EFFECT OF SCHIFF-BASES UPON THE GERMINATION OF OAT AND CAPSICUM

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Introduction

At the investigation of the complexes of Fe(III)Cl formed by the different aromatic Schiff-bases, a considerably great stability was observed in the case of a few compounds (1). Besides the usual phisicochemical investigations, it was desirable to introduce such experiments that would support the results obtained hitherto from an other aspect. According to some biological investigations a tuberculostatic effect wast observed with the (2) Schiff-base Cu complexes. There were no data found in literature about the effect of aso-methine complexes upon plants. Hence came the idea to investigate whether the plant decomposes such stable complexes in that way that it makes uses of their atoms or rather their atom groups for the contruction of its own structure.

After such considerations, four compounds were selected from the availabe stable complexes — the Fe/III/Cl complexes of the derivatives of o-fenilinediamine formed by aromatic aldehydes — as basic compounds, and their effect upon germination was observed. O-fenilenediamine derivatives were chosen in order to decrease the potential number of the isomers. It was established (3) that the potential isomers have an influence upon the efficiency of the effect of the compounds. The results showed quite a number of interesting things, therefore the writers of this paper found it justified to investigate the effect of the complex forming ligands upon the germination of oat and capsicum. The aim was not to find some new, growth stimulating substance, but merely to elucidate the problem connected with the assignement. During the experiment, work was performed with lesser seed number than usual and reliability calculation was not carried out.

Experimental

Used ligands are as follows:

I. o-Vanillin-o-phenylenediamine
II. Resorcylaldehyde-o-phenylenediamine
III. Resacetophenone-o-phenylenediamine
IV. Salicylaldehyde-o-phenylenediamine

Of the above compounds 0.5; 1,0; and 2,0 mg were measured in powder form, and put in layers as evenly as possible into smooth filterpapers placed in Petri dishes of 12 cm diameter. Fifty healthy oat-grains were placed uniformly in

to the prepared Petri dishes. They were moistened with distilled water, and the water loss was tested daily. (None of the compounds are solube in water). The seeds in the covered Petri dishes were germinated in a glass-house at 15 C° with the elimination of light. After the setting in of the experiment, the germination, its rate, and the growth of the seedlings was observed every 24 hours. Table I. shows, as an average of 4 series, the number of seeds that germinated out of the 50 during 24 hours.

Table I.

	I.	II.	III.	IV.	∅
0,5 mg	0 0 0 0	4 5 4 3	2 3 2 1	3 1 2 2	0
1,0 mg	2 3 2 1	2 2 2 1	1 1 1 1	0 0 0 0	
2,0 mg	0 0 0 0	1 2 1 0	0 0 0 0	0 0 0 0	

Table II. summarizes the results found after 48 hours. It also shows, besides the germinated plant-species, the number of those budlets that indicate the rate of growth.

Table II.

	I.		II.		III.		IV.		∅	
	a	b	a	b	a	b	a	b	a	b
0,5 mg	44	10	41	19	47	9	45	19	44	6
1,0 mg	46	22	49	20	27	10	49	9		
2,0 mg	40	16	47	17	25	8	47	11		

where a = rootlet and b = budlet

At the taking down of these data, comparative observations were also carried out between the control plants and those that were treated with compounds This observation was perfomed in such a manner, that the growth of the plants was evaluated on the basis of general impression as well.

The plants of those seeds that were treated with compound I. show a better growth than that of the control.

Seeds treated with compound II. grew well-developed roots, and the root-hairs were in good condition, and well-developed. Their growth is better compared to that of the control, but in comparison with (I) they are slightly underdeveloped. This can be established on the basis of the daily observation.

With the plants of seeds treated with compound III., a defenite backwardness could be observed in comparison with both the control and those that were treated with the above compounds. The seed-buds are shorter, and the sprout is stunded. It appears a plant that has defenitely been backward in growth.

The plants of seeds treated with compound IV. has developed well, they grew strong roots, the root-hairs are well grown and their development is the most vigorous compared to that of the others.

Table III. indicates the average height of the plants in cm.

Table III.

	I.	II.	III.	IV.	∅
0,5 mg	11 cm	13 cm	9 cm	10 cm	9 cm
1,0 mg	12 cm	11 cm	8 cm	11 cm	
2,0 mg	5 cm	10 cm	5 cm	12 cm	

Prior to ingathering phenological observations were carried out, and it was pointed out that the seeds, treated with 0,5 and 1,0 mg substance of compound I, showed a better growth, while treatment with 2,0 mg substance was stunded in growthe from that of the plants in the controlling experiment. (See Fig. 1.)

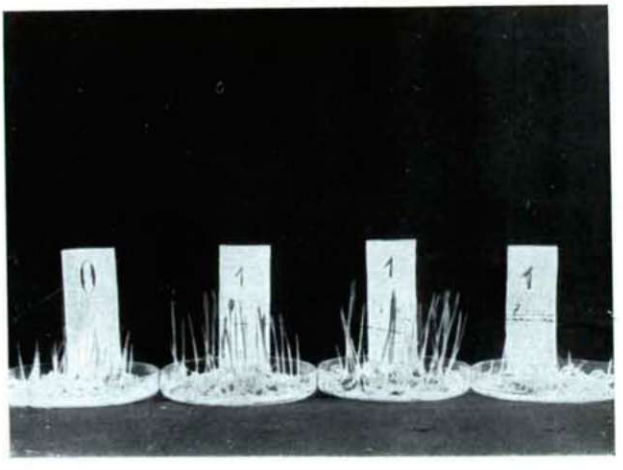


Fig. 1. ∅ = **control**; left to right from control:
1st = seeds treated with 0,5 mg of compound I;
2nd = seeds treated with 1 mg of compound I;
3rd = seeds treated with 2 mg of compound I;

The growth of the plants treated with compound II. is better in the cases of all the three doses than that of the control, but the degree of growth decreases with the increase of dosage.

After curing the plants with 0,5 mg of compound III. the result of the treatment was the same than that of the control, since plants treated with 1,0 and 2,0 mg of substance are very scarce, and are undeveloped and weak. The root seems to be stunted in growth as compared to that of other platns Root-hairs are defective. The plants have a yellow colouring. From the result of the growth of the plants treated with 1,0 and 2,0 mg of compound, it can be established, that in this concentration Schiff-bases 3 has a hindering effect upon the growth of the plants.

The results of growth of plants treated with compound IV. are better after each treatment. The morphological picture of the experimental oat improves in the ratio of increasing dosages. Treatments show a good disposition even individually, and a uniform proportional growth. The roots are nicely developed; there are, comparatively, many root-hairs. (See Fig. 2.)

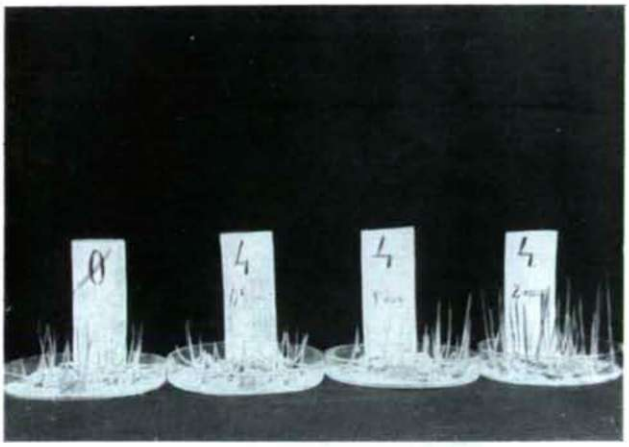


Fig. 2. ∅ = control; left to right from control:
1st = seeds treated with 0,5 mg of compound IV;
2nd = seeds treated with 1 mg of compound IV;
3rd = seeds treated with 2 mg of compound IV;

Ten days after the setting in of the experiment the plants were cut off right over the endosperm and fixed at 70 C°. They were air-dry conditioned, and after weighing their dry weights, the following results were obtained:

Table IV.

				Weight of plant	weight of 100 plants
1. 0,5 mg	44 db	germinated	plant	0,3207	0,7516
1,0 „	46 „	„	„	0,3580	0,7783
2,0 „	40 „	„	„	0,2190	0,5475
2. 0,5 mg	41 db	„	„	0,3490	0,8512
1,0 „	49 „	„	„	0,4159	0,8487
2,0 „	47 „	„	„	0,3480	0,7403
3. 0,5 mg	47 db	„	„	0,3154	0,6710
1,0 „	37 „	„	„	0,2781	0,7516
2,0 „	25 „	„	„	0,1106	0,4424
4. 0,5 mg	45 db	„	„	0,3446	0,7654
1,0 „	49 „	„	„	0,3430	0,7000
2,0 „	47 „	„	„	0,4630	0,9851
∅	45 db	„	„	0,3274	0,7651

The air-dried plants were pulverated, and destructed then the total P, N content was determined (Talaj és trágya vizsgálati módszerkönyv, Akadémia Kiadó, 1963) as well as the content of Fe (SNELL and SNELL: Colorimetric methods of analysis, Part III., D. VAN NOSTRAND, 1953. Third edition). The data are summarised in Table V.

Table V.

	N %	P %	Fe %
1. 0,5 mg	0,082 mg	0,620 mg	x
1,0 „	0,080	0,605	4,8
2,0 „	0,13	0,575	5,0
2. 0,5 mg	0,11	0,586	7,6
1,0 „	0,14	0,548	5,0
2,0 „	0,077	0,560	6,2
3. 0,5 mg	0,065	0,588	4,0
1,0 „	0,136	0,570	1,3
2,0 „	0,141	0,530	5,6
4. 0,5 mg	0,053	0,515	5,2
1,0 „	0,123	0,610	2,8
2,0 „	0,062	0,558	0,8
∅	0,087	0,574	1,6

(x = in small amouns)

Experimental (capsicum)

As a consequence of the results and phenomena observed with oat, such brand of seed was chosen that had a longer germinating time than that of the former. The relatively long germinating time of capsicum is well known, that is why it had been chosen for a test plant.

Experiments were carried out in the same manner and under same conditions as in the case of oat, with 4×100 seed number and with doses of 1, 2, 4 mg. Succesful observations could be achieved on the 11th day. From the available data, the results of only a few evaluations are reported in Tables (Table VI.).

Table VI.

	1 mg	2 mg	4 mg
I.	3 0 0 0	0 0 0 0	0 0 0 0
II.	2 2 5 3	2 0 3 2	1 7 4 5
III.	1 3 5 4	4 3 3 3	0 0 0 0
IV.	0 0 0 0		

No any special result could be observed with seeds treated with compound I; it is particlarly in accordance with the control. With compounds II. III. — in smaller degree though — but the germination began, hence germination of seeds was observed in every Petri dish. With compound III. of highest dosage, the seeds have not germinatéd With compound IV. a uniform positive effect was noticed in the sequence of increasing dosages.

On the control seed, the firts buds appeared at the 12th day, however, on seeds treated with compounds the development of even the cotyledons could be observed. (Table VII.).

Table VII.

	1 mg	2 mg	4 mg
I.	3 2 2 3	1 0 0 0	0 0 2 1
II.	5 6 8 7	4 3 4 4	10 6 5 6_1
III.	7 4 5 3	8 3 4 3	0 0 0 2
IV.	5 5 7 6	6_1 11 13 10	13 14 17 11_2
∅	2 1 0 0		

(indices of values represent the number of cotyledons).

Evaluation of Observations

Summarizing the results, the followings can be established. In the initial stage the small concentration leads to a more effenctive growth than that of the control plant. After a few days, the development of seeds treated with compound I. appears to be undergrown compared to that of the control, irrespective of the concentration. The same can be stated in the case of treatment with compound III. too, with the exception of treatment with 2 mg dosage. The dosage of 4 mg of compounds I. and III. has an inhibiting effect upon the germination of capsicum seeds. Treatment with compound IV. decreases the germination time in every concentration. It was on the 12th day of observation that on the seeds that were treated with a dosage of 4 mg of compound IV, the beggining of growth of the cotyledons could be noticed. Of the control plant, however, only 1 per cent germinated, as against the 15 per cent germination caused by 4 mg dosage of the previous compound. (See Fig. 3.)

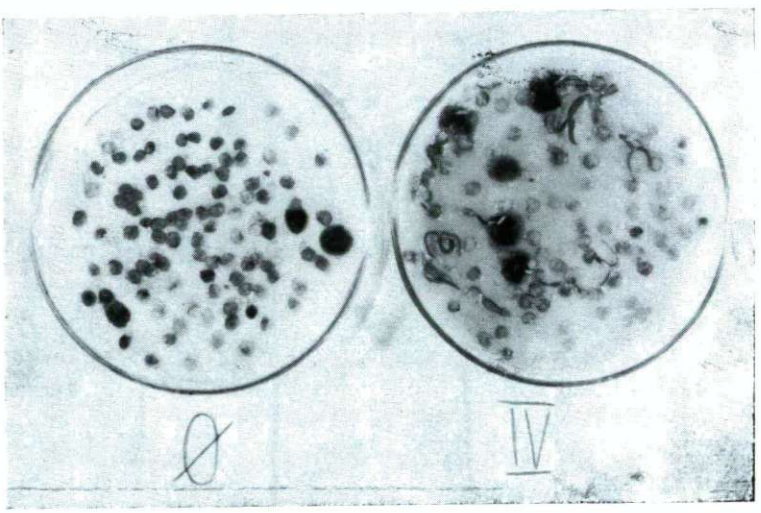


Fig. 3. ∅ = control; IV. = observation after 12 days of seeds treated with 4 mg of compound IV.

Seeds treated with compound I. became brown, they appeared as if the Schiff-base had coloured them from inside. The other 3 compounds caused no change of colour on the experimental stock. It was a remarkable phenomenon that after the germination on the root of the seeds treated with compounds, there were no hair-roots. The root was undeveloped and short-stemed. In many of them, a negative geotropism could be observed. Presumably, the plant falls down as a result of the weight of the cotyledon; this is natural, since the root cannot grip at the filter paper, but it could have been expected that the end of the root bends back. This, however, was not observed, for which, the bending of the root ends may give an explanation. Similar results were found (4) with chelating compounds, such as EDTA. These stimulate budding, and retard the growth of the root. This phenomenon is caused also by the auxins. It was noticed that after 24—28 hours a strong side-root development commenced at the juncture of the parts above ground and that of under ground.

The seed of brand „F. O. 3" hot red pepper, used by the authors of this paper, is registered as a badly and not easily germinating sort. In general, it gives 50 per cent germination seed in 16—18 days. With the experiments of those seeds that were treated with compound IV., germinating could be noticed as early as after 8—10 days.

The authors do not claim that compound IV. which defenitely posesses many good properties, and which shows a good result both with oat and capsicum, would stimulate growth. It seems likely, that the development of those plants that germinated earlier, is advanced, and this is what increases the growth of the plants. It is asserted, however, that it undoubtedly promotes the starting of germination. Presumably, the vigour of growth of the broad-leaves is increased by the energy due to the insufficient development of the root. However, it is a fact, that the germination of that capsicum seed which has a long germination time and is treated with compound IV., starts sooner than that of the untreated one.

Summarised results of the germination of the capsicum seed can be found in Table VIII; The values there were observed after the 13th day of the commencement of the experiment.

Table VIII.

	1 mg				2 mg				4 mg			
I.	6	2	2	3	7	0	0	0	1	5	2	3
	2			2								
II.	19	14	13	15	11	8	10	9	12	17	16	20
	3	2	5	4	2	2		1	5	5	4	4
III.	25	16	16	20	4	8	6	7	0	3	6	5
	2	2	3	5	2	2	3	5				
IV.	13	7	13	10	12	15	17	14	22	23	23	25
	3	2	3	3	4	2	6	5	5	7	9	9
∅	25	16	19	18								

(figures underneath represent the numbers of cotyledons)

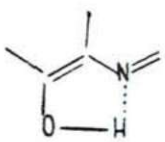
The figures sustain the statement that Sdhiff-bases increase the degree of germination; the results of the 20 per cent of control and that of 23 per cent of compound IV. also prove this, but they shorten the germination time.

Theoretical

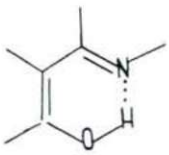
Relying on the results of the relatively few experimental material and of great variance, it would be a hazardous enterprise to reach a conclusion about the azomethines having an ifluence on the constituents building up the cells. The azomethenes, however, act upon oat and capsicum indentically, which makes the conclusion more assured. It is a well-known fact, that hydrogen bonds may exist in proteins whose energy content is very low and they may decompose for the effect of even a slight rise in temperature. On cooling, the hydrogen bond redevelops spontaneously, because the free energy change is small at the bond formation. Ion bond may form in proteins as a function of pH, when an electrostatic attraction and repulsion may occur between the dissociating groups. Besides these, convergent forces may be formed between the nonpolar side chains, and these may contribute to the stability of the cells that build up the molecules.

The Schiff-bases, used by the authors, are able to influence the formation of all the three bonds, since all of the aromatic aldehydes, used for the production of azomethenes, contain OH radical in ortho position, which is the criterium of the formation of hydrogen bonds. Besides, the nitrogen, found in the molecule, and which has a paired electron, is able to form a dative bond.

The tuberculostatic effect observed with animals is brought into connection with structure I. (2). This structure can be found with slight deviation in the applied compounds. (Structure II.)



I.



II.

Same results were observed in the case of the formation of the six-angular ring, too. In addition to these, there are relative electron dense and electron deficient places in the azomethene molecules, which are also able to bring about bonding with the proteins.

It has been proved spectroscopically that OH radicals, being in ortho position, have a tendency to form H bonds (5). With compounds II. and III., such radicals that are in para position are also found.

When applying the first three Schiff-bases, the possibility of the number of the hydrogen and dative bonds were measured, thus the equilibrium existing in the plants was disturbed. According to the results, this effect unfavourably influences the initial stage of the germination, which influence, however, becomes reduced later on.

Treatment with compound IV. promotes germination unanimously from the start all throungh the process, i. e. favourably influences it. In this compound there is no OH radical in para position, therefore, only the II. structural state is possible. Thus, at the two ends of the molecule a hydrogen bond cannot develope, only one of the other two bondings, which then is favourable for germination.

From the results of the experiment it is not possible to determine unanimously whether the plant decomposes the Schiff-bases or not. The concentration ratios influence the germination in a similar manner in negative or positive direction. The problem is still to be solved, that which concentration is that which affects the root or the stem in positive or negative direction.

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