Cytological Effects of Pesticides VI. Effect of the insecticide "Rogor" on the mitosis of *Vicia faba* and *Gossypium barbadense*

Soheir M. Amer and Odette R. Farah¹

National Research Centre, Dokki, Cairo, U.A.R.

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The insecticide o,o-dimethyl-N-methyl carbamidomethyl-dithiophosphate marketed under the name "Rogor" proved to be effective in controlling pests of : pea and beans (Sobol and Kozachenko 1964); berry crops (Tolstova 1964); mulberry trees (Kurdyukov 1963, Kurdyukov *et al.* 1964); apple trees (Shabanova *et al.* 1962); and cotton (Fadigas and Filho 1961 and Kumar *et al.* 1966). It seemed of interest to study the effect of "Rogor" on mitosis. The effect of "Rogor" on the mitosis of *Vicia faba* and *Gossypium barbadense* is discussed in the present study.

Materials and methods

Seeds of *Vicia faba* (v. Giza 1) and *Gossypium barbadense* (v. Ashmouni) were used in the present study. Two types of treatments were conducted: seed-soak, and root-treatments.

a. *Seed-soak-treatments*: Seeds were soaked for 24 hours in distilled water, then soaked for another 24 hours in the solution of the experimental agent. Two experiments were carried out in the case of *Vicia faba* seeds using pure and formulated "Rogor" (solution containing 40% active ingredient). The used concentrations were : 0.5%, 0.25%, 0.125%, and 0.0625%.

Cotton seeds were treated with formulated "Rogor" only. The used concentrations were: 0.25%, 0.125%, 0.0625, and 0.0313%.

Seeds were washed with water after the treatment, germinated in Petri dishes on filter paper moistened with distilled water, roots were cut when they reached 1.5-3 cm in length, fixed in acetic-alcohol (1: 3).

b. *Root-treatments* : Pure and formulated "Rogor" were used in the case of *Vicia faba*, and formulated "Rogor" in the case of cotton. The same concentrations were used as in the seed-soak-treatments. Such concentrations were selected after determining the lethal and subthreshold concentrations.

Roots of *Vicia faba* and *Gossypium barbadense* (1.5-3 cm in length) were immersed in the solution of the experimental agent for 4 hours, cut and fixed in acetic-alcohol (1: 3).

Recovery experiments were conducted where roots treated with the experimental agent were left to recover in distilled water for: 24, 48, and 72 hours; after the

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elapse of the recovery period roots were cut and fixed in acetic-alcohol (1:3).

Cotton roots were examined using the acetocarmine smear method, whereas, examination of bean roots was done from permanent root-tip squash preparations stained after Feulgen technique.

Results and discussion

Both pure and formulated "Rogor" inhibited cell division in *Vicia faba*, as it is evident from the effect of seed-soak and root-treatments on the mitotic index (Tables 1, 2).

Table 1. Mitotic index, percentage of the mitotic stages and percentage of abnormal mitoses in *Vicia faba* and *Gossypium barbadense* as affected by seed-soak-treatments (24 hours) with "Rogor" solution

Treatment	No. of counted cells	No. of dividing cells	Mitotic index	Prophase	Metaphase		Ana-telophase	
				%	%	%abn.	%	%abn.
a- <i>Vicia faba</i>								
Pure "Rogor"								
1-0.5%	29783	547	18.4	41.88	24.25	56.2	33.87	40.8
2-0.25%	21777	411	18.9	41.61	21.65	17.9	36.74	12.6
3-0.125%	14108	448	31.8	45.31	24.11	15.7	30.58	9.5
4-0.0625%	6665	417	62.6	33.57	29.50	15.4	36.93	5.8
5-Control	4400	414	94.1	45.89	24.15	3.0	29.96	3.2
Formulated "Rogor"								
1-0.25%	17231	137	8.0	34.47	33.98	17.1	31.55	36.9
2-Control	8366	408	48.8	39.71	25.25	1.8	35.05	0.7
3-0.125%	15069	191	12.7	50.79	17.28	9.1	31.94	19.7
4-0.0625%	9641	405	42.0	42.22	24.20	4.1	33.58	5.9
5-Control	8268	529	63.9	43.48	24.01	1.6	32.51	4.6
6-Mean of all controls	8317	469	56.4	41.79	24.52	1.7	33.69	2.7
b- <i>Gossypium barbadense</i>								
Formulated "Rogor"								
1-0.25%	—	511	—	40.31	24.66	30.16	35.03	9.50
2-0.125%	—	400	—	42.25	25.75	11.65	32.00	4.69
3-0.0625%	—	464	—	38.79	22.63	11.43	38.58	1.68
4-0.0313%	—	483	—	35.40	26.92	8.46	37.68	2.75
5-Control	—	457	—	33.92	33.70	3.90	32.39	2.03

The percentage of the occurring abnormalities in the meta- and ana-telophase stages decreased by decreasing the concentration of the agent in the case of both *Vicia faba* and *Gossypium barbadense* (Tables 1, 2).

Treatments of bean roots with 0.5% pure and formulated "Rogor" and of cotton roots with 0.125% formulated "Rogor" caused accumulation of the metaphase stage when compared with the control (Table 2).

Comparing the effect of "Rogor" on both *Vicia faba* and *Gossypium barbadense*,

Table 2. Mitotic index, percentage of the mitotic stages and percentage of abnormal mitoses in *Vicia faba* and *Gossypium barbadense* as affected by root-treatments (4 hours) with "Rogor" solutions

Treatment	No. of counted cells	No. of dividing cells	Mitotic index	Prophase		Metaphase		Ana-telophase	
				%	% abn.	%	% abn.	%	% abn.
a-<i>Vicia faba</i>									
<i>Pure "Rogor"</i>									
1-0.5%	13868	84	6.1	13.10	0.0	46.43	41.0	40.48	41.2
2-0.25%	13709	174	12.7	42.53	0.0	23.56	22.0	33.91	6.8
3-0.125%	18030	374	20.7	38.50	0.0	26.20	16.3	35.29	6.8
4-0.625%	21420	464	21.7	49.14	0.0	23.92	8.1	26.94	4.8
5-Control	4594	344	74.9	46.79	0.0	22.08	1.7	31.13	1.8
6-0.5% and re- covery for 24 hrs	4892	399	81.6	46.24	0.0	23.71	9.9	30.05	11.7
7-Control	5368	613	114.2	48.12	0.3	21.70	2.3	30.18	1.08
8-0.5% and re- covery for 48 hrs	7883	655	83.1	41.54	0.0	26.55	4.8	31.91	2.01
9-Control	6449	536	83.1	52.43	0.0	20.90	2.7	26.68	2.80
<i>Formulated "Rogor"</i>									
1-0.5%	6937	581	83.8	40.79	4.6	38.21	81.1	21.00	95.00
2-0.25%	6060	508	83.8	46.26	2.1	34.65	52.3	19.09	94.80
3-0.125%	6733	570	84.7	42.19	1.0	42.62	43.9	15.19	73.10
4-0.0625%	6583	557	84.6	62.22	0.3	24.07	12.3	13.70	24.30
5-Control	10633	947	89.1	50.33	0.0	21.73	1.0	27.94	2.40
6-0.5% and re- covery for 24 hrs*	5635	530	94.1	25.55	2.4	43.61	47.9	30.84	59.60
7-Control	5368	613	114.2	48.12	0.3	21.70	2.3	30.18	1.08
8-0.25% and re- covery for 24 hrs	3169	372	117.4	36.02	1.5	41.13	35.9	22.85	88.20
9-Control	2957	469	158.6	46.91	0.0	21.75	2.9	31.34	1.40
10-0.25% and re- covery for 48 hrs	6037	415	68.7	44.10	0.0	26.75	9.0	29.16	11.60
11-Control	4218	487	115.5	44.35	0.0	29.16	2.1	26.49	3.10
12-0.25% and re- covery for 72 hrs	5278	386	73.1	44.56	0.6	26.68	2.9	28.76	6.30
13-Control	5366	497	92.6	49.50	0.0	21.13	0.9	29.38	1.40
b-<i>Gossypium barbadense</i>									
<i>Formulated "Rogor"</i>									
1-0.125%	—	229	—	46.72	17.76	45.85	64.76	7.42	41.18
2-0.0625%	—	317	—	35.33	18.75	61.83	50.0	2.84	33.33
3-0.0313%	—	448	—	41.52	3.76	40.18	40.0	18.30	21.95
4-0.016%	—	724	—	41.30	2.01	49.31	22.13	9.39	13.24
5-0.008%	—	386	—	44.82	0.0	45.34	4.57	9.84	7.89
6-Control	—	514	—	42.02	0.0	40.08	2.91	17.90	1.09

* Roots died after 24 hours.

it was found that seed-soak-treatments with 0.25% formulated "Rogor" effected about 17% and 11% abnormal mitoses in beans and cotton respectively (Table 3); cotton, on the other hand, was found to be more sensitive to the agent than beans when root-treatments were conducted (Table 4).

The percentage of abnormal mitoses in beans, induced by formulated "Rogor" after root-treatment was much higher than that induced by the pure substance (Table 4).

The effect of pure "Rogor" diminished by dilution more rapidly than the formulated substance (Table 4). Recovery experiments showed also that roots treated with pure "Rogor" recovered faster than those treated with the formulated substance (Table 4).

Table 1. Percentage of the different types of abnormalities occurring in the mitosis of *Vicia faba* and *Gossypium barbadense* after seed-soak-treatment (24 hours) with "Rogor" solutions

Treatment	% abn. mitoses	Percentage of different types abnormalities					
		Dist.	Lag.	Frag.	Bridge	Stick	multi-polar
a- <i>Vicia faba</i> . Pure "Rogor"							
1-0.5%	27.46*	81.0	14.6	3.7	0.7	—	—
2-0.25%	8.52*	62.9	28.6	2.9	5.7	—	—
3-0.125%	6.70	80.0	20.0	—	—	—	—
4-0.0625%	6.71	60.7	25.0	7.1	3.6	3.6	—
Formulated "Rogor"							
1-0.25%	17.48**	50.0	44.4	2.8	—	—	2.8
2-0.125%	7.85**	46.7	26.7	26.7	—	—	—
3-0.0625%	2.96	58.3	41.7	—	—	—	—
b- <i>Gossypium barbadense</i> Formulated "Rogor"							
1-0.25%	10.76**	74.5	25.5	—	—	—	—
2-0.125%	4.5	72.2	27.8	—	—	—	—
3-0.0625%	3.23	53.3	46.7	—	—	—	—
4-0.0313%	3.31	12.5	87.5	—	—	—	—

* Significant at 0.05 level.

** Significant at 0.01 level.

Disturbed prophases, meta- and anaphases (Figs. 1-5) comprised the most dominant type of anomalies induced by "Rogor" even after recovery for 72 hours (Table 4).

In the case of disturbed prophase (Fig. 1) the arrangement of chromatin threads was abnormal; similar abnormal prophases were observed by Kabarity (1966) after treatment of *Allium cepa* roots with cardiazol and methylbenzoate.

The chromosomes spread irregularly all over the cell in the case of disturbed meta- and ana-telophases (Figs. 2-5) such type may be due to the disturbance of the spindle apparatus. Disturbed type dominated also in both *Vicia faba* and *Gossypium barbadense* after seed soak- and root-treatments with "Sevin" (Amer *et al.* 1971).

Star meta- and anaphases were observed (e.g. Figs. 6, 7); such type was also

observed after treatment of *Allium cepa* roots with pure "Sevin" (Amer 1965) and considered as being a fore-step of the complete disturbance of the spindle (Amer 1966).

Lagging of chromosomes was observed in the meta- and anaphase stages (Figs.

Table 4. Percentage of the different types of abnormalities occurring in the mitosis of *Vicia faba* and *Gossypium barbadense* as affected by root treatments (4 hours) with "Rogor" solutions

Treatment	% abno. mitoses	Percentage of different types of abnormalities						
		Dist.	Lag.	Frag.	Bridge	Stick	Multi- polar	Contr- act
a-<i>Vicia faba</i> Pure "Rogor"								
1-0.5%	35.71**	70.0	10.0	—	—	—	20.0	—
2-0.25%	7.47**	92.3	7.7	—	—	—	—	—
3-0.125%	6.68*	84.0	8.0	—	4.0	4.0	—	—
4-0.0625%	3.23	86.7	13.3	—	—	—	—	—
5-0.5% and re- covery for 24 hrs	5.87	60.0	24.0	12.0	—	—	4.0	—
6-0.5% and re- covery for 48 hrs	1.90	66.7	22.2	—	—	—	11.1	—
Formulated "Rogor"								
1-0.5%	52.84**	92.2	—	—	—	7.5	0.3	—
2-0.25%	37.20*	95.2	0.5	—	—	3.2	1.1	—
3-0.125%	30.23*	94.9	1.9	—	—	0.5	2.8	—
4-0.0625%	6.48	85.7	8.6	2.9	2.9	—	—	—
5-0.5% and re- covery for 24 hrs ⁺	39.88**	74.2	2.3	2.3	—	19.6	1.6	—
6-0.25% and re- covery for 24 hrs	35.48**	89.4	—	—	—	10.6	—	—
7-0.25% and re- covery for 48 hrs	5.78	29.2	62.5	4.1	4.1	—	—	—
8-0.25% and re- covery for 72 hrs	2.90	63.6	36.4	—	—	—	—	—
b-<i>Gossypium barba-</i> <i>dense</i> Formulated "Rogor"								
1-0.125%	41.05**	64.9	1.1	—	—	—	—	34.0
2-0.0625%	38.48**	25.4	5.7	—	—	—	—	68.9
3-0.0313%	21.65**	48.5	11.3	—	—	—	—	40.2
4-0.016%	12.98*	71.3	6.4	—	—	—	—	22.3
5-0.008%	2.85	72.7	27.3	—	—	—	—	—

⁺ Roots died after 24 hours.

* Significant at 0.05 level.

** Significant at 0.01 level.

8, 9), such type was induced by all the used concentrations however, in different percentage (Table 4).

Marked chromosome contraction in the metaphase stage was observed in a considerable percentage in cotton (Table 4). Such phenomenon was observed after treatments with isopropyl phenyl carbamate (Storey and Mann 1967) ortho- and

Figs. 1-7. 1-3: Disturbed type. 1, disturbed prophase in *Gossypium barbadense* after root-treatment with 0.125% formulated "Rogor". 2, disturbed metaphase. 3, a group of disturbed anaphase in *Vicia faba* after root-treatment with 0.25% formulated "Rogor". 4-5, disturbed metaphase in *Gossypium barbadense* after seed-soak-treatment with 0.25% formulated "Rogor", and root-

para-nitrophenols (Amer and Ali 1969).

Multipolar anaphases (e.g. Fig. 10) were observed in *Vicia faba* only in a considerable percentage after some treatments with "Rogor" (Table 4). In this connection it may be mentioned that, Doxey (1949) in his study of the effect of isopropyl-phenyl-carbamate on mitosis in *Secale cereale* and *Allium cepa* observed multipolar spindles in onion only.

Figs. 8-12. 8, metaphase with lagging chromosome in *Gossypium barbadense* after seed-soak-treatment with 0.25% formulated "Rogor". 9, anaphase with lagging chromosomes in *Vicia faba* after root-treatment with 0.5% pure "Rogor". 10, four-polar anaphase in *Vicia faba* after root-treatment with 0.125% formulated "Rogor". 11, anaphase. 12, telophase bridges in *Vicia faba* after root-treatment with 0.0625% pure "Rogor", and seed-soak-treatment with 0.125% formulated "Rogor".

Less common phenomena were: chromosome stickiness, ana- and telophase bridges. (Figs. 11, 12), and chromosome fragmentation. Such types of anomalies were observed in *Vicia faba* after some treatments with the agents (Table 4).

Summary

The effects of the insecticide o.o-dimethyl-N-methyl-carbamido methyl dithiophosphate "Rogor" were studied on root-mitosis of *Vicia faba* and *Gossypium* treatment with 0.125% formulated "Rogor". 6, star-metaphase "lower", and star-anaphase "upper" in *Vicia faba* after root-treatment with 0.125% formulated "Rogor". 7, star-anaphase "upper" and disturbed-metaphase "lower" in *Vicia faba* after root-treatment with 0.5% formulated "Rogor".

barbadense. Two types of treatments were conducted: seed-soak, and root-treatments. Both pure and formulated "Rogor" effected a great decrease in the mitotic index of *Vicia faba* when compared with the control. The percentage of abnormal mitoses in beans induced by formulated "Rogor" was much higher than that induced by the pure substance. Recovery experiments showed that roots treated with pure "Rogor" recovered more rapidly than those treated with the formulated substance. Disturbed prophase, meta- and anaphases comprise the most dominant type of anomalies in both beans and cotton, lagging chromosomes were also observed in both plants. Chromosome contraction was observed only in cotton. Chromosome stickiness, chromosome fragmentation, anaphase bridges, and multipolar anaphases were observed only in *Vicia faba*.

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