Cytological Effect of Pesticides VIII. Meiotic effects of the insecticide Methamidophos¹

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The organophosphorous insecticide Methamidophos is widely used to control pests which attack different crops e.g. cabbages (Chuo 1973), broad bean (EI-Kifl *et al.* 1975), cotton (Hassan *et al.* 1975), and potato (Kolbe 1978). In Egypt it is widely used in the fields of, tomato, potato, pepper, cucurbit, cucumber, melon, water melon, phaseolus, cotton, broad bean and soybean (Programs of pest control, U.A.R., 1972 and 1973, 1976 and 1977 and 1980 and 1981).

Methamidophos induced a statistically significant percentage of cells with chromosome aberrations in root-mitosis of *Vicia faba*, after both root- and seed-soak-treatments (Amer and Farah 1985). It seemed highly indicated to study the effect of this well known insecticide on the meiosis of *Vicia faba*.

Materials and methods

Seeds of *Vicia faba* (v. Giza 2) and pure Methamidophos were used in the present study. Plants were sprayed at the seedling and the flowering stages with aqueous Methamidophos solution (500 ppm). The following treatments were conducted:

a—Spraying at the seedling stage: two weeks old plants were sprayed for four successive days.

b—Spraying at the flowering stage: five weeks old plants were sprayed:

1—Once.

2—Twice for two successive days.

3—Four times for four successive days.

c—Another group of plants was sprayed twice: at the seedling stage and at the flowering stage.

Each treatment consisted of 30 plants. The plant received 7 ml of the insecticidal solution/each spray. Control plants were sprayed similarly with distilled water. Flower buds were gathered 24 hour after the last spray, fixed in acetic acid: ethyl alcohol (1: 3), preserved in 70% ethyl alcohol, and examined using the aceto-carmin smear method. Abnormalities were counted in the 1st and 2nd meiotic divisions and tetrad stage.

Results and discussion

All the conducted treatments with Methamidophos induced a significant percentage of abnormal PMCs./plant (Table 1). Spraying plants at the flowering stage with the insecticide induced a high percentage of abnormal PMCs which increased as the number of sprays with the insecticide increased and reached about 21% after spraying plants for 4 successive days. A statistically significant percentage of abnormal PMCs was still observed in the plants which were sprayed at the seedling stage (Table 1). This may indicate the slow recovery of the induced abnormal PMCs.

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The percentage of the induced PMCs varied to a great extent from plant to plant within plants treated similarly with Mathamidophos. Such phenomenon was observed in *Vicia faba* plants when sprayed with the insecticides: phosvel and dursban (Amer and Farah 1980, 1983), and may be attributed either to the difference in the type of abnormalities induced in the initial cells and its effects on the viability of these cells or to differences in physiological conditions at the time of treatment (Wuu and Grant 1967).

Table 2 represents the percentage of abnormal PMCs in the different meiotic stages. A

Table 1. Percentage of abnormal PMCs/*Vicia faba* plant after different treatments with 500 ppm Methamidophos solution

Treatments	No. invest. plants	% of abnormal PMCs /plant								Mean % abn. $\pm$ S.E. PMCs
a	7	3.42,	3.50,	3.95,	9.71,	8.55,	11.11,	5.20		6.49** $\pm$ 1.22
b (1)	6	21.70,	8.94,	7.10,	4.61,	2.25,	5.78			8.39* $\pm$ 2.82
b (2)	5	15.63,	6.65,	9.22,	12.99,	6.08				10.11** $\pm$ 1.84
b (3)	6	41.35,	18.39,	22.99,	9.87,	19.04,	12.95			20.77** $\pm$ 4.54
c	7	8.0,	0.75,	6.71,	34.58,	6.29,	8.81,	14.37		11.36* $\pm$ 4.16
Control	7	0.58,	1.14,	2.92,	0.80,	1.17,	0.00,	0.64		1.03 $\pm$ 0.35

Compare Materials and methods for treatments a, b (1-3) and c.

* Significant at 0.05 level (t-test).

** Significant at 0.01 level (t-test).

Figs. 1-6. 1-3: Sticky metaphase I. 1, metaphase I with sticky chromosomes and univalents. 2, sticky metaphase II. 3, after spraying *Vicia faba* plants with 500 ppm. Methamidophos: once at seedling and once at flowering stage (Figs. 1, 2), and for 4 successive days at the seedling stage (Fig. 3). 4-6: Anaphase I showing difficulty of chromosome separation. 4, bridge-fragment configurations. 5, anaphase II with lagging chromosome. 6, after spraying *Vicia faba* plants with 500 ppm. Methamidophos solution for 4 successive days at the flowering stage.

Table 2. Number and percentage of abnormal PMCs in the different meiotic stages and tetrads of *Vicia faba* plants after different treatments with 500 ppm. Methamidophos solution

Treatments	1st meiotic division						2nd meiotic division						Tetrads					
	Metaphase			Anaphase			Telophase			Metaphase			Anaphase			Telophase		
	No.	% abn.	No.	% abn.	No.	% abn.	No.	% abn.	No.	% abn.	No.	% abn.	No.	% abn.	No.	% abn.	No.	% abn.
a	306	2.29	78	17.95	346	0.29	159	22.64	112	18.75	485	0.21	1251	0.16				
b (1)	466	10.94	136	38.97	550	0.18	252	10.32	108	25.00	408	0.49	677	0.59				
b (2)	367	19.35	112	11.61	347	0.86	301	20.60	66	33.33	335	0.30	910	0.11				
b (3)	769	31.21	80	41.25	712	1.12	205	43.90	82	28.05	374	1.07	654	0.31				
c	250	24.80	98	29.59	336	0.89	366	21.31	124	43.55	531	0.38	1458	0.07				
Control	681	1.47	217	3.69	745	0.40	269	1.86	118	1.69	651	0.15	1423	0.00				

Compare Materials and methods for treatments a, b (1-3) and c.

high percentage of abnormal PMCs was still observed in anaphase II, however, the frequency of abnormal cells in the tetrad stage decreased to a great extent.

The most dominant type of chromosome alterations induced in PMCs after all the treatments with Methamidophos was chromosome stickiness which, sometimes resulted in the formation of sticky bridges in anaphases (Table 3 and Figs. 1–5). Amer and Ali (1983) concluded that, chromosome stickiness is a general effect induced in PMCs, after treatment with different chemical agents. Such phenomenon was observed in the meiosis of *Vicia faba* after treatment with other organophosphorous insecticides e.g. phosvel (Amer and Farah 1980), dursban (Amer and Farah 1983) and trichlorphon (Amer and Ali 1983).

Disturbed meta- and anaphases where the chromosomes spread irregularly over the cell were observed in a considerable percentage after the different treatments with Methamidophos (Table 3). The irregular spreading of chromosomes may be attributed to the disturbance of the spindle apparatus (Amer and Ali 1974).

Lagging chromosomes were observed in a considerable percentage (e.g. Fig. 6 and Table 3). Micronuclei were observed in the different meiotic stages after some treatments with Methamidophos and may be resulted either from lagging chromosomes or chromosome fragmentation (Table 3). Anaphase bridges (e.g. Fig. 5), the presence of univalents in diakinesis and multipolar telophase II were observed in a low percentage (Table 3).

It is well known that fragments and bridges lead to structural changes in the chromosomes. Lagging of chromosomes and their presence as univalents may result in aneuploidy (Schulz-Schaeffer 1980). As is evident from Table 3, these chromosome alterations were observed in a low percentage which indicates the low mutagenic effect of Methamidophos in *Vicia faba*.

Table 3. Percentage of the occurring abnormalities in the meiosis of *Vicia faba* after different treatments with 500 ppm. Methamidophos solution

Treatments	No. counted PMCs	No. abn. PMCs	% of the different types of abnormalities relative to the number of abnormal PMCs							
			Stick.	Dist.	Lag.	Frag.	Brid.	Micro n.	Multi pol.	Univ.
a	1486	80	50.00	32.5	11.25	1.25	2.50	0.00	2.50	0.00
b (1)	1920	160	43.13	40.63	6.25	1.25	6.88	0.63	0.00	1.25
b (2)	1528	172	66.28	19.19	6.40	4.07	1.74	1.74	0.58	0.00
b (3)	2225	398	83.67	6.28	3.52	2.76	0.50	1.26	0.00	2.01
c	1705	228	77.19	13.16	3.95	0.88	2.63	0.00	0.88	1.32

Compare Materials and Methods for treatments a, b (1–3) and c.

Summary

The effect of the pure insecticide "Methamidophos" (O,S-dimethyl phosphoramidothioate) on the meiosis of *Vicia faba* has been studied. *Vicia faba* plants were sprayed for different periods of time (1, 2 or 4 days) at the seedling and flowering stages with the insecticidal solution (500 ppm). All the conducted treatments affected a significant percentage of abnormal PMCs/plant.

Stickiness of the chromosomes dominated, and only few PMCs with chromosome fragments were observed. Such phenomena indicate the toxicity of Methamidophos and its low mutagenic potential.

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