Cytological Effects of Pesticides

IX. Effects of the phosphonothioate insecticide Leptophos on *Vicia faba*

Soheir M. Amer and Odette R. Farah

Laboratory of Genetics and Cytology, National Research Centre,
Dokki, Cairo, Egypt

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Leptophos¹ (O-4-bromo-2, 5-dichloro-phenyl) O-methyl phenyl-thiophosphonate) is an insecticide of moderate mammalian toxicity, and is marketed under the name "Phosvel". It is used for the control of pests on: cabbage (Workman and Greene 1970), tobacco (Dominick 1972), sweet corn, peppers (Mc Clanahan and Founk 1972), peppermint (Berry 1975), and rice (Row *et al.* 1976). Phosvel is used in Egypt for the control of the cotton leaf worm (*Spodoptera littoralis*), spiny boll worm (*Earias insulana*), and pink boll worm (*Pectinophora gossypiella*) (Program of pest control, U. A. R., 1970/1971; 1972/1973; and 1974/1975).

Townsend and Specht (1975) reported that Leptophos was detected in the soil three months after application (68%). It seemed, therefore, interesting to study the effect of the insecticide on root-mitosis. The present study aims to elucidate the effect of Leptophos on root-mitosis of *Vicia faba* after seed-soak and root-treatments.

Materials and methods

Compare part VI of this series (Amer and Farah 1974a). Seeds of *Vicia faba* (v. Giza 2) and the insecticide Leptophos were used in the present study. The used concentrations were : saturated, 0.5, 0.25, and 0.125 of the saturated solution. Three replicates were performed for each treatment. The effect of "Leptophos" has been studied on: root-mitosis, seed-germination, root- and shoot-lengths after seed-soak and root-treatments.

Results and discussion

All the conducted treatments with Leptophos solutions affected a significant percentage of abnormal mitoses/replicate (Table 1). The percentage of abnormal mitoses increased as the time of treatment or the concentration of the experimental agent increased (Table 1). It may worth mentioning that, treatment of barley

¹ Leptophos

(*Hordeum vulgare*) seeds, and *Vicia faba* root-tips with other pesticides produced a high percentage of cells with chromosome abnormalities (Wuu and Grant 1966, 1967).

Whereas, the percentage of abnormal mitoses is high in the meta- and anaphase stages, yet the percentage of abnormal interphase cells is too low (Table 2).

Table 1. Percentage of abnormal mitoses/replicate after seed-soak-, and root-treatments of *Vicia faba* with pure Leptophos solutions

Treatment	No. of invest. repl. +	% of abnormal mitoses/replicate	Mean % of abn. $\pm$ S. E. mitoses
I. Seed-soak-treatment with sat. soln. for:			
1 24 hrs.	3	22.08, 16.31, 15.73	18.04** $\pm$ 2.03
Control	3	2.03, 2.63, 0.00	1.55 $\pm$ 0.80
2 48 hrs.	3	20.12, 29.77, 33.00	27.63** $\pm$ 3.87
Control	3	1.32, 0.39, 1.05	0.92 $\pm$ 0.28
3 72 hrs.	3	35.69, 49.81, 36.86	40.79** $\pm$ 4.51
Control	3	0.00, 1.41, 3.67	1.69 $\pm$ 1.07
II. Root-treatment for 4 hrs. with:			
1 Sat. soln.	3	12.46, 13.25, 23.38	16.36* $\pm$ 3.52
2 0.5 sat. soln.	3	11.21, 14.63, 10.18	12.01** $\pm$ 1.33
3 0.25 sat. soln.	3	7.24, 9.56, 13.22	10.01* $\pm$ 1.73
4 0.125 sat. soln.	3	4.02, 5.59, 5.58	5.06** $\pm$ 0.54
Control	3	0.88, 0.22, 0.15	0.42 $\pm$ 0.23

+ Three roots were investigated for each replicate.

* Significant from control at 0.05 level (t-test).

** " " " 0.01 " " .

Table 2. Percentage of abnormal mitoses and interphase cells in *Vicia faba* roots as affected by seed-soak-, and root-treatments with pure Leptophos solutions

Treatment	Prophase % abn.	Metaphase % abn.	Ana-, telophase % abn.	Interphase cells % abn.
I. Seed-soak-treatment with sat. soln. for:				
1 24 hrs.	2.92	24.64	29.58	0.50
Control	0.00	2.67	2.76	0.19
2 48 hrs.	6.67	31.58	40.00	3.12
Control	0.00	0.79	1.68	3.58
3 72 hrs.	3.70	36.36	76.00	0.83
Control	0.00	2.86	3.23	0.31
II. Root-treatment for 4 hrs. with:				
1 Sat. soln.	2.15	29.39	35.76	0.46
2 0.5 sat. soln.	2.76	29.71	24.12	0.16
3 0.25 sat. soln.	1.31	16.58	21.23	0.35
4 0.125 sat. soln.	1.46	8.83	7.88	0.17
Control	0.00	0.52	0.96	0.06

Table 3. Number of counted dividing and interphase cells and mean mitotic index/replicate in *Vicia faba* roots as affected by seed-soak, and root-treatments with pure Leptophos solutions

Treatment	No. of invest. repl.	No. of dividing cells	No. of counted interphase cells	Mean mitotic index/ repl.	± S. E.
I. Seed-soak-treatment					
With sat. soln. for:					
1 24 hrs.	3	617	9245	62.57	± 4.91
Control	3	608	9123	62.41	± 3.47
2 48 hrs.	3	50	9075	5.45*	± 3.37
Control	3	575	9493	56.29	± 13.32
3 72 hrs.	3	74	9303	7.79	± 2.69
Control	3	137	9895	13.85	± 4.27
II. Root-treatment for 4 hrs. with:					
1 Sat. soln.	3	1201	33285	34.78	± 4.51
2 0.5 sat. soln.	3	1294	33617	37.08	± 0.63
3 0.25 sat. soln.	3	1616	40187	39.37	± 5.36
4 0.125 sat. soln.	3	1335	33153	37.89	± 8.62
Control	3	1580	33729	44.68	± 6.28

* Significant from control at 0.05 level (t-test).

This may be an indication that, most of the abnormal metaphases continued their mitotic cycle till the ana- and telophase stages whereas, they either recovered or did not continue their mitotic cycle.

Both seed-soak and root-treatments with the insecticidal solutions affected a decrease in the mitotic index when compared with the control (Table 3).

Disturbed pro-, meta-, and anaphases (where the chromosomes spread irregularly in the cell e.g. Fig. 1) comprised the most dominant type of anomalies observed after treatments with Leptophos solutions (Table 4). In the case of disturbed prophases the arrangement of the chromatin threads was abnormal (compare Kabarity 1966). Such phenomenon has been also observed in the mitosis of: *Allium cepa*, after root-treatments with "Sevin" (Amer 1965), *Vicia faba*, and *Gossypium barbadense*, after seed-soak and root-treatments with "Rogor" and isopropyl N-phenyl carbamate (Amer and Farah, 1974 a, b).

Other anomalies e.g. prophase-metaphase (Fig. 2), metaphases with contracted chromosomes, and unequal separation of the chromosomes at the anaphase stage were observed in some cells and classified under disturbed type in Table 4.

Lagging chromosomes (e.g. Fig. 3) were observed in a considerable percentage after the different treatments with the insecticide (Table 4).

As could be seen from Table 4, chromosome fragmentation, tetraploid metaphases, chromosome stickiness, multipolar anaphases (e.g. Fig. 4), and anaphase bridges were observed in a low percentage.

Few abnormal interphase cells were observed after the different treatments with Leptophos (Table 2). However, micro-nucleated cells (e.g. Fig. 5) comprised the dominant type.

Figs. 1-5: Disturbed anaphase. 1, prophase-metaphase. 2, anaphase with lagging chromosomes. 3, tripolar anaphase. 4, interphase cell with micro-nucleus. 5, after, seed-soak-treatment for 48 hours (Figs. 1, 5); and root-treatment for 4 hours (Figs. 2-4) with saturated pure Leptophos solution.

Table 4. Percentage of the different types of abnormalities occurring in the mitosis of *Vicia faba* roots, after seed-soak, and root-treatments with pure Leptophos solutions

Treatment	Mitoses		Percentage of the different types of abnormalities						
	No.	No. abn.	% Dist.	% Lag.	% Frag.	% Poly-ploid	% Bridges	% Multi-pol.	% Stick.
I. Seed-soak-treatment with sat. soln. for :									
1 24 hrs.	660	121	76.86	13.22	7.44	—	—	—	2.48
2 48 hrs.	50	14	85.71	7.14	—	—	—	—	7.14
3 72 hrs.	74	28	67.86	25.00	7.14	—	—	—	—
II. Root-treatment for 4 hrs. with:									
1 Sat. soln.	1201	208	78.85	17.79	1.44	1.44	0.48	—	—
2 0.5 sat. soln.	1294	155	89.03	9.68	0.65	0.65	—	—	—
3 0.25 sat. soln.	1637	161	81.99	8.70	1.24	1.24	4.97	0.62	1.24
4 0.125 sat. soln.	1335	64	87.50	10.94	—	—	1.56	—	—

Table 5. The effect of seed-soak, and root-treatments with pure Leptophos solutions on: seed-germination, root- and shoot-growth of *Vicia faba*

Treatment	Age: 4 days		Age: 8 days		Average growth rate in 4 days for:		Age: 12 days		Average growth rate in 4 days for:	
	% of germination as related to the control	Shoot	% of germination as related to the control	Shoot	Root	Shoot	% of germination as related to the control	Root	Shoot	
I. Seed-soak-treatment										
1	with sat. soln. for:									
24 hrs.	102.32		100.00		0.60	—	100.00	0.35	0.96	
Control	100.00		100.00		0.60	—	100.00	0.43	1.02	
2	93.02		95.56		0.70	—	95.56	0.08	1.20	
Control	100.00		100.00		1.96	—	100.00	0.83	1.40	
3	88.89		91.62		1.20	—	91.62	0.49	0.92	
Control	100.00		100.00		2.52	—	100.00	1.16	2.34	
II. Root-treatment for										
4 hrs. with:										
1	—		—		0.41	1.63	—	0.38	0.91	
Sat. soln.	—		—		0.55	1.97	—	0.51	1.04	
2	—		—		0.54	1.49	—	0.50	1.33	
0.5 sat. soln.	—		—		0.72	2.56	—	0.55	1.00	
3	—		—		0.19	2.06	—	0.29	1.03	
0.125 sat. soln.	—		—							
Control	—		—							

Seed-soak treatment with Leptophos solution for 72 hours affected a slight inhibition of seed germination (Table 5). It may be mentioned in this respect, that, the insecticide *N*-methyl-*l*-naphthyl carbamate "Sevin" affected also slight inhibition of germination of *Vicia faba* seeds after being soaked in the insecticidal solution for 2 days (Amer *et al.* 1971).

As is evident from the average growth rate in 4 days (Table 5), soaking the seeds in a saturated solution of Leptophos for 72 hours, caused marked inhibition of root- and shoot-growth. Root-treatments with the insecticide, on the other hand, had no harmful effect on both root- and shoot-growth (Table 5). Such inhibition in root-growth may be due to the inhibition of cell division as can be seen from Table. 1.

Summary

The effects of the insecticide O-4-bromo-2,5-dichloro-phenyl O-methyl phenylthiophosphonate "Leptophos" has been studied on: root-mitosis, seed-germination, root- and shoot-lengths of *Vicia faba*. Two types of treatments were conducted: seed-soak and root-treatments. Both treatments affected a significant percentage of abnormal mitoses. Seed-soak treatments for 48 and 72 hours affected marked inhibition of cell division. Disturbed prophase, meta- and anaphases (where the chromosomes spread irregularly in the cell) comprised the main type of the observed anomalies. Lagging chromosomes and chromosome fragmentation were also observed.

Soaking the seeds in a saturated solution of Leptophos for 72 hours caused marked inhibition of root- and shoot-growth, which was attributed to the inhibition of cell division.

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