EFFECT OF Fe(III)Cl COMPLEXES OF A FEW AROMATIC SCHIFF-BASES UPON GERMINATION OF OAT AND CAPSICUM SEED

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Introduction

In a previous publication (1) the authors published data on the affect of Schiff-bases of o-phenylenediamine formed with aromatic aldehydes upon the germination of oat and capsicum seeds. Compound IV. (Salycylaldehyde--o-phenylenediamine) favourably influences the germination time of both seeds.

It was established (2) that the growth control of complex formers such as EDTA, could be achieved by bonding the iron, calcium and cobalt ions of the structure in inactive form. The authors started experiments on oat and capsicum seeds with the complexes of Fe(III) Cl Schiff-base for the elucidation of the open question left in their first paper, and for deciding that if complexes are added to the seeds, in wich direction does this influence germination or rather growth.

Experimental

The results of the complex forming ligands made the using up of the following complexes justified:

I. o-Vanillin-o-phenylenedinamineFe(III)Cl
II. Resorcylaldehyde-o-phenylenediamineFe(III)Cl
III. Resacetophenone-o-phenylenediamineFe(III)Cl
IV. Salicylaldehyde-o-phenylenediamineFe(III)Cl.

The application of these compounds precludes the possibility of isomers, because the o-phenylenediamine group forms a five-angular ring with the iron ion which determines the structure of the molecule unequivocally (See Fig. 1). As is well-known, the possible isomers can have an influence on germination and growth (3).

For four parallel experiments, 5, 10, and 20 mg of the complexes were spread over filter papers placed in appropriate Petri dishes. The same amount of complex, that was used at the experiments with Schiff-base, was not sufficient for the even layering on the filter paper. Fifty healthy oat-grains were put in the so prepared Petri disches. Then they were maistened with distilled water and germinated in a glass-house at 18° C with the exclusion of light. Water was replaced every 24 hours. Observations were carried out for germination, growth of plants, and for investigation of internal content [P(4), N(5), Fe(6) determination].

Evaluation of results

The first succesful observation was carried out after 48 hours. Results can be found in Table I.

Table I.

	I.	II.	III.	IV.	∅
5 mg	17	11	16	9	
10 mg	13	9	10	6	9
20 mg	9	5	5	2	

(Figures represent the number of sprouts)

Observations carried out after 96 hours can be put down as follows:

Ten mg dosage of compound I. yielded the most favourable effect out of all the other compounds and of various amounts. These plants left behind in height and growth as well those of the control and those that were treated with other compounds. Dosages promote growth in the order of 10, 20, and 5.

Lenght of the plants whose seeds were treated with compound II. exceeds the height of the control ones, the disposition, however, was not uniform. There were relatively many seeds backward in growth. As regards effects, there was no deviation among the different dosages.

Plants of seeds treated with compound III. showed a better growth in the case of dosage of 5 mg than those of the controls. Their stems were bulkier and stronger. Dosage treatment of 10 and 20 mg also bring about a more vigorous growth, but this appears only in the thickness of the stems, since the heights of the plants are smaller than those of the control.

Plants of seeds treated with compound IV. in the case of a 5 mg dosage show a better growth than those of the comparative ones. They developed good roots. Dosage of 10 mg decreases the time of growth. As for the dosage of 20 mg., it definitely hinders the growth of the plants.

The influence of the above four complexes upon the germination indicates the following order in the period of observation:

Table II.

5 mg	III.	>	II.	>	IV.	>	I.	>	∅
10 mg	I.	>	∅	>	II.	>	IV.	>	III.
20 mg	II.	>	∅	>	III.	>	I.	>	IV.

With 5 mg dosage, it is compoud III., with 10 mg it is compoud I., and with that of 20 mg it is compoud II. that shows the most favourable influence. Observations carried out after 120 hours make the results obtained so far more definite and can be put down as follows:

Plants sprouting from seeds treated with compound I. show a better growth than those of the control, and the effect of this complex is better than those of all the other complexes. The various dosages have near the same effect, except, perhaps, dosage of 10 mg, where the growth was more balanced.

With plants sprouted from seeds treated with compound II. it is the dosage of 5 mg that shows a better result than that of the control. This, however, cannot be seen in the heights of the plants, but rather in their disposition. The root is abundantly well-developed and strong. With increase of dosages of the compounds the effect declines.

The root of plants from seeds treated with 5 mg of compound III. are long and densely reticulated. The height of the stems are identical with those of the control. In the case of dosage of 10 and 20 mg, the root is stunted. The latter dosage almost completely destroys the root, its surface is brown coloured. With this dosage the plants are entangled and unstable. The roots are unable to hold the growing plant. The hindering effect is in direct proportion to the increase of the dosage.

The development of plants sprouted from seeds treated with compound IV. are identical with those of the control; the initial differences have completely disappeared. Development of the root in case of the 5 mg dosage is better than that of the control. The effect exerted upon the development of the root is in inverse proportion to the increase of dosage.

The effect of the complexes upon the growth of the plants on the basis of the results observed after 120 hours is as follaws:

Table III.

5 mg	I.	>	III.	>	II.	>	∅	>	IV.
10 mg	I.	>	∅	>	II.	>	IV.	>	III.
20 mg	I.	>	∅	>	II.	>	IV.	>	III.

All the applied dosages of compound I. show the most favourable effect.

Observations carried out prior to ingathering (after 192 hrs.) can be summarized as follows.

The growth of the control plants is normal, the root formation is uniform and abundant. The roots completely enmesh the Petri dish and they grip well onto the filter paper. Pathological changes can be observed neither on the root, nor on the leaves. The leaves have normal green colour.

Development of the plants of seeds treated with compound. I. changes depending on dosages. In the case of 5 mg and 10 mg, the root formation is normal, about the same as that of the control plants. Malformation in the roots cannot be observed. These plants somewhat overtook the controls in growth. Pathological change cannot be observed. Their colour is identical with that of the controls. Dosage of 20 mg has a hindering effect upon the development of the root, anomalous change, however, cannot be observed. The longitudinal growth of the root is hindered, but the lenght of the plants reaches that of the control even so.

Plants of seeds treated with 5 mg of compound II. show a normal root formation, which appears to be the same as that of the control. Development of the stem is uneven; as a general impression, it is the same than that of the control. Treatment with 10 mg dosage results in a more abundant root formation. Anomalous twisting can be seen in some places which apparently does not affect the growth of the plants. Their development is somewhat better than that of the control. As a result of the two treatment, pathological change cannot be observed. Treatment with dosage of 20 mg does not disturb very much the growth of the roots, but they develop anomalously, become strongly twisted, and grow thin in some places, or even die off. As regards growth, the plant hardly falls behind that of the control. Treatment does not affect the colour of the plant.

Plants of seeds treated with 5 mg of compound III. show a moderate root formation, which seemingly is better than that of the control. Traces of root distortion can be seen. Growth of the stem is better than that of the control its colour is fresher, too. Dosage of 10 mg results in defective root formation and the developing roots are also wiry. Some of them die off, then formation of new roots begins. The plant, however, does not get stunted; treatment with dosage of 20 mg results in an even lesser root formation. The roots turn brown and after reaching a length of 1 to 2 cm the tip of the root dies off. Similar phenomenon was observed (2, 7, 8,) at the root development of various plants treated with 2,2-diclorpropionacid, EDTA, and with derivatives of indole. etc. In later period of the development of the plants new roots of side direction developed in place of the dead ones. The plant hardly falls behind in growth from that of the control; its colour, however, is fresher and greener. The root does not support — does not grip — therefore, the plant falls down.

Plant of seeds treated with 5 mg of compound IV. show a good root development, which is somewhat stronger than that of the controls and they have a good grip. The growth of the plant is also slightly stronger than that of the controls, but in colour it does not differ from those. Pathological change cannot be observed. The same observation applies on treatment with dosage of 10 mg as at the previous one; the plant colour, however, is a shade greener. The treatment with 20 mg dosage causes irregular root formation where the roots develop anomalously and, in places, they become twisted. The development of the plants is also uneven. As a whole, they are weaker,

Table IV.

		I.	II.	III.	IV.
I.	Compound	5 mg	41	0,3628 g	0,8638 g
	„	10 mg	48	0,3846 g	0,7692 g
	„	20 mg	42	0,3816 g	0,9086 g
II.	Compound	5 mg	37	0,2920 g	0,6790 g
	„	10 mg	37	0,2746 g	0,7421 g
	„	20 mg	34	0,2510 g	0,7382 g
III.	Compound	5 mg	40	0,2154 g	0,6110 g
	„	10 mg	39	0,2184 g	0,5600 g
	„	20 mg	40	0,2228 g	0,5570 g
IV.	Compound	5 mg	37	0,2328 g	0,6292 g
	„	10 mg	38	0,2270 g	0,5974 g
	„	20 mg	39	0,2068 g	0,5170 g
∅			40	0,3074 g	0,7149 g

too, than those of the controls; their colour is the darkest among all the other treated ones.

After the above observations the plants were cut off right above the endosperm, then fixed and dried. The number of buds, the total air-dried weight and the value of g calculated for 100 seeds, are summarized in table IV.

Experimental (capsicum)

Experiments with capsicum were carried out with 4×100 seed number and dosages of 5, 10, and 2 0mg in a similar manner as with oat.

Succesful observations could be made on the 12th day. Data are summarized in Table V.

Table V.

	5 mg				10 mg				20 mg			
I.	3	5	4	3	2	1	3	3	3	2	4	2
II.	2	1	1	1	4	2	3	3	4	7	3	5
III.	3	3	3	3	5	5	6	4	2	4	5	3
IV.	1	1	1	1	3	3	3	4	3	5	5	4
∅	2	2	3	2								

From the data a relatively small degree of negative effect of the dosage of 5 mg of compound II. and IV. shows up strikingly.

The following data were collected on the basis of observations carried out on the 16th day and are summarized in Table VI.

Table VI.

	5 mg				10 mg				20 mg			
I.	40	45	35	40	38	34	33	37	40	46	38	45
II.	48	46	39	42	40	35	43	41	44	46	39	42
III.	31	32	30	29	30	30	29	30	31	34	28	30
IV.	27	28	30	32	34	29	34	30	23	27	31	25
∅	22	30	34	26								

Plants of seeds treated with compound I. show a more vigorous growth than those of the control ones in case of every concentration.

Those that were treated with compound II. and using dosages of 5 and 20 mg are well-developed and stronger than those of the control; dosage of 10 mg does not cause any difference.

Plants of seeds treated with compound III. show identical development with that of the control in every case of the different dosages.

Those plants whose seeds were treated with compound IV. have an identical development as those of the control in cases of dosages of 5 and 10 mg; 20 mg, however, hinders their growth.

The last observation was carried out at the time of ingathering i. e. 24 days after the setting up of the experiment. Data are summarized in Table VII. where the number of germinated seeds are presented. The figures underneath represent the number of those seeds that developed cotyledons as well.

Table VII.

	5 mg				10 mg				20 mg			
I.	46	49	44	45	42	40	44	46	44	47	48	49
	19	18	17	16	14	14	16	15	17	15	16	14
II.	48	46	41	43	40	38	44	45	46	48	40	42
	16	12	11	10	5	2	4	3	2	5	2	3
III.	32	34	33	35	33	34	35	35	39	38	35	37
	1	4	1	2		2	3	1	5	2	1	
IV.	29	30	34	36	35	32	36	32	26	29	34	29
	1	1	3	2	5	2	2	4				
∅	39	33	34	35								
	3	2		3								

Plants of seeds treated with compound I. are nicely developed and show a uniform growth. They have strong root-hairs and abundantly developed roots.

Plants of seeds treated with compound II. are more abundant and stronger than thos of the control ones. The roots are short and with dosage of 20 mg, development of root-hair does not occur, only that of the main root, but even that is short.

Plants of seeds treated with compound III. are weaker in growth than those of the control. Their root had died off, they appear as if the compound would have blocked the development of the plant.

Those plants whose seeds were treated with compound IV. are weakly developed. Though, the results of treatment with dosage of 5 mg hardly differ from those of the control, the dosage of 10 and 20 mg, however stops growth. After the commencement of germination the plant does not develope.

The plants were fixed and dried after ingathering. Dry-weight data of the four parallel measurements are summarized in Table VIII.

Table VIII.

I.	Compound	5 mg	0,2502 g
	,,	10 mg	0,1890 g
	,,	20 mg	0,2302 g
II.	Compound	5 mg	0,1856 g
	,,	10 mg	0,1870 g
	,,	20 mg	0,1858 g
III.	Compound	5 mg	0,1630 g
	,,	10 mg	0,1540 g
	,,	20 mg	0,1110 g
IV.	Compound	5 mg	0,1320 g
	,,	10 mg	0,1580 g
	,,	20 mg	0,1206 g
∅			0,1464 g

In order to decide whether the plant takes up the compounds salicylaldehyde-o-phenylenediamine Fe^{59}(III) Cl radioactive complexes were added to the oat seeds. When the plants of these oat seeds were 10 cm high they were cut off at the part where they were green, then they were dried and their weight was measured. After this they were crushed and the count per minute was also measured by a scaler. Reproducable count was obtained, the value of which — after deduction of the background (60—70 c. p. m.) — proves the presence of iron in the plant.

The effect of the four complexes upon phosphate enzyme of the soil was measured. Experiments were set in according to the (B. TARNGER) method. Four mg of the complex compound was added to the measured soil sample. The results can be seen in Table IX.

Table IX.

Ø	2,9	3,0	3,0	2,9	mg %
I.	4,0	4,2	4,0	4,1	„
II.	3,0	3,0	2,9	3,0	„
III.	3,1	3,0	3,0	2,8	„
IV.	3,0	2,9	3,0	3,0	„

The values, unequivocally, support the outstanding effect of compound I.

Theoretical

From the theoretical data it can be established that the salicylaldehyde-o-phenylenediamine Fe (III)Cl (IV) compound has the least effect upon growth. At the investigation of the ligands this complex former showed the greatest activity. The result of the investigation is in agreement with the statements hitherto. Growth is promoted by the polar part of the applied compound.

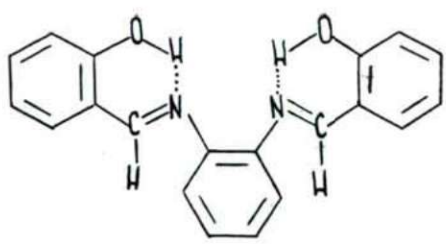

Figure 1.

If this structure is altered then the effect does not occur; this result corresponds with the observations of other researchers (9). If they exchanged the OH radical for that of methyl one, the so formed compound did not exert any influence upon growth, or rather upon the living organism. When carrying out the experiments the authors of this paper ceased the existence of the OH radical by producing complexes and in doing so the hydrogen of the OH radical broke off and the oxygen directly bonded to Me. With compound IV. the activating effect, which exists with the Schiff-base, ceases, moreover, inhibition occurs. From among the complexes the o-vanillin-o-phenylenediamine Fe(III)Cl (I) compound undoubtedly increases growth and decreases the time of germination. With this compound the Schiff-base part was ineffective. The active character of the complex may be associated with its electron dense and electron deficient places existing within itself.

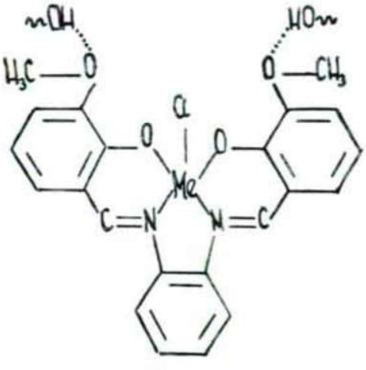

Figure 2.

The metoxy group in compound I. being in ortho position is able to produce hydrogen bonding with its unused electron, thus being in a position to bond to the protein molecule. Therefore, the plant integrates the relatively big molecule of the complex into its organism without decomposition. In complex I. there are two such metoxy groups located on one side of the molecule (in cis form).

Establishment of the results of investigations hitherto.

1. The complexes are very stable (10). These compounds do not dissolve in alcoholic solution of 0,1n hydrochloric acid and 0,1n sodium hydroxide.

2. The complexes do not dissolve, nor do the Schiff-bases in aqueous solutions and thus neither do they in the press-fluid of plant tissues.

3. If the plant dissolved complex I., then the above structure and the activating effect would cease to exist.

4. Since Schiff-base I. is effective, while complex IV. is not, therefore it is presumable that both of them have to integrate into the plant without dissolution.

5. The plant takes up the complexes; the iron isotopes were succesfully traced. It follows from the findings comprised in the above points, that the complex can get into the organism of the plant without dissolution.

6. That the electrofil radical in ortho position has an activating effect, it is proved by the data of enzym investigations. If the complex had dissolved, the results would not be uniform.

7. Complex I. is effective, receiving this character from the electrofil radical in ortho position. As long as the electrofil radical can be found at the Schiff-base, it is active, when, however, the above radical gets bonded (at the formation of complex IV.) the compound becomes inactive. Though the electrofil radicals of Schiff-base IV. and complex I. are not the same, their effect upon the germination of the seeds are nearly identical.

8. From the experimental data the role of the electrofil radical seems to be very important, which is in contrast with the establishments made for the complex forming ligands (2).

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