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ALTERATIONS IN NUCLEIC ACIDS, PROTEIN CONTENT AND MITOTIC DIVISION OF *Vicia faba* ROOT TIP CELLS AS AFFECTED BY MALATHION AND TAMARON INSECTICIDES

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BY

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SUMMARY

The effect of malathion and tamaron insecticides on *Vicia faba* root tip cells was studied. The results showed that malathion increased MI% after 4 and 6 hrs treatments, while tamaron decreased MI% severely. Both insecticides altered mitotic phases percentages and induced a number of chromosomal anomalies.

The two insecticides with the concentrations and time durations applied increased protein content of the root tips. Malathion decreased DNA content, while tamaron showed nearly the same values as the control.

INTRODUCTION

Chemical pest control is widely spread due to various reasons, among them are, the ease of preparation and the somewhat low costs as compared with other methods of control, such as biological control, modifications of methods of cultivation, crop rotation and the extensive plant breeding studies.

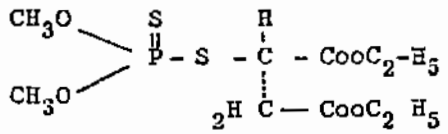
The vital processes of the crop itself may be affected by the use of pesticides. The most serious injury is that on the cytological level, where the DNA molecule and consequently the gene is altered. Regular application of pesticides to crop plants has been cited as a possible source of genetic damage leading to instability (Unrau & Larter, 1952; Suneson & Jones, 1960; Liang et al., 1969; Lee et al., 1974; Adam, 1975 and Reddy & Rao, 1982).

Here in this study we tried to test the effect of two organophosphorus insecticides namely malathion and tamaron on mitosis and some metabolic processes of Vicia faba root tip cells.

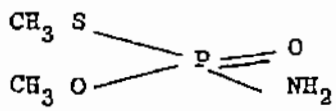
MATERIALS AND METHODS

The test plant was Vicia faba (Var. Giza 2). The chemical formulae of the two used insecticides were as follows and they are considered phorsphoric acid esters.

Malathion



Tamaron



The active ingredients in these insicticides have a number of properties in common :

1. Combined activity against chewing and sucking insects.
2. Inhibition of cholinesterase, a vital enzyme present in blood and tissue.

The germinated seeds (1.5-2 cm radicle length) were immersed in aqueous solutions of the insecticides at concentrations of 250, 500 and 1000 ppm in addition to the control (radicles immersed in tap water) for 2, 4, 6 & 8 hrs. The treated radicles were then washed with water, fixed in Carnoy's fixative (Ethyl alcohol: Glacial acetic acid; 3:1), then stained with leuco basic fuchsin, then smeared in 45% glacial acetic acid and examined.

Nucleic acids were extracted according to Guinn (1966) method and according to Ogur & Rosen (1950). RNA and DNA were determined by using UV. Spectrophotometer at 260 mμ.

Protein was determined photometrically by folinphenol reagent as reported by Lowery et al., (1951).

RESULTS AND DISCUSSION

The data recorded in the following tables showed that both insecticides malathion and tamaron has a preprophasic and a post-prophasic action although they acted differently on Vicia faba root tip cells.

Malathion treatments increased MI% especially after 4 & 6 hrs, while tamaron decreased MI% as compared to the control (Table 1). It is clear from the table also that the effect of tamaron in reducing cell division was more pronounced by increasing concentration, this may indicate that tamaron interfere with the normal sequence of cell cycle to reduce the number of cells starting to divide at interphase. It is also possible to suggest that the reduction in mitotic activity was due to the inhibition of the DNA synthesis which is considered one of the major prerequisites for a cell to divide.

Table (1) Mitotic index of Vicia faba roots treated with different malathion and tamaron concentration under different exposure times.

Time of exposure (hrs.)	Control	Malathion (p p m)			Tamaron (p p m)		
		250	500	1000	250	500	1000
2	53.28	57.21	53.43	44.29	54.28	40.90	28.30
4	52.66	57.77	59.86	61.83	33.11	44.15	31.50
6	50.75	59.93	59.73	64.37	32.86	37.65	30.87
8	56.76	Toxic	Toxic	Toxic	20.53	38.12	45.32

In this connection, Heiner (1971) found that diethyl sulphate caused a blockage of DNA synthesis which in turn induced a substantial mitotic delay. Also, Davids (1973) reported mitotic index inhibition was accompanied by DNA synthesis inhibition and

similar relation was reported by Swietlinska et al., (1974).

On the other hand, the application of malathion tended to increase the mitotic index of root tip's of Vicia faba. Since that increase was not accompanied by an increase in DNA content, the effect of the insecticide could not be attributed to a consequent effect on DNA molecule.

In this respect, the reduced mitotic rate may in part be explained by chromosome aberrations and/or an altered metabolic rate - the data in Table (2) showed that protein contents were sharply increased by tamaron treatments.

Table (2) Protein, RNA and DNA concentrations in Vicia faba root tips treated with malathion and tamaron pesticides at different exposure times.

Treatments	Exposure time (hr.)	Protein mg/100 g F.w	RNA mg/100 g F.w	DNA mg/100 g F.w
Control	2	285	75.00	26.00
	4	265	82.50	30.00
	6	320	93.75	29.50
	8	430	100.00	31.00
Malathion 1000 ppm	2	255	58.75	27.00
	4	280	68.75	28.25
	6	335	61.25	21.50
	8	435	80.00	15.25
Tamaron 1000 ppm	2	345	75.00	26.50
	4	375	83.75	28.25
	6	400	80.00	28.25
	8	510	100.00	27.00

F.W. = Fresh weight

In addition, Vant-Hoff (1968) explained the drop in mitotic activity which is not accompanied by inhibition of DNA synthesis by an increase in the G_2 period. Also Shalaby et al. (1986) reported the same trend where MI changes were not correlated with changes in DNA content in Vicia faba root tip cells treated with growth regulators under different salinity conditions.

Changes in mitotic activity was recorded and discussed by some authors as Shehab & Adam (1981 & 1983); Lazar & Keul (1983); Adam & Rashad (1984) and Amer et al., (1987).

Concerning the percentages of the different mitotic phases Table (3) shows that the general effect of malathion and tamaron in increasing the percentage of metaphase and ana-telaphase was accompanied by a rise in protein content of the treated root tips (Table, 2). This may be explained on the basis that spindle formation required active protein synthesis to form the micro-tubules (Spindle Sub-units). This data showed that tamaron effect was more pronounced in this respect.

Table (3) Percentages of abnormalities in each phase for Vicia faba roots treated with different malathion and tamaron concentrations under different exposure times.

Mitotic phases	Time of treat-ment (hrs.)	Control	Malathion (p p m) 250	500	1000	Tamaron (p p m) 250	500	1000
Prophase	2	85.50	77.96	73.17	77.94	77.73	86.20	63.52
	4	87.58	75.69	52.67	72.59	66.26	88.45	59.73
	6	86.91	62.59	51.20	80.54	87.69	85.53	58.93
	8	85.34	Toxic	Toxic	Toxic	75.00	77.24	58.26
Metaphase	2	9.48	16.85	21.85	14.50	3.39	5.99	29.37
	4	8.40	17.79	29.44	18.62	26.90	3.63	20.64
	6	8.22	21.27	30.37	15.46	4.70	13.16	24.98
	8	9.30	Toxic	Toxic	Toxic	18.40	14.97	21.61
Ana-telophase	2	5.02	5.19	4.98	7.56	18.28	10.68	7.11
	4	3.52	6.52	17.89	8.79	6.84	7.92	19.63
	6	4.87	16.13	18.43	4.00	7.61	2.53	16.09
	8	5.36	Toxic	Toxic	Toxic	6.60	7.79	20.13

Malathion and tamaron induced a number of mitotic abnormalities (Table, 4). The percentages of total abnormalities were higher in case of malathion than tamaron treatments as shown in Table (4).

Table (4) Percentage of total abnormalities in Vicia faba root tips treated with different malathion and tamaron concentrations under different exposure times.

Time of treatment (hrs.)	Control	Malathion (p p m)			Tamaron (p p m)		
		250	500	1000	250	500	1000
2	10.43	34.26	32.31	64.92	34.06	27.26	21.63
4	10.47	34.87	44.73	32.89	23.72	28.98	22.64
6	10.97	38.75	45.26	33.31	21.80	27.34	27.35
8	11.84	Toxic	Toxic	Toxic	12.62	30.44	34.38

The most dominant types of abnormalities scored (Table, 5) were the following in a descending order:
Stickiness (Fig., 1), abnormal prophases, Spindle disturbance (Figs, 2 & 3). In addition malathion and tamaron induced other types of clastogenic anomalies such as lagging (Fig, 4), bridges (Fig., 5), despiralization, binucleate cell (Fig.6) contraction and Asynchronization of chromosome movements.

The types of abnormalities scord in this investigation resembles those reported by Ravindran(1971), Shaikh& Godward (1972) (1972), Anantha (1980), Amer & Mikhael (1983), Somashekar & Goda (1984) and Amer et al., (1987).

The data showed that toxicity appeared after 8 hrs treatment with malathion only that means that malathion was more toxic to Vicia faba root tip cells than tamaron. In support of this view was the high percentages of total abnormalities brought about by malathion as compared with those resulted from tamaron treatments.

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Table (5)- Percentage of different abnormality types in Vicia faba roots treated with malathion and tamaron concentrations under different exposure times.

Insecticide (p p m)		Exposure time (hrs.)	Types of abnormalities								
			Stickiness	Abnormal prophase	Spindle. dis.	Despiralizatio-n	Lagging	Bridge	Break	Asynchronizat-ion	Contract-ion
Malathion	250	2	45.71	25.22	15.54	16.32	2.09	1.13	—	—	—
		4	51.32	22.29	10.98	11.16	3.25	0.99	—	—	—
		6	48.48	17.18	17.28	9.08	4.93	3.14	—	—	—
		8	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	500	2	55.11	25.57	5.84	7.78	3.57	1.05	0.46	—	0.65
		4	48.93	17.87	12.66	8.71	7.20	1.35	0.29	0.38	2.44
		6	45.18	19.91	11.40	11.78	8.66	1.39	—	—	1.67
		8	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	1000	2	41.92	34.36	17.5	14.42	2.70	0.45	0.52	—	—
		4	43.33	30.46	10.46	13.38	0.85	1.03	—	—	0.45
		6	41.23	33.33	8.34	15.07	0.45	1.05	—	—	0.45
		8	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
Tamaron	250	2	66.69	10.83	12.99	6.08	0.96	0.95	0.5	1.43	—
		4	24.42	41.84	10.93	12.09	0.84	—	0.88	—	—
		6	80.99	10.44	4.75	3.78	—	—	—	—	—
		8	60.77	14.47	12.77	12.50	—	—	—	—	—
	500	2	72.47	8.57	2.62	4.50	1.09	—	—	0.44	0.36
		4	75.98	13.33	3.61	4.25	1.13	1.06	—	1.18	—
		6	65.09	26.65	6.04	1.65	2.91	—	—	—	—
		8	54.39	25.50	9.98	7.53	4.11	—	—	—	0.42
	1000	2	54.84	14.50	14.56	10.46	—	1.19	—	—	2.02
		4	49.80	15.55	11.56	13.84	5.93	2.68	0.66	—	—
		6	47.27	22.33	12.08	7.92	6.35	3.70	—	—	—
		8	53.09	19.70	11.64	8.09	4.91	2.57	—	—	—

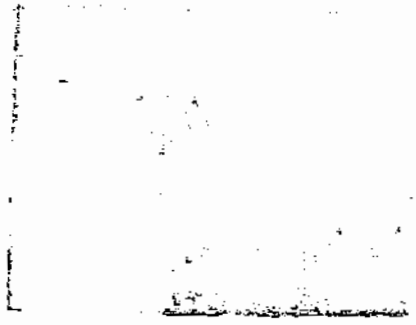

Fig. (1)
Sticky and irregular prophase after treatment with malathion 250 ppm for 2 hrs.

Fig. (2)
Partial C-metaphase after treatment with tamaron 500 ppm for 2 hrs.

Fig. (3)
Disturbed anaphase after treatment with tamaron 250 ppm for 2 hrs.

Fig. (4)
Lagging chromosome after treatment with malathion 1000 ppm for 2 hrs.

Fig. (5)
Double bridged anaphase after treatment with malathion 250 ppm for 2 hrs.

Fig. (6)
Binucleate (micro) cell-after treatment with malathion 500 ppm for 4 hrs.

Also, RNA and DNA contents were decreased by malathion application, while they were more or less the same in case of tamaron.

So we can come to the conclussion that the insecticidal treatments with the used concentrations and time durations affected Vicia faba root tip cells preprophasic (DNA, RNA and protein synthesis period) and postprophasic (chromosome and spindle movements, formation and function).

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