.9		19.7	16.6	14.8	12.5
4-(p-ethoxyphenylazo)-m-phenylenediamine		55.3	47.3	40.9	28.8	4.1 / 5.5		20.3	19.0	15.5	11.1
3, 5-Dimethylpyrazole		54.9	46.6	39.6	31.5	4.2 / 5.6		19.5	16.8	13.9	11.7
1-Nitroso-2-naphthol-3, 6-disulfonic acid (disodium salt)		57.4	49.9	45.2	35.1	4.7 / 6.3		19.2	16.2	15.9	12.7
Diphenylamine		49.3	48.3	39.9	29.4	3.3 / 4.4		16.2	15.8	15.0	14.0

*Average of ten seedlings.

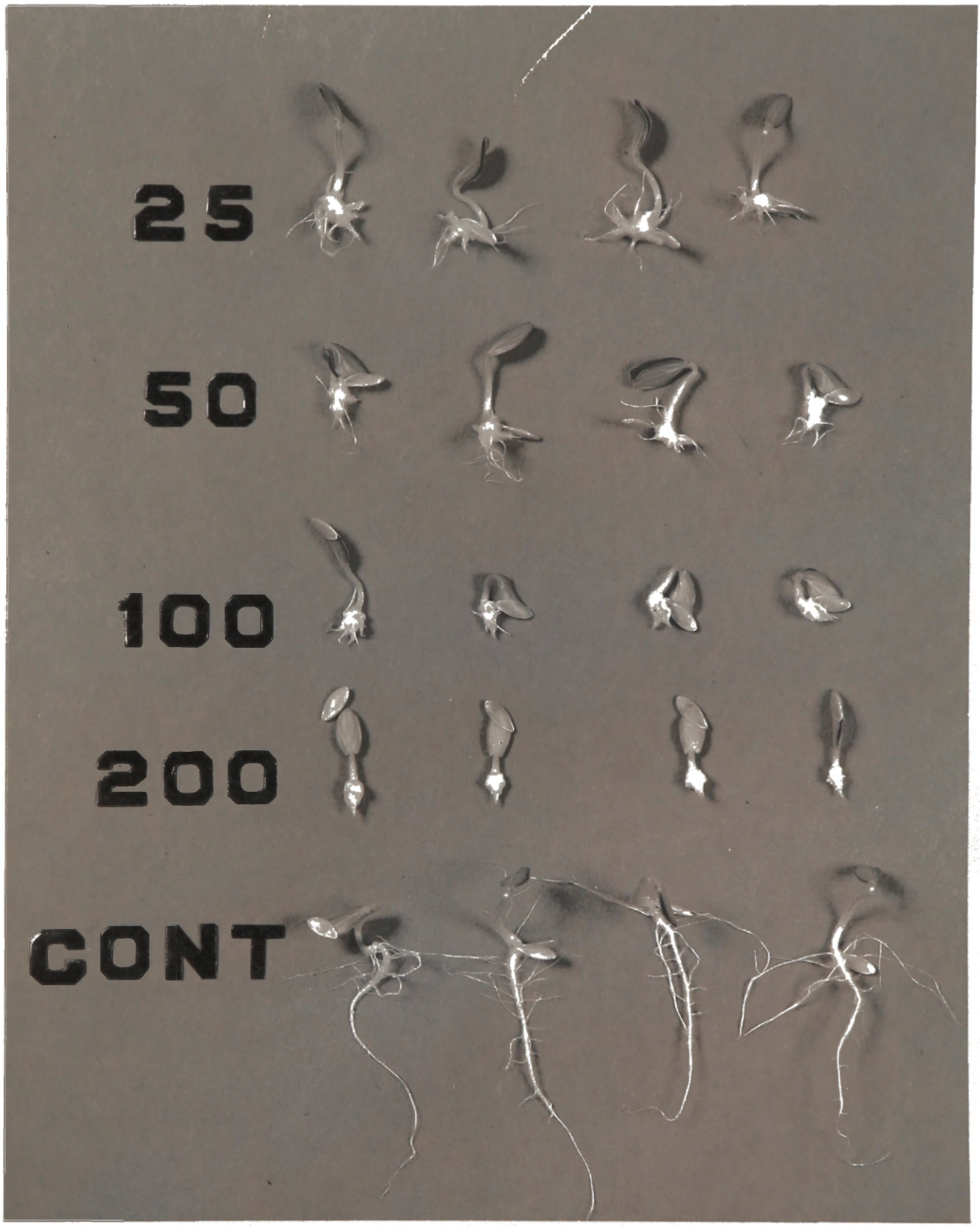


Figure 5. Marketer cucumber seedlings grown in solutions of 25, 50, 100 and 200 ppm of 1-naphthoic acid for four days.

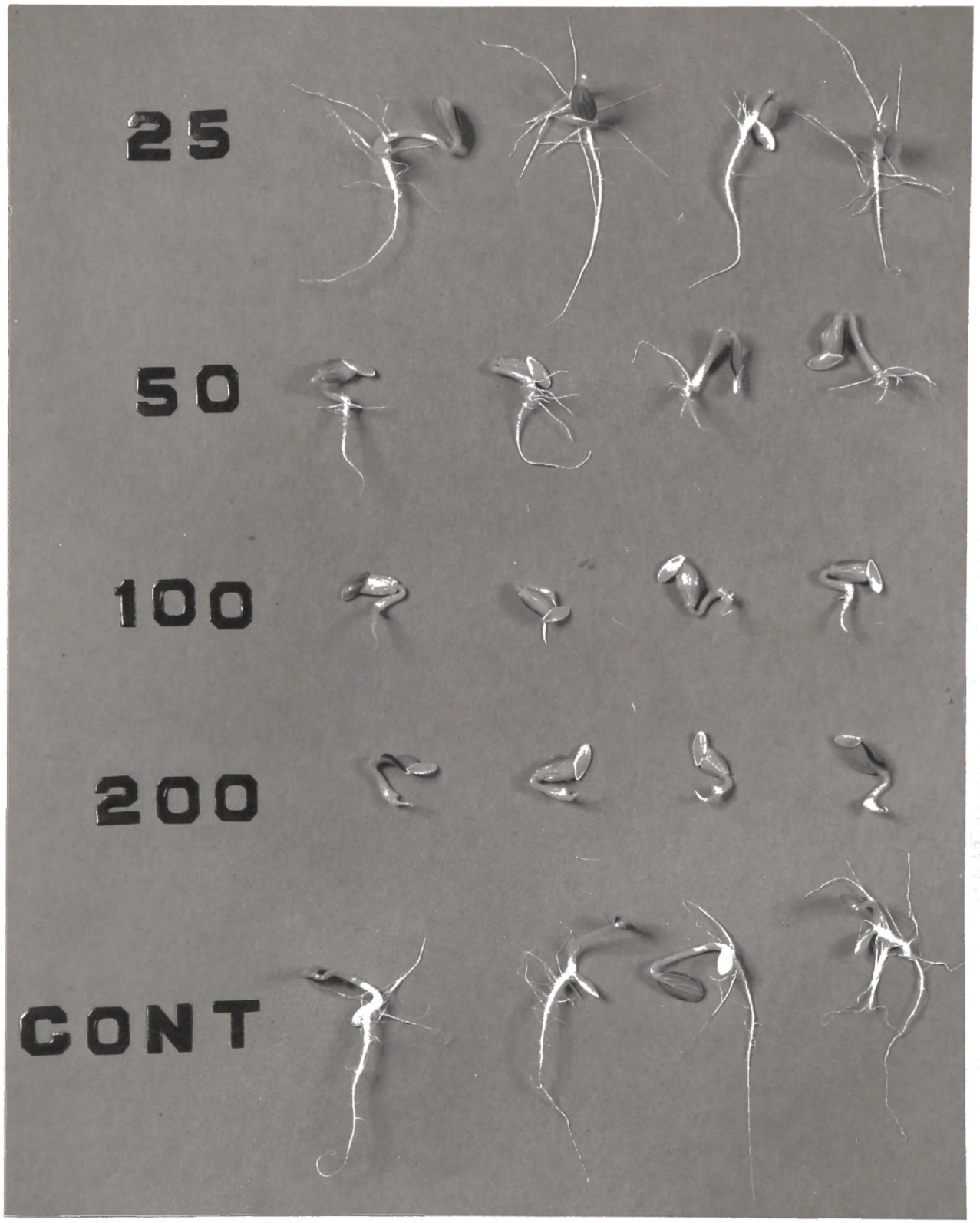


Figure 6. Marketer cucumber seedlings grown in solutions of 25, 50, 100 and 200 ppm of quinaldinic acid for four days.

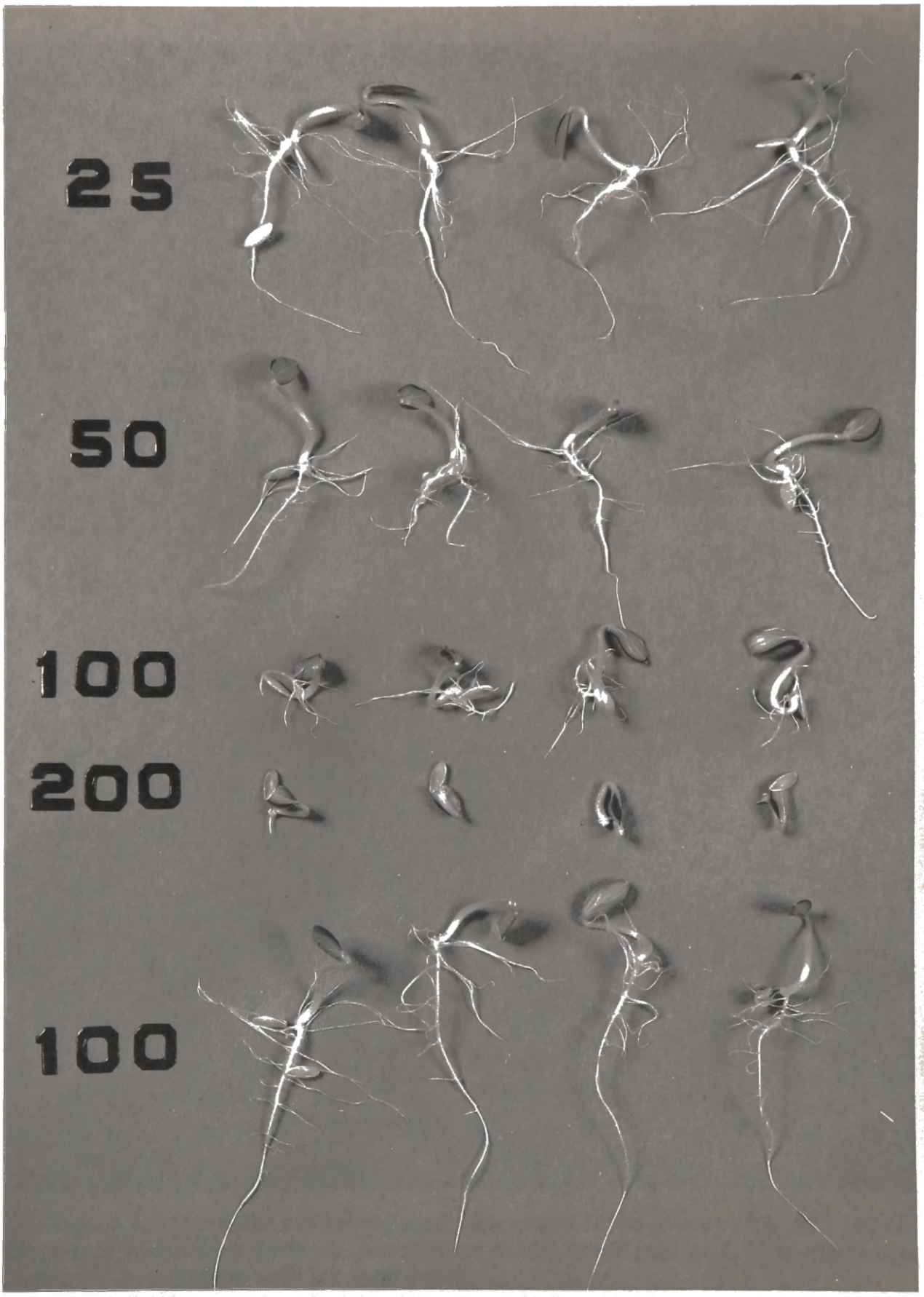


Figure 7. Marketer cucumber seedlings grown in solutions of 25, 50, 100 and 200 ppm of 2-naphthoic acid and 100 ppm of 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid for four days.

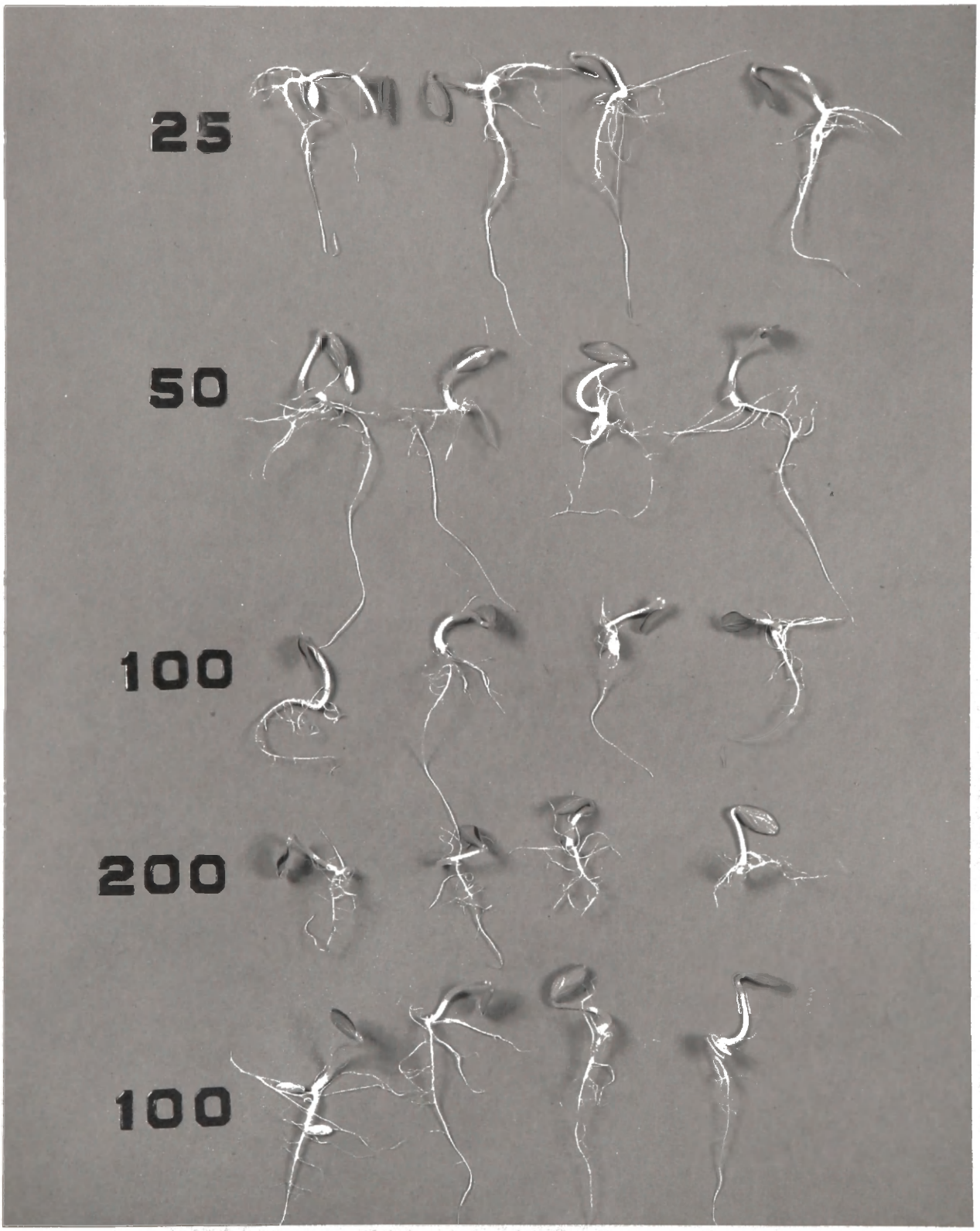


Figure 8. Marketer cucumber seedlings grown in solutions of 25, 50, 100 and 200 ppm of 4-chloro-1-hydroxy-2-naphthoic acid and 100 ppm of 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid for four days.

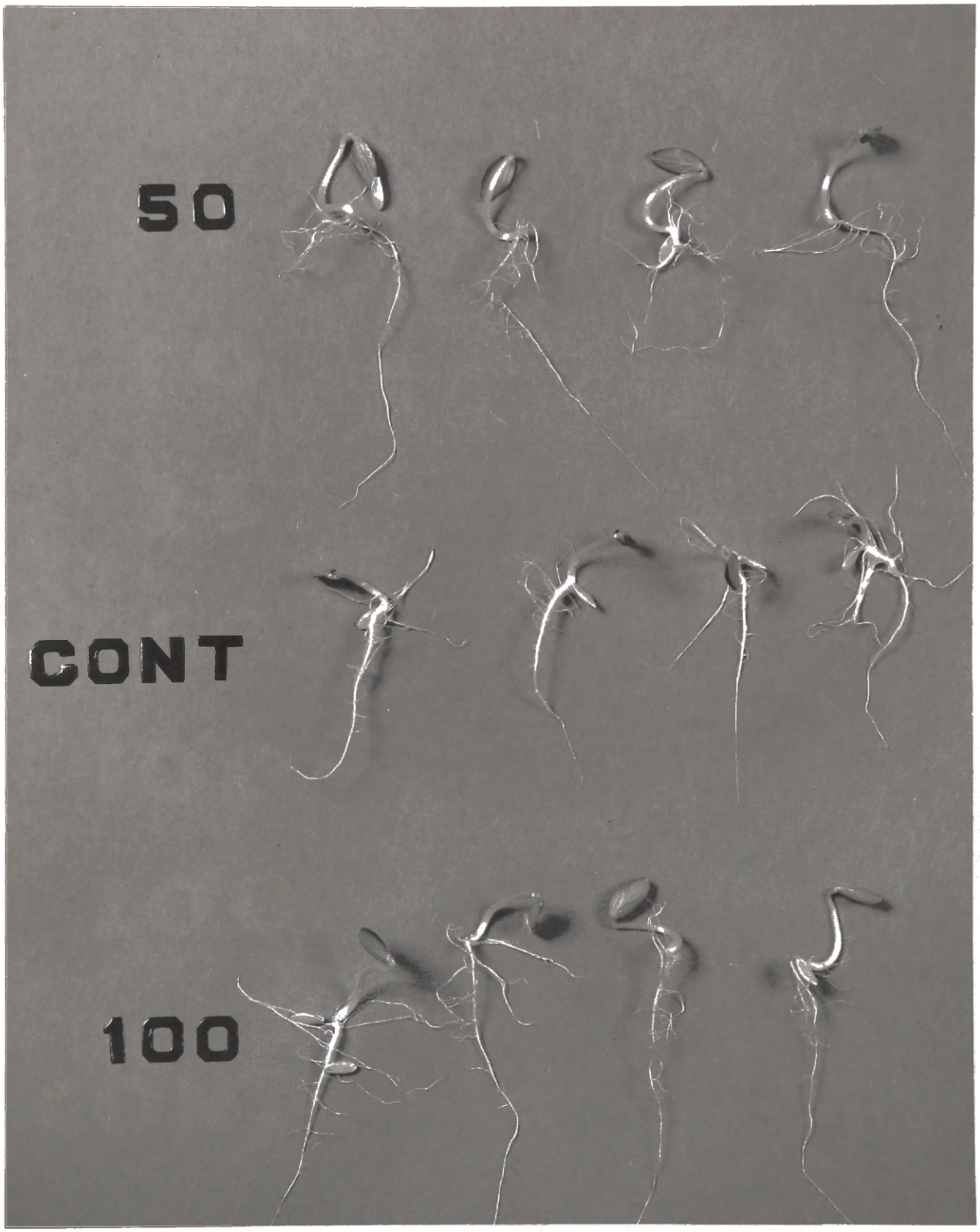


Figure 9. Marketer cucumber seedlings grown in 50 ppm solutions of 4-chloro-1-hydroxy-2-naphthoic acid and 100 ppm of 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid for four days.

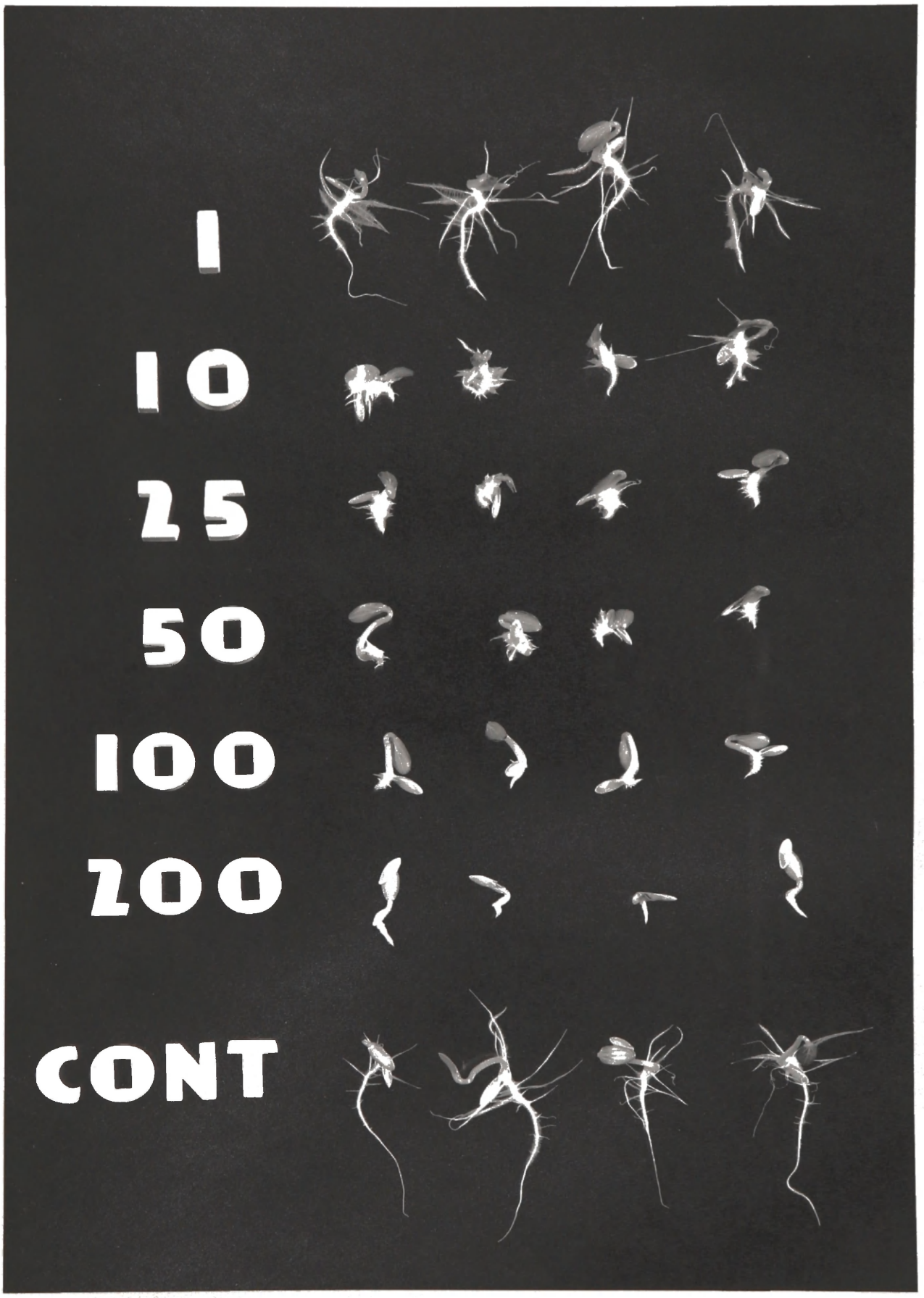


Figure 10. Marketer cucumber seedlings grown in solutions of 1, 10, 25, 50, 100 and 200 ppm of 6-quinolinecarboxylic acid for four days.

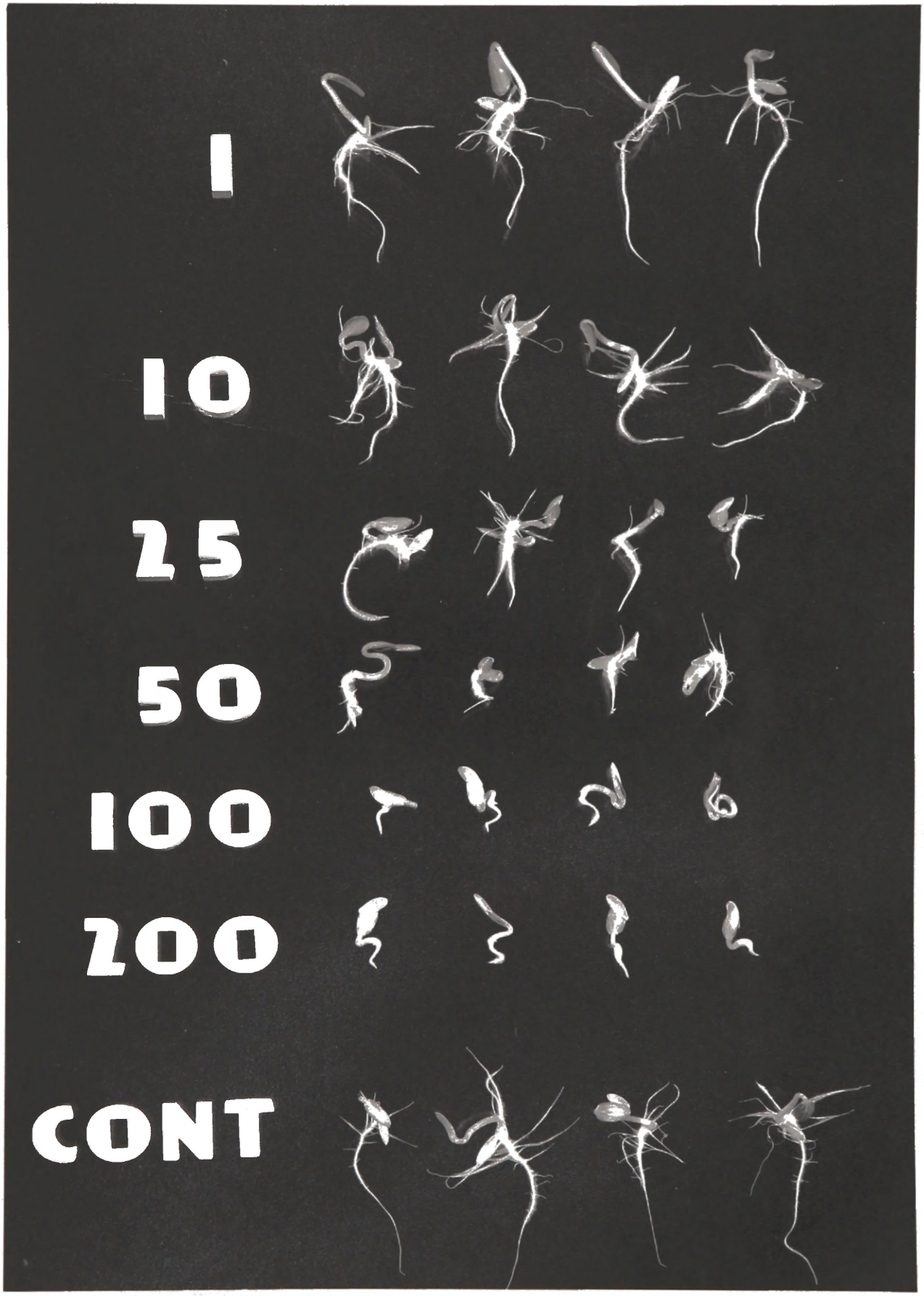


Figure 11. Marketer cucumber seedlings grown in solutions of 1, 10, 25, 50, 100 and 200 ppm of 2-picolinic acid for four days.

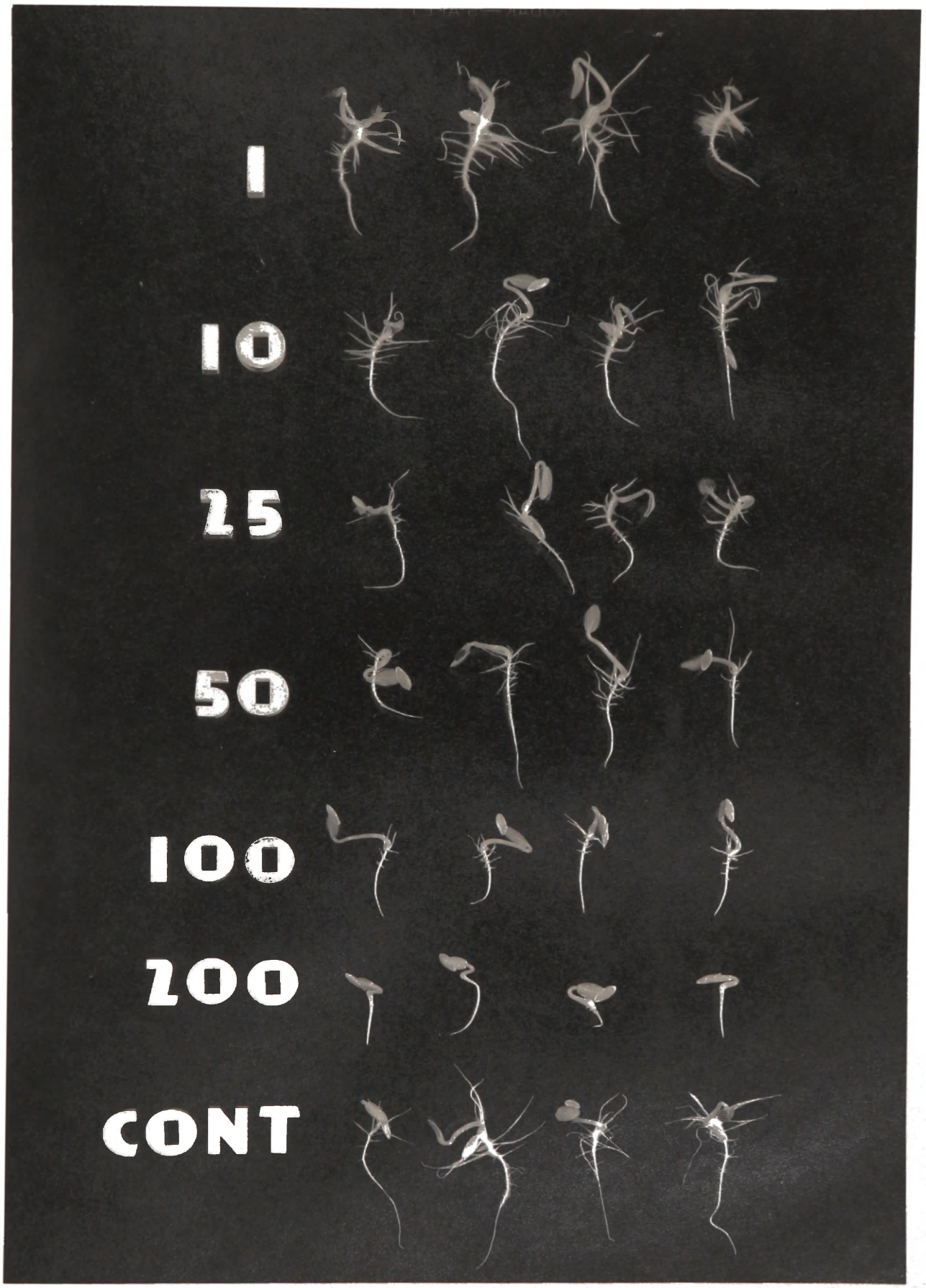


Figure 12. Marketer cucumber seedlings grown in solutions of 1, 10, 25, 50, 100 and 200 ppm of 2-hydroxy-3-methylenebenzoic acid for four days.

Discussion

The compounds reported in Tables 1 to 9 are aromatic organic chemicals containing an hydroxy group, or an acid group, or both Also modification in structure involving other substituents and heterocyclic nitrogen were included

In view of the general root inhibition encountered at 200 ppm with almost all compounds, it was believed that this was the upper limit for correctly evaluating biological activity. The effectiveness of a compound in inhibiting root growth of cucumber was determined by its action at concentrations of between 1 and 100 ppm. It was found that many of the naphthoic acid compounds produced general root inhibition, some at concentrations as low as 1 ppm. The inhibiting activity appeared to be correlated with the position of the carboxyl group on the ring structure It was more active when in the one position than in the two position.

The activity of naphthoic acid was greatly modified by the introduction of an hydroxyl group on the ring structure This tended to produce root stimulation instead of root inhibition The activity was thus reversed by introduction of an hydroxyl group When this hydroxyl group was in the one, and three positions, the greatest root stimulation occurred

The introduction of chlorine into the hydroxyl naphthoic structure did not appear to greatly modify the action of the compound in concentrations between 1 and 100 ppm

If the fused ring nucleus contains only hydroxyl group and no carboxyl group, general root stimulation occurs. Thus, it appears, when a naphthalene ring contains an hydroxyl radical the compound is likely to have a stimulating effect upon cucumber root growth.

It should be mentioned that naphthaleneacetic acid is an important growth regulator and is very effective in inhibiting root growth of cucumber at low concentrations 1-naphthoic acid is similar in structure to naphthalene-acetic acid, except that it has only a carboxyl group whereas in naphthaleneacetic acid the carboxyl group is attached through methylene on the ring nucleus.

It is also of interest to note that if the basic ring structure is changed by substituting a nitrogen for carbon in the ring structure, it produces a hetero-cyclic nitrogen compound with activity similar to naphthaleneacetic acid If, on the other hand, an hydroxyl group is added to the heterocycle nitrogen compound, then the activity is reversed.

Roots are generally inhibited in their growth by adding auxin, although sometimes very low concentrations may slightly increase root growth. Burstrom (17) has suggested two histologically different reactions which are involved in the response of roots applied with auxin First, is the time interval between cell division in the meristem and second, is initiation of cell elongation Burstrom (17) advocated that this interval may be shortened by low concentration of auxin. The second sensitive phase in root growth is the rate of cell

elongation, which is decreased by the addition of auxin. In his later paper Burstrom (18) explained how responses of externally added auxin and related compounds can be distinguished: - (1) by positive action on the first part of cell elongation process, (2) an inhibiting action in cell elongation process, (3) an anti-auxin action exerted by certain compounds, and (4) unspecific toxic action of both auxins and anti-auxins.

On the basis of the above hypothesis, two very promising compounds were studied in this investigation. They were 1-naphthoic acid and 6-quinolinecarboxylic acid (Figures 5 and 10). These compounds had inhibited root growth in a very characteristic manner which appeared to be identical to that induced by auxin. Other compounds found to inhibit root growth significantly were 2-naphthoic acid, 6-hydroxy-2-naphthoic acid, quinaldinic acid, picolinic acid, 5-chlorosalicylic acid, and 1-hydroxy-2-acetonaphthone.

Burstrom (20) observed that anti-auxins, indole and phenoxyisobutric acid derivaties caused an increase of more than 100 percent in the root length by increasing cell elongation without changing cell multiplication. In this investigation, 2-naphthoic acid with hydroxy at 1, 3 position and substituted chlorine, 1-naphthol, 2-naphthol, 2,4-dichloro-1-naphthol, 8-quinolinol and 1-hydroxy-2-naphthalene sulfonic acid (sodium salt), 4,4'-methylenebis-3-hydroxy-2-naphthoic acid and its disodium salt have increased root growth remarkably. It is, therefore, suggested from their behavior on growth that they might have

anti-auxin properties

The compounds reported in Tables 10 to 13 are essentially metal chelating compounds Certain of these compounds, 1-amino-4-hydroxyanthraquinone, resorcinol, benzotriazol 2, 2'-biquinoline, diethyldithiocarbamic acid, 2, 4-dihydroxyacetophenone, mercapto-acetic acid, and thiazole yellow had significantly increased root growth at the lowest concentration under investigation It is possible that these compounds act by removing certain toxic substances that may be present in roots and thereby encouraging growth. It is also possible that these materials are acting as anti-auxins

Martell et al. (44) advocated that sometimes chelating agents may cause death of organisms by inhibiting a metal-enzyme function, if it is powerful enough to compete with the enzyme for the metal This might explain why certain of the chelating compounds had inhibited root growth.

Activity of Organic Compounds on the Abscission of Coleus blumei Petioles

Review of Literature

The detachment, or abscission of various plant organs is a well-known phenomenon. The separation of immature floral parts, leaves, foliage branches, and fruits of the plant is of considerable interest The regulation of abscission by chemicals has now become an agricultural practice A great number of chemicals have been found to be effective for retarding pre-harvest drop of orchard fruits, and inducing abscission of blossom thinning of heavy setting varieties The various uses of abscission regulators have been extensively reviewed by Tukey (73), Audus (9), and Tharp (65).

Laibach (37) suspected that auxin played a part in the phenomenon of organ abscission. He observed that application of orchid pollenia retarded abscission of debladed petioles. In 1936, LaRue (39) demonstrated that application of synthetic auxins delayed leaf fall in Coleus blumei. He applied lanolin paste containing 50 ppm of indoleacetic acid to the tips of debladed petioles of coleus plants, and observed that the treated petioles stayed on longer than the controls. These results were repeatedly confirmed by Addicott and Lynch (2), Gardner and Cooper (21), Myers (50), Wetmore et al (86). Beal and Whiting (12) observed that the abscission of stems of Mirabilis jalapa could be inhibited by applying indoleacetic acid in lanolin to the cut surface of a branch.

Abscission accelerants have been investigated (3), and found that, in general, their mechanism of action is similar. Many of these materials are toxic to the leaves, either killing or injuring them, thus accelerating organ abscission without seriously affecting other parts of the plants. The known accelerants are certain unsaturated hydrocarbons, anti-auxins, enzyme inhibitors, defoliants, and fruit thinners.

Crocker (19) reported that ethylene among the unsaturated hydrocarbons is the most potent abscission accelerator. Gawadi and Avery (22) remarked that only one derivative, ethylene chlorohydrin is active in accelerating abscission.

Bonner and Bandurski (14) postulated that an anti-auxin which competes biochemically with auxin for a site on an apoenzyme might induce abscission. Weintraub et al. (84), Whiting and Murray (87) found that 2, 3, 5-triiodobenzoic acid and other halogen substituted benzoic acids applied to the apical bud of a bean seedling induced abscission of the lateral buds. It was further reported by Weintraub et al. (84) that abscission was prevented by simultaneous application of indoleacetic acid and 2, 3, 5-triiodobenzoic acid. Hall (24) in cotton petiole abscission studies, noticed that trans-cinnamic acid accelerated abscission, and this acceleration was reduced by simultaneous application of indoleacetic acid. Several maleimides, which accelerate the abscission of peach leaves, have been reported by van Overbeek et al. (75).

The physiological mechanism of action on abscission is not clearly understood, however, many theories have been postulated Only two theories, the hormone ethylene balance theory of Gawadi et al (22), and the auxin gradient theory of Addicott et al. (4) have been supported comprehensively

The hormone ethylene balance theory proposes that leaf abscission is regulated by the balance of hormone and ethylene in the leaf It was proposed by Gawadi et al. (22) and supported by Hall (24) Gawadi et al (22) remarked that leaf auxin diminished with maturity and assumed that ethylene was produced in leaves and served to accelerate their abscission. He concluded that the interaction of the opposing factors, auxin and ethylene regulate abscission. Hall (24) working with debladed petioles of coleus and cotton found that auxin prevented the acceleration of abscission by ethylene and ethylene chlorohydrin.

Many of the recent advances in abscission physiology shows that auxin has a dominant role in controlling the intervals of leaf abscission (Wetmore et al , 86). Shoji et al. (57) suggested, by measuring the auxin concentration distal and proximal to the abscission zone, that auxin gradient across a zone was more important in the regulation of abscission than the auxin concentration in the abscising organ. Considering all available information, Addicott et al. (4) proposed a theory of auxin gradient They remarked that rate of abscission is regulated by the auxin gradient across

the abscission zone, abscission does not occur when the gradient is steep, that is, high on the distal side and low on the proximal side of the abscission layer If the auxin is high on the proximal side and low on the distal side, abscission would occur This theory was supported by the investigations of Rossetter et al. (56), Jacobs (34) They found that the acceleration of neighboring young leaves on the abscission of debladed petioles is attributed to the auxin produced in the leaves.

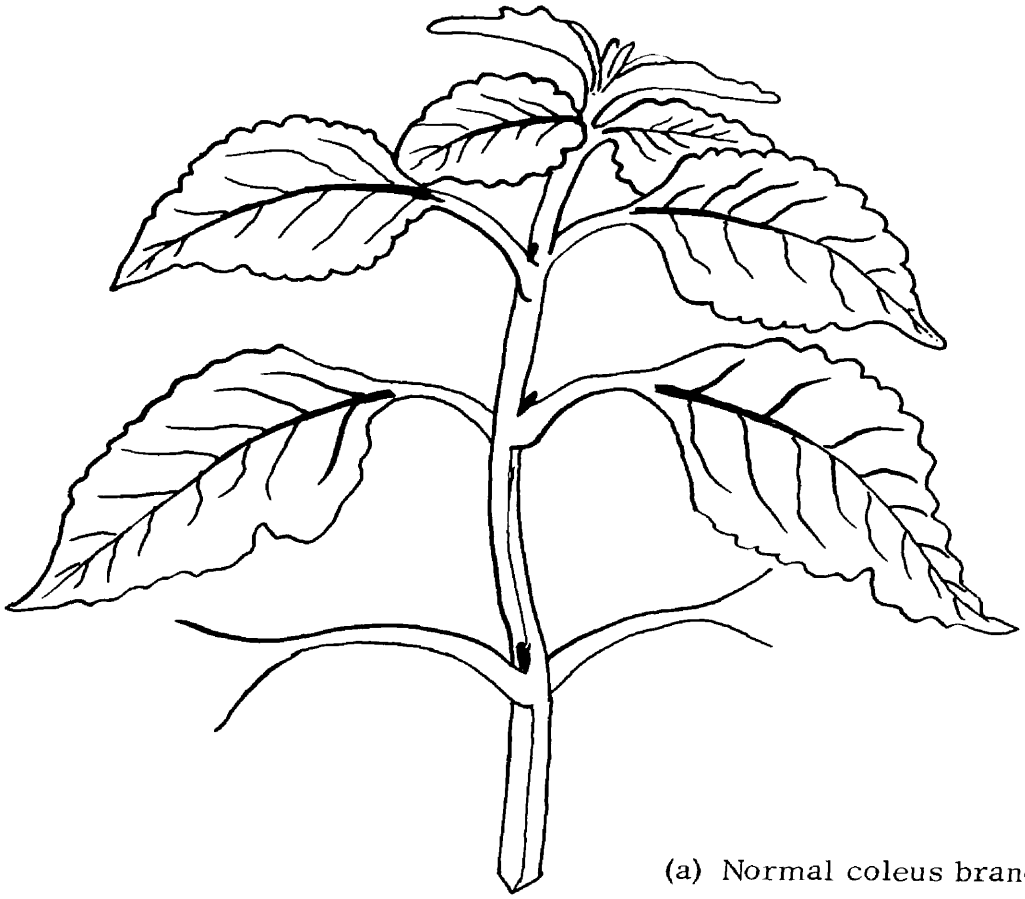
Since many of the organic compounds, which were used in the cucumber root test, had stimulated root growth, it was thought desirable to test their relative response on abscission to further determine their action as anti-auxins Hence, a series of experiments were initiated to determine their effect on Coleus blumei petiole abscission.

Methods and Materials

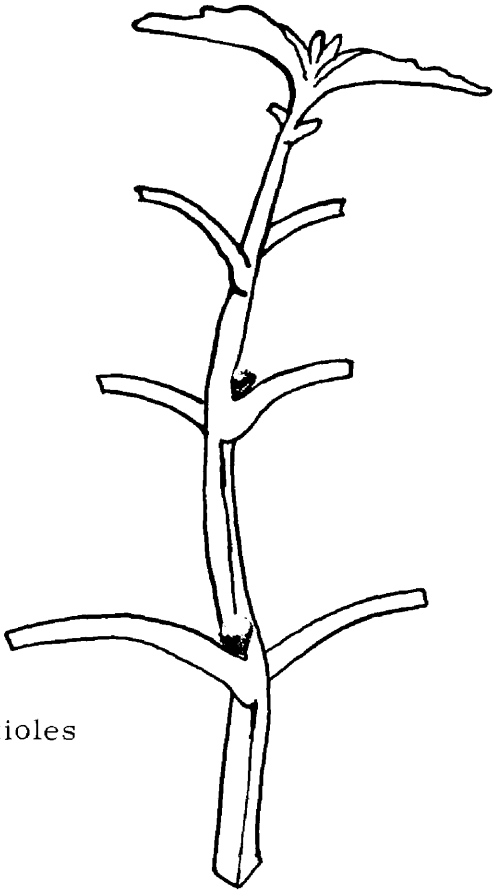
Coleus blumei L var Christmas Gem was used throughout the course of investigations. The experimental plants were grown from cuttings which were taken from clonal material The plants were grown under greenhouse conditions during the period from April to September, 1955 with temperature fluctuations of 65° to 80° F.

Abscission Test

Seventy single-stemmed upright plants were graded for size and the leaf blades were cut off in the manner shown in Figure 13b. The tips of the petioles of different lots of plants were then covered with lanolin paste containing 10, 100 and 1000 ppm of 2, 3, 5-triiodobenzoic acid (TIBA), 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, 1-hydroxy-2-naphthoic acid, and 1-naphthoic acid Pure lanolin paste was used as control and a lanolin paste with 100 ppm of naphthaleneacetic acid (NAA) was used as a standard growth regulator check. Concentrations of the compounds mentioned above were dissolved in a small quantity of ethyl alcohol before mixing into warm lanolin. Every precaution was taken to apply the lanolin paste uniformly on the tips Five plants were used for each treatment. The number of petioles which did absciss with slight pressure were recorded after every twelve hours The percentage drop of petioles was determined for each treatment (Table 14)



(a) Normal coleus branch.



(b) Branch with debladed petioles

Figure 13. Method of preparation of coleus plants

Simultaneous Application of Naphthaleneacetic Acid (NAA) and Organic Compounds

Auxin usually delays the abscission of coleus petioles (Gardner et al. 21) and it is also evident from our previous abscission test experiment that naphthaleneacetic acid (NAA) has effectively delayed the drops of petioles. Therefore, a series of experiments were initiated to investigate whether the compounds which were found effective in enhancing the abscission in the previous test, might have some interacting effect on the translocation and action of naphthaleneacetic acid. The following procedures were adopted for the investigations:

Mixed application: Treatment used for the simultaneous application of abscission compounds are as follows:

1. Naphthaleneacetic acid 100 ppm
2. Naphthaleneacetic acid 100 ppm + 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 10 ppm
3. Naphthaleneacetic acid 100 ppm + 3-hydroxy-2-naphthoic acid 100 ppm
4. Naphthaleneacetic acid 100 ppm + 1-hydroxy-2-naphthoic acid 1000 ppm
5. Naphthaleneacetic acid 100 ppm + 2, 3, 5-triiodobenzoic acid 1000 ppm
6. 2, 3, 5-triiodobenzoic acid 1000 ppm.
7. 2, 3, 5-triiodobenzoic acid 1000 ppm + 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 10 ppm

8 2, 3, 5-triiodobenzoic acid 1000 ppm + 3-hydroxy-2-naphthoic acid 100 ppm

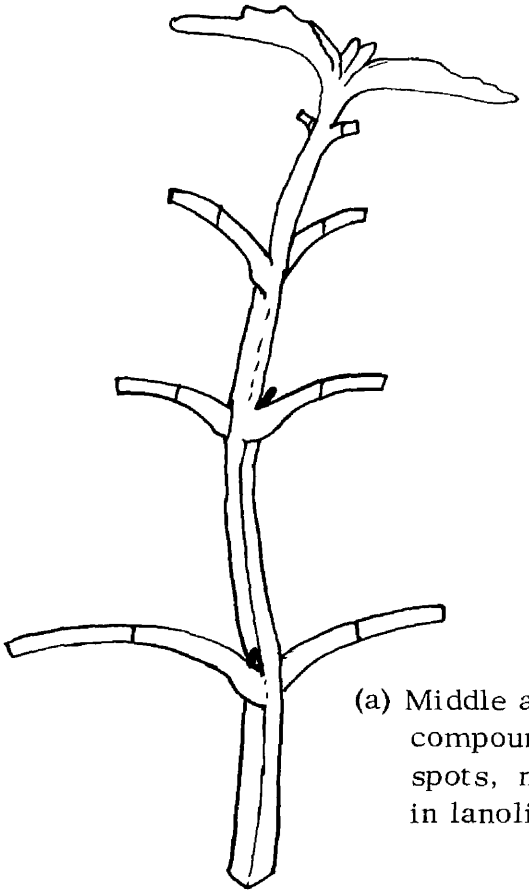
9 2, 3, 5-triiodobenzoic acid 1000 ppm + 1-hydroxy-2-naphthoic acid 1000 ppm

10 Lanolin only (check)

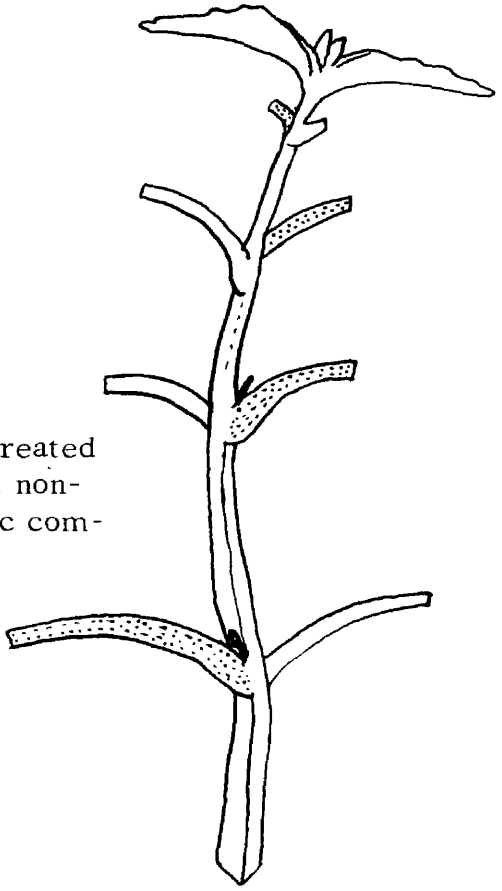
The above treatments were mixed in lanolin and were applied on debladed petioles Records were taken after every twelve hours and the percentage rate of petiole drops was used as an index for inhibition or acceleration of abscission (Table 15)

Middle application: (Figure 14a) A lanolin paste containing naphthaleneacetic acid 100 ppm was placed on the tips of the debladed petioles and a ring of lanolin pastes containing 10 ppm of 4, 4'-methylene-bis-3-hydroxy-2-naphthoic acid; 100 ppm of 3-hydroxy-2-naphthoic acid; 1000 ppm of 1-hydroxy-2-naphthoic acid; and 1000 ppm of 2, 3, 5-triiodo-benzoic acid applied around the petioles between the abscission zone and the tips as shown in Figure 14a A ring of plain lanolin was applied to the other group of plants which served as control. Three plants were used in each treatment The rate of abscission was the criteria used to determine the effect if the materials are modifying the action of naphthaleneacetic acid

Spiral application: (Figure 14b) In this experiment the method of designating leaf numbers is the same as used by Jacobs (33) Wetmore et al



(a) Middle application of organic compounds applied at indicated spots, naphthaleneacetic acid in lanolin paste applied at tips.



(b) Stippled debladed petioles treated with naphthaleneacetic acid; non-stippled treated with organic compounds in lanolin.

Figure 14. Methods of treatment of coleus plants

(86) has also used a spiral arrangement of deblading leaves in their abscission studies The debladed petioles were treated spirally, as shown in Figure 14b The purpose of this investigation was to determine the possible interaction of naphthaleneacetic acid and abscission accelerators when applied at a different location on the plant For example, the debladed petioles on the plant were treated in such a manner that petiole tip which was treated with 100 ppm of naphthaleneacetic acid had a petiole treated with 10 ppm of 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid just above or below and opposite it The other chemicals, 100 ppm of 3-hydroxy-2-naphthoic acid, 1000 ppm of 1-hydroxy-2-naphthoic acid, and 1000 ppm of 2, 3, 5-triiodobenzoic acid were tested with naphthaleneacetic acid in a similar manner A plain lanolin was used as control. Three plants were used for each treatment. The rate of abscission was recorded after every twelve hours, and used as a criterion to determine the effect of materials in modifying the action of naphthaleneacetic acid.

Results

In attempting to ascertain the activity for abscission response, 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid, 1-hydroxy-2-naphthoic acid, and 3-hydroxy-2-naphthoic acid compounds were tested (Table 14) A known anti-auxin, 2, 3, 5-triiodobenzoic (TIBA) and naphthaleneacetic acid (NAA) an auxin, were included as standard compounds in each experiment as a

check on the consistency of response. It was observed that 1-hydroxy-2-naphthoic acid at 1000 ppm had injured the tissues of debladed petiole tips immediately following application. The other compounds used were not injurious to the plants, and had a significant effect on accelerating abscission thus indicating that these substances are acting as anti-auxins

Exploratory tests were carried out with the same compounds to investigate their simultaneous application (Table 15). When the anti-auxin 2, 3, 5-triiodobenzoic acid was applied simultaneously with the above mentioned compounds, it was found that acceleration of abscission was enhanced, thus indicating that these materials had a synergistic effect with 2, 3, 5-triiodobenzoic acid. The most effective material in this experiment was 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid followed by 3-hydroxy-2-naphthoic acid and 1-hydroxy-2-naphthoic acid. It was also noticed that 1-hydroxy-2-naphthoic acid was toxic to the plant tissues when applied with 2, 3, 5-triiodobenzoic acid.

When naphthaleneacetic acid, an auxin simultaneously applied with these materials, it was found that the accelerators, 4, 4'-methylenebis-3-hydroxy-2-naphthoic, 3-hydroxy-2-naphthoic, 1-hydroxy-2-naphthoic, 2, 3, 5-triiodobenzoic acids, reduced the effectiveness of NAA in preventing abscission, thus again indicating their anti-auxin properties

In another experiment the abscission accelerators were applied at a

point midway between the cut surface and the abscission layer (Table 16) Naphthaleneacetic acid, which was applied to the cut surface might thus be less effective because of interference by an anti-auxin and have its translocation and action modified. This was found to be true when an anti-auxin or abscission accelerator was applied in such a manner, the action of naphthaleneacetic acid was reduced.

In still another experiment to determine the effectiveness of the anti-auxin on modifying the action of naphthaleneacetic acid, an experiment was initiated in which the anti-auxin or abscission accelerators were applied using a spiral arrangement of the debladed leaves (Table 16). It was observed that the abscission accelerator under these conditions also modified the action of naphthaleneacetic acid. It is evident from the results that these compounds have anti-auxin properties when investigated in different ways.

1-naphthoic acid which worked like naphthaleneacetic acid on cucumber roots was tested for its activity on abscission. It was found that at 10 ppm the abscission was delayed, however at 1000 ppm it had considerably accelerated the abscission (Table 17) At a low concentration it may be acting as an auxin, while at high concentration 1000 ppm it may have some injurious effect on certain tissues and hasten abscission.

TABLE 14

The Percent Abscission Rate of Debladed Coleus Petioles as Influenced by Organic Compounds Applied in Lanolin to the Tips of Petioles.

Concentrations of Organic Compounds in Lanolin	Percent Abscission Rate of Debladed Petioles						
	Hours After Treatment						
	48	60	72	84	96	108	120
Plain lanolin	--	--	24.2	33.3	51.5	75.7	100.0
Naphthaleneacetic acid (NAA) 100 ppm	--	--	--	--	24.1	65.5	86.2
2, 3, 5-Tri-iodobenzoic acid (TIBA) 10 ppm	--	--	28.0	34.6	69.2	84.6	100.0
2, 3, 5-Tri-iodobenzoic acid (TIBA) 100 ppm	3.1	6.5	31.3	68.8	81.3	100.0	--
2, 3, 5-Tri-iodobenzoic acid (TIBA) 1000 ppm	9.0	25.8	60.6	78.8	90.9	100.0	
4,4'-Methylenebis, 3-hydroxy-2-naphthoic acid 10 ppm	3.0	9.0	48.5	51.5	75.8	81.2	100.0
4, 4'-Methylenebis, 3-hydroxy-2-naphthoic acid 100 ppm	3.4	10 9	48.8	75.9	86.2	100 0	
4, 4'-Methylenebis, 3-hydroxy-2-naphthoic acid 1000 ppm	--	--	50.0	76.8	84.2	100.0	--
1-Hydroxy-2-naphthoic acid 10 ppm	--	9.5	30.0	36.6	56.6	78.9	100.0
1-Hydroxy-2-naphthoic acid 100 ppm	--	15.6	32.1	46.4	64.3	80.0	100 0
1-Hydroxy-2-naphthoic acid 1000 ppm	6.0	30.2	69.7	75.8	87.9	100.0	--
3-Hydroxy-2-naphthoic acid 10 ppm	--	8.1	46.7	73.3	80.0	86.7	100.0
3-Hydroxy-2-naphthoic acid 100 ppm	--	10.6	55.2	75.6	86.2	100.0	--
3-Hydroxy-2-naphthoic acid 1000 ppm	--	11.0	56.7	78 0	86.6	100.0	--

TABLE 15

The Percent Abscission Rate of Debladed Coleus Petioles as Influenced by Organic Compounds Mixed with Auxin and Anti-Auxin in Lanolin Applied to the Tips of Petioles.

Concentrations of Compounds in Lanolin	Percent Abscission Rate of Debladed Petioles								
	Hours After Treatment								
	60	72	84	96	108	120	132	144	156
Plain lanolin	--	--	40.7	47.7	77.7	85.2	92.6	100.0	--
2, 3, 5-Tri-iodobenzoic acid (TIBA) 1000 ppm	--	28.6	42.9	64.3	84.3	92.9	100.0	--	--
TIBA 1000 ppm plus 4, 4'-methylenebis-3-hydroxy, 2-naphthoic acid 10 ppm		56.6	63.3	76.6	96.6	96.6	100.0		
TIBA 1000 ppm plus 3-hydroxy-2-naphthoic acid 100 ppm	--	39.3	64.5	64 5	92.3	96.3	100.0		
TIBA 1000 ppm plus 1-hydroxy-2-naphthoic acid 1000 ppm	3.2	35.5	64.5	67.4	84.2	94.2	100.0	--	--
Naphthaleneacetic acid (NAA) 100 ppm	--	--	--	--	--	--	38.5	65.4	65.4
NAA 100 ppm plus TIBA 1000 ppm	--	--	7.1	14.3	60.7	60.7	67.9	82 1	92.8
NAA 100 ppm plus 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 10 ppm						19.2	43.0	66.0	68.5
NAA 100 ppm plus 3-hydroxy-2-naphthoic acid 100 ppm			--		11.5	11 5	40.0	65.8	67.7
NAA 100 ppm plus 1-hydroxy-2-naphthoic acid 1000 ppm	--	--	--	45.8	66.6	70.8	75.0	79.2	83.3

* Compounds were mixed in lanolin before application

TABLE 16

The Translocation Rate of Naphthaleneacetic Acid Applied to the Tips of Debladed Coleus Petioles as Affected by the Application of Various Organic Compounds in Lanolin in Different Patterns as Indicated by the Percent Rate of Abscission

Organic Compounds	Percent Abscission Rate of Debladed Petioles							
	Hours After Treatment							
	72	84	96	108	120	132	144	156
Spiral application								
Naphthaleneacetic acid (NAA) 100 ppm with plain lanolin			22.2	22.2	33.3	50.0	72.2	72.2
NAA 100 ppm with Tri-iodobenzoic acid (TIBA) 1000 ppm	12.5	12.5	25.0	37.5	62.5	62.5	81.3	100.0
NAA 100 ppm with 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 10 ppm		--	30.0	35.0	50.0	50.0	85.0	85.0
NAA 100 ppm with 3-hydroxy-2-naphthoic acid 100 ppm			33.3	38.8	61.1	61.1	77 7	83.3
NAA 100 ppm with 1-hydroxy-2-naphthoic acid 1000 ppm	--	--	23.5	30.0	50.0	50.0	80 0	85.0
Middle application								
NAA 100 ppm with plain lanolin	--	--	--	--	11.8	29.4	62.3	72 3
NAA 100 ppm with TIBA 1000 ppm	--	--	--	33.3	50.0	61.1	72.2	83.3
NAA 100 ppm with 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 10 ppm		--			70.0	70.0	80.0	80.0
NAA 100 ppm with 3-hydroxy-2-naphthoic acid 100 ppm					66.6	66 6	83.3	83.3
NAA 100 ppm with 1-hydroxy-2-naphthoic acid 1000 ppm	--	--	--	--	65.0	65.0	70.0	75 0

TABLE 17

The Percent Abscission Rate of Debladed Coleus Petioles as Influenced by 1-Naphthoic Acid Applied in Lanolin to Tips of Petioles.

Concentrations of Compounds in Lanolin	Percent Abscission Rate of Debladed Petioles					
	Hours After Treatment					
	72	84	96	108	120	132
Plain lanolin	25.9	44.4	55.5	60.6	89.3	100.0
Naphthaleneacetic acid (NAA) 100 ppm	--		--	--	25.9	50.0
1-Naphthoic acid 10 ppm	8.3	16.7	37.5	45.8	83.3	92.0
1-Naphthoic acid 100 ppm	11.1	48.1	70.4	70.4	85.2	100.0
1-Naphthoic acid 1000 ppm	60.0	64.0	92.0	100.0	--	--

Biological Activity of Organic Compounds on the Rooting of Cuttings

Review of Literature

The use of growth regulators to promote rooting in the plant propagation industry is very recent. Before the discovery of auxin, many chemical compounds had been reported to increase the rooting of cuttings, such as permanganate by Curtis (20), and carbon monoxide by Zimmerman et al. (89) Van der lek (74) made extensive investigations into the factors involved in the rooting of cuttings. He reported that in currant, poplar, grapes, and willow the intensity of root production was directly correlated with the rate of bud development. He suggested the formation of hormone or hormones in the developing buds, and its tranlocation to the base of the cutting where it initiated the roots. In 1929, Went (85) observed that non-specific, heat resisting substances could be extracted from leaves of barley, which, when applied to cuttings, promoted the development of new roots. Later, Thimann and Went (67) discovered that auxin exerts a primary control over root formation. Recently, a great number of chemicals primarily different derivatibes and formulation of aryloxy-alkyl-carboxylic acid and aryl-alky-carboxylic acids were tried on a large variety of different species and under different conditions. These works have been extensively discussed by Tukey (73), Thimann and Behnke (69), Audus (9), Leopole (41), Avery and Johnson (10)

An understanding of the mechanism by which growth substances stimulate the rooting of cuttings emerged very recently The initial step in the formation of roots is the differentiation of meristems into root primordia By analysing the different parts of cuttings of young bean plants, whose cut surfaces were treated with indoleacetic acid, Stuart (62) and Alexander (6) found carbohydrates and nitrogenous food materials were translocated to the treated area These substances might stimulate the root primordia Skoog (58, 59) demonstrated that the type of differentiation that occurs in the meristem is dependent upon the proportion of auxin to other substances. He postulated that the ratio of auxin to other plant constituents should be high for encouraging root primordia.

In attempting to correlate chemical structure with biological activity, certain compounds were found whose action closely resembled that of naphthaleneacetic acid at least for the cucumber root test It was thought desirable to extend the investigations of these compounds on plants other than cucumber, and hence an experiment was set up to determine their activity on the rooting of cuttings

Methods and Materials

Two compounds, 1-naphthoic and 6-quinolinecarboxylic acids were discovered which were similar in response to naphthaleneacetic acid on cucumber root growth. These were used to determine their activity on

the rooting of cuttings One hundred and sixty-five cuttings of Coleus blumei L var Christmas Gem were used for the study The chemicals were first dissolved in small quantities of ethyl alcohol to facilitate their solubility in water The concentrations used were 1, 10, 50, 100 and 1000 ppm Fifteen cuttings were used in each treatment placed in a beaker containing 50 cc of the solution and allowed to soak for 22 hours After treatment, the cuttings were planted in a greenhouse bench. Three replications were used containing five cuttings in each treatment

After 15 days the cuttings were taken out and graded to a subjective evaluation of number of roots in which the following scale was employed:

(1) Heavy rooting

(2) Good rooting

(3) Fair rooting

(4) Poor rooting

(5) Very poor rooting

Results

The data indicate that 1-naphthoic acid and 6-quinolinecarboxylic acid has considerably stimulated the root growth on Coleus cuttings in all concentrations (Table 18) The maximum stimulation of root growth was attained at 100 ppm concentration of 1-naphthoic acid and at 1000 ppm of 6-quinolinecarboxylic acid

TABLE 18

The Percent Rooting of Coleus Cuttings Fifteen Days after Treatment with Various Concentrations of Organic Compounds.

Concentrations of Organic Compounds	Average Percent of Rooting on Cuttings Grades*				
	I	II	III	IV	V
Control	--	33.3	46.7	20.0	--
1-Naphthoic acid - 1 ppm	--	66.7	33.3		
1-Naphthoic acid - 10 ppm	20.0	60.0	20.0		
1-Naphthoic acid - 50 ppm	53.4	46.6	--	--	
1-Naphthoic acid - 100 ppm	80.0	20.0	--	--	
1-Naphthoic acid - 1000 ppm	33.3	60.0	6.7		
6-Quinolinecarboxylic acid - 1 ppm	--	40.0	60.0		
6-Quinolinecarboxylic acid - 10 ppm	13.3	66.7	20.0		
6-Quinolinecarboxylic acid - 50 ppm	26.6	60.0	13.4		
6-Quinolinecarboxylic acid - 100 ppm	46.6	53.4	--		
6-Quinolinecarboxylic acid - 1000 ppm	86.6	13.7	--	--	--

*Grading refers to a subjective evaluation of numbers of roots in which the following scale was employed:

I - Heavy rooting
II - Good rooting
III - Fair rooting
IV - Poor rooting
V - Very poor rooting

Growth of Tomato Plants as Affected by Transplanting Treatments with Organic Chemicals

Review of Literature

Vegetable crops are often affected by a severe shock in transplanting as roots are usually disturbed and exposed. If the environmental conditions are adverse during the transplanting period, plants may be permanently injured or even killed To minimize these chances, the starter solution (66) and puddling method (72) have been advocated with all their advantages for best crop yields. However, these methods do not markedly prevent water losses from the plant and the sticking of clay paste to the roots (puddling method) can cause severe injury Hamner et al. (28) have devised a simple, yet inexpensive method of transplanting This method is a substitute for the puddling method and it involves the dipping of roots in a mixture made up of finely divided paper pulp and peat moss in suitable proportions. They reported that cranberry bean plants so treated did not suffer transplanting shock and subsequently made superior growth, as compared to untreated plants Other trials conducted by Hamner and his co-workers in the greenhouse and field strongly suggested that it is desirable to incorporate commercial fertilizers and growth regulators with the paper pulp and peat mixture McCall, Rai and Hamner (45) in their preliminary trial, using paper and peat moss with fertilizer as a transplanting aid for tomato plants, have

found that greater growth of both tops and roots resulted from such treatments

It is evident that this new method may have beneficial effects in the transplanting of many vegetable and floricultural crops which have very sensitive root systems. Therefore, experiments were conducted with tomato plants using paper pulp and peat moss as a carrier for some promising organic compounds to determine whether or not they showed promise as root promoters.

Methods and Materials

The response of many chemicals was found similar to auxins and anti-auxins on cucumber root growth. Therefore, it was thought desirable to evaluate some of the promising compounds in horticultural practices. Hence, experiments were initiated in field and greenhouse in which the roots of the tomato seedlings were treated with various chemicals at the time of transplanting.

Tomato Transplants - Greenhouse Studies

Two experiments involving treatments of transplants were simultaneously started in the greenhouse with tomato seedlings. Uniform seedlings, approximately seven centimeters in height were selected for the various treatments.

In the first experiment, the concentrations of 1-naphthoic, 6-quinolinecarboxylic and 1-hydroxy-2-naphthoic acids used were: 50, 100 and 1000 ppm.

Five seedlings were used in each treatment placed in beakers containing 50 cc of solution, and allowed to soak for 16 hours. After treatment the seedlings were transplanted in 9-inch pots containing sterilized soil and placed on a greenhouse bench and grown under greenhouse conditions for 15 days. The temperature ranged from 65° to 70° F at night, and from 75° to 80° F during the day in the greenhouse.

In order to determine the beneficial effects of paper and peat moss as a media for chemical application, another experiment was set up in the greenhouse. In this experiment, the same procedure was adopted as devised by Hamner et al. (28). Using a Waring Blendor, a mixture of 1/4 paper and 3/4 peat moss was made, this was used as the substrate for the various chemicals. Treatments were prepared using 1-naphthoic, 6-quinolinecarboxylic, and 1-hydroxy-2-naphthoic acids at concentrations of 50, 100 and 1000 ppm. Tap water only was used as check. The roots of tomato seedlings were dipped in the various slurries so that the pulverized mixture would adhere to the roots. The seedlings were then planted in 9-inch pots containing sterilized soil.

Fifteen days after transplanting, the plants of both experiments were harvested at the same time and their fresh weights were recorded (Table 19)

Tomato Transplants - Field Studies

Tomato, var Detroit early seedlings were raised in the greenhouse When the seedlings were eight weeks old, healthy and uniform plants were selected and treated August 6, 1955

The following treatments were considered:

(1) Paper pulp + peat moss.

(2) Paper pulp + peat moss + 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 50 ppm.

(3) Paper pulp + peat moss + 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 100 ppm

(4) Paper pulp + peat moss + 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 200 ppm

Essentially, the same procedure was adopted for preparing the mixture of paper pulp and peat moss as was advocated by Hamner et al. (28) One ounce of paper pulp and three ounces of peat moss were first mixed by hand Then this mixture was placed in a Waring Blendor with 500 mililiters of tap water The Waring Blendor was operated for about 15 minutes until a pulp of thick consistency was obtained In case of treatments 2, 3 and 4, 500 mililiters of solution of various concentrations were used in place of water

The roots of the plants were dipped in mixtures so that the pulp thoroughly covered the roots

The plants so treated were planted in rows 4 feet by 4 feet in the field where the treatments were arranged in a random fashion Eight plants were set per plot, and four plots for each treatment. Due to extremely dry weather conditions, it was thought desirable to water each transplanted plant No additional water was given to the plant throughout the experiment After 20 days the height of each plant in every treatment was measured The plants were harvested after 37 days in the field and fresh weights of vines were recorded (Table 20).

Results

Greenhouse Studies

When tomato seedlings were soaked in water solutions of the various chemicals, no marked stimulation of vine growth was observed (Table 19) On the contrary, growth was considerably inhibited with treatment at 100 ppm of all compounds and all seedlings were killed at 1000 ppm of 1-naphthoic acid and 1-hydroxy-2-naphthoic acid. In experiment 2, in which chemicals were applied through the media of paper and peat moss, significant increase in vine growth was recorded with treatment at 50 ppm of 1-naphthoic acid and 6-quinolinecarboxylic acid (Table 19) The seedlings were injured with 1000 ppm of 1-naphthoic acid and 1-hydroxy-2-maphthoic acid. The results indicate that the paper and peat moss mixture had a buffering action on the chemicals and the toxic action observed in the first experiment at 100 ppm

with all the concentrations did not occur. However, this buffering effect was not sufficient to overcome the toxic effect of the higher concentrations

It is evident that the mixture of peat moss, paper pulp and growth regulator show promise as a commercial method for aiding in transplanting of tomato seedlings.

Field Studies

Addition of 50 and 100 ppm of 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid to a mixture of paper and peat moss has significantly increased length of vines 20 days after transplanting (Table 20) Also, after 37 days of transplanting, significant increase in weight of tops was recorded with a treatment of 50 ppm of the chemical in paper and peat moss (Table 20).

Results indicate that high concentration of chemicals damaged the tender roots and consequently reduced the water absorption capacity of the roots

TABLE 19

The Effect of Methods of Transplanting Treatment on Fresh Weight of Tomato Vines

Organic Chemicals	* Experiment No. 1				** Experiment No 2			
	*** Average Fresh Weight (gms)				*** Average Fresh Weight (gms)			
	Conc. Organic Chemicals (ppm)				Conc. Organic Chemicals (ppm)			
	0	50	100	1000	0	50	100	1000
Control	16.80				15.60			
1-Naphthoic acid		18.00	10.02	--		23.20	15.20	
6-Quinolinecarboxylic acid		17.20	11.60	7.80		24.40	21.60	11.20
1-Hydroxy-2-naphthoic acid		14.40	9.60	--		15.20	12.80	--
L.S.D at 5% level	3.39				4.50			
L.S.D at 1% level	4.55				6.04			

* Soaked for sixteen hours before transplanting.

** Seedlings soaked in a mixture of paper and peat moss with different concentration of organic chemicals.

*** Average of five plants.

TABLE 20

The Effect of Methods of Transplanting Treatments on the Growth of Tomato Vines

Treatment	*Average Height of Tops per Plant (Inches) After 20 Days of Transplanting	*Average Fresh Weight of Tops per Plant (Pounds) After 37 Days of of Transplanting
Paper pulp and peat moss	10.7	0.36
Paper pulp and peat moss plus 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 50 ppm	12.1	0.62
Paper pulp and peat moss plus 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 100 ppm	11.8	0.41
Paper pulp and peat moss plus 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 200 ppm	10.9	0.38
L.S.D. at 5% level	0.8	0 19
L.S D. at 1% level	1.1	0 27

*Average of four replications.

Mixture of one-quarter paper pulp and three-quarters peat moss.

The Treatment of Seedling Plants with Organic Chemicals

Review of Literature

Plant growth regulators applied in very dilute solution to roots or shoots is absorbed by the plants If suitable concentrations are used, characteristic growth responses may occur. Hitchcock and Zimmerman (31) reported that synthetic plant growth regulators applied as solutions to the soil of plants growing in pots were absorbed by the roots, and responses such as epinasty of leaves and formation of roots on the stem of the plant occurred. Pearse (53) sprayed young tomato plants with a 0.1 percent solution of phenylacetic acid and indolebutyric acid and noted increased height of the plants Grace (23) reported that growth of young seedlings of tomato, nasturtium and salvia, watered daily with nutrient solution containing synthetic growth substances, was increased considerably

There are, however, several results that have been advocated in which the synthetic compounds were not able to show their significance on the subsequent growth Templeman (64) carried out pot culture experiments in which solutions of indoleacetic acid, a-naphthaleneacetic, skatole and ascorbic acid were applied to plants by spraying the foliage, and by watering the sand. No significant results were found. Hamner (26) observed that adding alpha-naphthaleneacetamide to the nutrient solution

for growing red kidney bean plants resulted in less top growth, but increased root growth In later papers Hamner (30) reported that no particular benefits were achieved by adding small quantities of phenyleacetic acid or naphthaleneacetamide to the plants grown in pots Swartz (63) reported alpha-naphthaleneacetic acid, contained in a complete nutrient solution failed to stimulate the growth of chrysanthemum, marigold or cosmos seedlings Zimmerman (88) stated that there is at present no established proof that any of the synthetic growth regulators stimulate the growth of plants.

A series of experiments were conducted by treating different growing plants with various concentrations of organic compounds found effective in retarding or elongating cucumber roots to determine if these newer materials might be effective These treatments, also included many chelating compounds, which were found to stimulate cucumber root growth and it was believed that their chemical structure resembled that of the anti-auxins

Methods and Materials

Foliage Application

Tomato (var Early Detroit) seedlings grown under greenhouse conditions[1] were used as experimental material Seedlings, approximately 8 cm in height were transplanted to 9-inch pots filled with sterilized rich soil

[1]The greenhouse conditions were similar to those used for the abscission studies of coleus.

Ten days after transplanting, the seedlings were sprayed, using a hand sprayer, with 1000, 5000, and 10,000 ppm of 1-naphthoic acid, 6-quinolenecarboxylic acid, 1-hydroxy-2-naphthoic acid Five plants were sprayed under each treatment. Fifteen days after treatment, the plants were harvested. The fresh weights of vines were recorded (Table 21)

Hamner (29) reported that 1, 4-naphthalenediol is very active in inhibiting cucumber roots even in minute doses. Therefore, it was thought desirable to test the activity of inhibiting growth of seedling organs of sensitive plants Tomato (Lycopersicum esculentum var Early Detroit) and coleus (Coleus blumei L. var Christmas Gem), grown in the greenhouse were used for investigation. The seedlings were transplanted to 6-inch pots containing rich sterilized soil. Tomato and coleus seedlings, approximately 9 inches and 6 inches in height respectively, were sprayed with a hand sprayer, using 1000 ppm, 5000 ppm, and 10,000 ppm of 1, 4-naphthalenediol. Six plants of tomato and four plants of coleus were sprayed under each treatment. Pictures of the seedling (Figure 15) were taken 10 days after spraying and the plants were harvested 23 days after treatment. The fresh weights of tops were recorded (Table 22)

Results

It is evident from the data that no marked differences in growth occurred due to different treatments However, when tomato plants were sprayed with

TABLE 21

The Average Fresh Weight in Grams of Tomato Plants Fifteen Days After Treatment with Various Concentrations of Organic Chemicals.

Concentrations of Organic Compounds	*Average Fresh Weight (gms) per Plant
Control	17.2
1-Naphthoic acid - 1000 ppm	22.8
1-Naphthoic acid - 5000 ppm	15.2
1-Naphthoic acid - 10000 ppm	14.0
6-Quinolinecarboxylic acid - 1000 ppm	19.2
6-Quinolinecarboxylic acid - 5000 ppm	18.4
6-Quinolinecarboxylic acid - 10000 ppm	12 0
1-Hydroxy-2-naphthoic acid - 1000 ppm	18.0
1-Hydroxy-2-naphthoic acid - 5000 ppm	10.4
1-Hydroxy-2-naphthoic acid - 10000 ppm	8.8
L. S. D. at 5% level	4.5
L. S. D. at 1% level	6.0

*Average of five plants.

TABLE 22

The Effect of Various Concentrations of 1, 4-Naphthalenediol upon the Growth of Seedling Tomato and Coleus Plants.

Concentrations	Average Fresh Weight (gms)	
	Tomato**	Coleus***
Control	21.71	23.88
1000 ppm	12.21	17.38
5000 ppm	8.04	11.06
10000 ppm	4.63	6.81

*Data recorded after twenty-three days of spray of chemical.

**Average of six plants in case of tomato seedlings.

***Average of four plants in case of coleus.

Figure 15. Response of foliar application of 1000, 5000, and 10, 000 ppm of 1, 4-naphthalenediol on coleus and tomato plants after ten days of treatment.

1000 ppm of 1-naphthoic acid, some increased growth was noted This was significant at the 5% L S D level

Sprays of 1, 4-naphthalenediol at all concentrations considerably inhibited the growth of coleus and tomato seedlings Plants were killed at increasing concentrations, as is evident from Figure 15 Results indicate that 1, 4-naphthalenediol was markedly toxic to plant tissues and therefore might be of some value in using as a herbicide

Methods and Materials

Soil Application

Several chelating compounds which stimulated the root growth of cucumber seedlings were investigated to determine their activity on tomato Tomato (Lycopersicum esculentum var Early Detroit) seedlings approximately 6 cm in height were transplanted in 9-inch pots containing soil lacking in organic matter and nutrients. It was believed that any beneficial effect of these chemicals might be exaggerated in this type of soil One week after the seedlings were transplanted, solutions of 25 and 100 ppm of various chelating compounds were applied to the soil Each pot was soaked with 500 mililiters of solution Five plants were kept under each treatment. Control plants were also irrigated with the same amount of tap water. Twenty days after soil application, the plants were harvested and the fresh weights of vines were recorded (Table 23).

Results

Tomato vine growth was significantly stimulated with 25 ppm of 4-(p-ethoxyphenylazo)-m-phenylenediamine, as compared to the control No marked differences in fresh weight were found with other compounds (Table 23)

TABLE 23

The Effect of Soil Application of Various Chelating Compounds Upon the Growth of Seedling Tomato Plants.

Chelating Compounds	Average Fresh Weight per Plant (gms) Conc. Chelating Compounds (ppm)		
	0	25	100
Control	8 75		
1-Amino-4-hydroxyanthraquinone		12. 15	6. 20
1-Amino-2-naphthol-4-sulfonic acid		11. 10	7. 45
Resorcinol		8. 80	10 55
Diethyldithiocarbamic acid		11. 00	10. 75
2, 4-Dihydroxyacetophenone		11. 55	9. 45
4-(p-nitrophenylazo) resorcinol		12. 40	8 85
p, p-Methylenebis(N, N-dimethylaniline)		8. 85	14. 15
Diphenylglyoxime		10. 25	11. 15
2, 2, 4, 4, 6, 6-Hexanitrodiphenylamine		9. 50	11. 25
4-(p-ethoxyphenylazo)-m-phenylenediamine		13. 90	12. 70
L. S. D. at 5% level	3. 48		
L. S. D. at 1% level	4. 62		

* Data taken twenty days after treatment.

Blossom and Fruit Thinning of Fruit Trees with Organic Chemicals

Review of Literature

Excessive flowering in one year accompanied by heavy fruiting results in biennial bearing in many fruit trees To alleviate this situation, hand thinning of flowers and young fruits has been practiced by growers This is an expensive and tedious process. Auchter and Roberts (7) first demonstrated that chemical sprays could be used effectively to thin apple blossoms. Later Burkholder and McCown (16) noticed that auxins, such as naphthaleneacetic acid, would reduce the fruit set of apples Following these discoveries, many synthetic chemicals were effectively utilized to thin flowers and fruits The use of these chemicals has been summarized by Tukey (73), Leopold (41), Audus (9), Batjer and Hoffman (11).

The degree of thinning flowers or young fruits resulting from the use of chemicals is greatly influenced by tree vigor, time of application, environmental conditions, and variety Southwick et al (61), and Langer (38) advocated that no satisfactory method of thinning peaches has yet been established. Leopold (41) demonstrated that thinning of blossoms and young fruits by auxins appears to be due to three physiological factors: (1) prevention of natural pollination; (2) abortion of young embryo; and (3) direct acceleration of abscission by alteration of the auxin gradient at the abscission zone

Experiments were conducted to determine if actidione, sodium azide, and 1, 4-naphthalenediol would be effective in thinning apples, peaches and cherries on the Horticultural farm of Michigan State University, East Lansing, in the spring of 1955.

Methods and Materials

Peaches

Trees of equal vigor of two different varieties, Halehaven and Redhaven, were used for the investigations. Aqueous sprays at 50 and 250 ppm of 1, 4-naphthalenediol and sodium azide were applied to the limbs during the middle of the day on April 28, 1955. Sprays of actidione at 2. 5 and 10 ppm were also used Each treatment was applied to the limbs which were selected in a random fashion on five different trees of each variety The trees were at the peak of full bloom. The number of flowers and flower buds on the branches at the time of spraying was recorded Observations were taken each week on the abscission of flowers.

Cherries

Montmorency sour cherry trees in full bloom were sprayed with 50, 250, 500 and 750 ppm of 1, 4-naphthalenediol, 2 5 ppm and 10 ppm of actidione, and 5, 15, 30 and 60 ppm of 2, 4, 5-trichlorophenoxy propionic acid on May 3, 1955 Each treatment was applied on a small limb which was selected

in random fashion on five trees Since sodium azide was found to be considerably promising in thinning blossoms in experiments of peach trees, it was thought desirable to determine its effectiveness on sour cherry blossoms Hence, other lots of cherry trees were sprayed with 500 ppm of sodium azide at early petal fall on May 5, 1955 The treatment was replicated five times on different trees. The branches were tagged and their flowers were counted.

Apples

Three varieties of apples (Delicious, in full bloom; Wealthy, at early petal fall; and McIntosh, at late petal fall) were sprayed with 25, and 50 ppm of 1, 4-naphthalenediol; and 50 and 250 ppm of sodium azide on May 5, 1955. Three trees of each variety were selected for treatment. Small limbs selected in random fashion on these trees were used The number of flowers on the treated branches were counted at the time of sprayings and records were kept in order to compare the effect of the chemicals.

Results

No conclusive results were obtained due to a late freeze All fruits on the tree were frozen and damaged. However, our early observation indicated that sodium azide at all concentrations had effectively thinned the blossoms on peaches. However, the greater thinning was noticed

when 250 ppm of the chemical was used Slight differences were recorded with 1, 4-naphthalenediol and actidione at the higher concentrations. The Delicious apple variety was markedly affected by sodium azide, as compared to McIntosh and Wealthy varieties The flowers were not injured at high concentrations. The activity of the compounds varied somewhat with the dosage applied By personal observations, it was found that sodium azide was more promising in blossom thinning than was 1, 4-naphthalenediol.

No marked differences were recorded with cherry trees However, a considerable number of flowers were injured with 500 ppm of sodium azide and 750 ppm of 1, 4-naphthalenediol.

GENERAL DISCUSSION

An attempt has been made to determine whether or not there is any correlation between structure and activity of various organic compounds which were tested

As a result of this research, many new growth regulators have been discovered, and perhaps several important leads have been uncovered

It has been found that many naphthoic acid compounds are active as growth regulators. These compounds are similar in structure to naphthaleneacetic acid, except the side chain consists only of a carboxyl group, whereas in naphthaleneacetic acid the carboxyl group is attached through methylene on the ring nucleus. If the carboxyl group is in the 1-position, the compound is more active, as compared to when it is in the 2-position When an hydroxyl group is added in different positions on the ring structure, for example 1 and 3 positions the activity is reversed, and apparently the compounds act as an anti-auxin, this is indicated by their action in stimulating root elongation, and on hastening abscission It is believed that the introduction of an hydroxyl group might effect the two point attachment of the chemical on the protein substrate, as explained by Muir and Hansch (49)

Substituting nitrogen for carbon in the ring structure apparently does not change the activity of the compounds, since heterocyclic nitrogen

compounds have also been found to act as an auxin, and when hydroxyl groups are added to the heterocyclic nitrogen compound, it then acts as an anti-auxin.

The structures of these materials has suggested also that perhaps the activity might be correlated with their ability to act as metal chelates. Many chelating materials were tested, and some were found to be acting in promoting root growth and also top growth of certain plants. It is believed that perhaps these chemicals were removing excessive quantities of metal from the roots, and thus increasing the metabolic activities. The fact that some of these chelating agents increase root growth might also indicate that they are acting as an anti-auxin. It is thought possible that many of our so-called anti-auxins act like chelating agents and complex some metal enzyme system.

In the course of the study other materials seemingly unrelated were actidione, 1, 4-naphthalenediol, and sodium azide These compounds had inhibited the root growth of cucumber seedlings; therefore, it was thought that these compounds perhaps be active as blossom thinners. These materials were sprayed on peaches, apples and cherries It was observed that sodium azide is very promising for flower thinning.

1, 4-naphthalenediol is also an inhibitor of root growth of cucumber and was observed active as a toxic compound for the growing plants It is, therefore, suggested that this compound has a property for herbicidal use.

SUMMARY

Aromatic organic compounds containing an hydroxyl group or an acid group, or both, other substituents and heterocycle nitrogen were investigated for their activities upon cucumber roots. A number of metal chelating compounds were also tested for their activities Promising compounds were then tested on various crops to determine the possibilities for their horticultural use

1. Compounds that most effectively stimulated root growth of cucumber seedlings grown in petri dishes were: 1-hydroxy-2- naphthoic acid, 3-hydroxy-2-naphthoic acid, 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid and its disodium salt, 1-naphthol,2, 4-dichloro-1-naphthol, 4, chloro-1-hydroxy-2-naphthoic acid. Slight response upon root elongation was observed with 2-naphthol, 8-quinolinol, benzoic acid, and 1-hydroxy-2-naphthalene sulfonic acid (sodium salt).

2. Compounds, 1-naphthoic and 6-quinolinecarboxylic acids inhibited root growth of cucumber seedlings in a characteristic manner similar to the responseof naphthaleneacetic acid on cucumber roots Other active root inhibiting compounds were quinaldinic, 2-picolinic, 2-naphthoic acids, and 1-hydroxy-2-acetonaphthone

3 No additive response upon cucumber root elongation occurred when urea and KH_2PO_4 were simultaneously applied with 4. 4'-methylenebis-3-hydroxy-2-naphthoic acid in petri dishes

4 Metal chelate compounds found to stimulate cucumber root growth significantly at the 25 ppm dilution were: resorcinol, benzotriazol, 2, 2'-biquinoline, diethyldithiocarbamic acid, 2, 4-dihydroxyacetophenone and mercaptoacetic acid Slight effect was observed with 1-amino-4-hydroxy-anthraquinone, thiazole yellow, and furil dioxime compounds in petri dishes

5 Among the metal chelate compounds tested that strongly inhibited cucumber root growth were phenyl-y-thiohydrantoic acid, phenylglyoxal aldoxime and 2, 2, 4, 4, 6, 6-hexanitrodiphenylamine. Somewhat less inhibition was found with 1, 5-(p-dimethylaminebenzylidene) and 2, 3-butanedione oximethiosemi-carbazone

6 Other metal chelating and other organic compounds under investigation had no marked effect on cucumber root growth. Somewhat toxic effect was noticed at higher concentrations.

7 Concentrations of 10, 100 and 1000 ppm of 2, 3, 5-triiodobenzoic, 4, 4'-methylenebis-3-hydroxy-2-naphthoic, 1-hydroxy-2-naphthoic, and 3-hydroxy-2-naphthoic acids applied in lanolin to the cut surface stimulated the abscission of debladed coleus petioles The rate of abscission was directly proportional to the dosage applied

8 When 2, 3, 5-triiododbenzoic acid 1000 ppm was applied simultaneously on the debladed petioles with either 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 10 ppm, 3-hydroxy-2-naphthoic acid 100 ppm, or 1-hydroxy-2-naphthoic

acid 1000 ppm increased stimulation of abscission was noted

9. Simultaneous application of naphthaleneacetic acid at 100 ppm with either 2, 3, 5-triiodobenzoic acid 1000 ppm, or 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 10 ppm, or 3-hydroxy-2-naphthoic acid 100 ppm, or 1-hydroxy-2-naphthoic acid 1000 was found to increase the abscission as compared to naphthaleneacetic acid when applied alone. It indicates that the action of naphthaleneacetic acid on abscission was counteracted by these materials showing that these compounds may be anti-auxins.

10. When 2, 3, 5-triiodobenzoic acid 1000 ppm, or 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid 10 ppm, or 3-hydroxy-2-naphthoic acid 100 ppm or 1-hydroxy-2-naphthoic acid 1000 ppm was applied midway between the abscission zone and the cut surface with naphthaleneacetic acid 100 ppm applied on the tips of debladed petioles, the response of naphthaleneacetic acid was reduced. A similar action was recorded when these materials were applied in a spiral fashion; thus indicating that these materials have anti-auxin properties.

11 The compound 1-naphthoic acid at a 10 ppm concentration applied on the cut surface of debladed petioles delayed the abscission of petioles of coleus. However, 1000 ppm concentration was found to accelerate the abscission.

12 Soaking coleus cuttings in a 100 ppm solution of 1-naphthoic acid and a 1000 ppm solution of 6-quinolinecarboxylic acid before rooting, in-

creased the percentage of cuttings which rooted.

13 No stimulation on growth occurred with 1-naphthoic, 6-quinolinecarboxylic, and 1-hydroxy-2-naphthoic acids when the seedlings were soaked for 16 hours before transplanting, but they were injured at higher concentrations. However, when these chemicals were applied through the media of paper and peat moss, vine growth was stimulated except in the plants treated with 1-hydroxy-2-naphthoic acid.

14. Tomato plant roots treated with 50 ppm of 4, 4'-methylenebis-3-hydroxy-2-naphthoic acid through the media of paper and peat moss and transplanted in the open field exhibited greater fresh weight of vines as compared to control.

15. Spray of 1000 ppm of 1-naphthoic acid on tomato plants stimulated the vine growth. Dilution of 10, 000 ppm of 6-quinolinecarboxylic and 1-hydroxy-2-naphthoic acids considerably reduced the growth of vines as compared to the control.

16. Coleus and tomato plants sprayed with 1000 ppm, 5000 ppm and 10, 000 ppm solutions of 1, 4-naphthalenediol were seriously injured. At high concentration, plants were killed.

17 Soil application with 25 ppm dilution of 4-(p-ethoxyphenylazo)-m-phenylenediamine gave significantly greater fresh weight of vines as compared to control Other metal chelate compounds under investigation had

no stimulating response upon vine growth.

18. Chemicals, 1, 4-naphthalenediol, sodium azide, actidione and 2, 4, 5-trichlorophenoxy propionic acid sprayed on peaches, cherries and apples gave no conclusive indications of their response due to late snow fall in spring. All fruits and flowers were damaged However, sodium azide sprayed with various dilutions on different fruit trees significantly reduced the number of blossoms on the treated limbs as compared to the control.

19 An attempt was made to correlate the chemical structure with the activity of the organic compounds tested.

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