

Effect of the herbicide "Treflan" on the mitosis of

Vicia faba

By

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INTRODUCTION

The toluidine herbicide "Treflan" [α,α,α , trifluoro 2,6-dinitro-N,N-dipropyl -P-toluidine (trifluralin)] (Fig. 1), controls most grass and broad leaf weeds, Ballada et al. (1971), Poole and Conover (1971), Harvey et al. (1972) and Baskakov et al. (1982). It used as a herbicide for weed control in soyabean (Kudinov 1980).

Continuous use of pesticides may cause its accumulation in the soil. The aim of the present study is to evaluate the genotoxic effect of pure Treflan on Vicia faba plant.

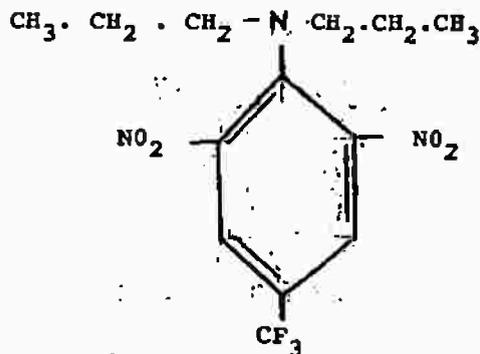


Fig. (3): Treflan

MATERIALS AND METHODS

Sterilized seeds of Vicia faba (var. Giza 2) and pure Treflan 100 % were used in this study. The herbicide was dissolved in distilled water.

Two types of treatments were conducted.

I. Irrigation :

Seeds were soaked for 24 hrs. in tap water then cultivated in 10 pots, (5 pots for treatment and 5 pots for control, each pot contains 5 plants, i.e. 25 seedling for each treatment and control). The plants were irrigated twice for one week once with the saturated "Treflan" solution (390 ppm) and the other with water. The other plants in 5 pots were irrigated twice with water and used as control. The roots were washed thoroughly with water cut and fixed, after one week only.

II. Seed-soaking :

Seeds were soaked in tap water for 24 hrs, then soaked in four different concentrations (390, 195, 97.50 and 48.75 ppm) of the herbicide solution for another 24 hrs, control seeds were soaked in distilled water. Seeds were germination in rolles of filter paper which were placed in containers with tap water at the bottom. Main roots were cut when they were about 2-3 cm in length and fixed in 1:3 acetic acid: ethanol for 24 hours and the roots were stored in 70 % ethyl alcohol in a refrigerator.

Five replicates (five roots) were performed for each treatment and the control (one root from each pot of irrigated seeds and one root from each roll of seed-soaked).

Examination of the roots was done in permanent root-tip squash preparations stained by the Feulgen technique.

Abnormalities were counted in the different mitotic stages (abnormal mitosis) and interphase cells. The data were analyzed according to the t-test.

RESULTS AND DISCUSSION

Both treatments with the herbicide did not affect the mitotic index in Vicia faba root tip-meristems (Table I). However, a statistically significant percentage of abnormal cells was observed in root mitosis after both treatments with the herbicide (Table II). It may be mentioned that other pesticides did not affect cell division but induced abnormal mitosis e.g. the insecticide "Dursban" and "Methamidophos" (Amer and Farah, 1983, 1985).

Most of the abnormal cells were observed in the metaphase and anafelophase stages (Table II). Hussein et al. (1984) found also that the highest frequency of abnormal cells in Allium cepa root-tips treated with "Trifluralin" were observed in the metaphase and telophase stages.

Treflan-treatments induced cells with chromosome abnormalities which increased in number as the concentration of the herbicide increased (Table II).

Disturbed pro,meta-and anaphases where the chromosomes spread all over the cell comprised the main type of the induced abnormalities after the two treatments (Table III, Fig. 2). The arrangement

of chromatin threads was abnormal in the case of disturbed metaphases (Compare Kabarity, 1966). Disturbed meta- and anaphases may be due to the disturbance of the spindle apparatus, and so the chromosomes spread irregularly all over the cell (Amer and Ali, 1969), and (Selim et al. 1981). Disturbed metaphases with contracted chromosome were observed (e.g. Fig. 3). Such phenomenon was observed after treatment with the herbicide isopropyl phenyl carbamate (Story and Mann, 1967), Ortho- and Paranitrophenols (Amer and Ali, 1969).

Prophase-metaphase stage where, the chromosomes retained their arrangement as in prophase stage were also observed, and classified under disturbed type (Amer and Ali 1986). Lagging and sticky chromosomes were observed in a considerable percentage (Table III, Fig. 4).

It seems that the effect of "Treflan" on the cells differs in the different plants. Grigorenko et al. (1986) mentioned that when maize grains were soaked in a solution of "Treflan" the main aberrations were chromosome fragments and bridges. In the present study fragments and bridges were observed in a low percentage in the two treatments (Table III, Fig. 5). Micronuclei were observed in a low percentage in seed-soaked treatment only. In the present study polyploid cells were not observed as Kabarity and Nahas, (1979) found in *Allium* root tip treatments with "Treflan". Sawamura and Jackson (1968) found also that Trifluralin was more toxic to Vicia faba than to Tradescantia cells.

SUMMARY

The effect of pure, Treflan has been studied on root-mitosis of Vicia faba plant (var. Giza 2). Two types of treatments were conducted after the seeds were soaked in tap water for 24 hours. I. Seeds were cultivated in pots then irrigated twice/week once with Treflan solution (390 ppm) and the other with water. Control seeds were irrigated twice/week with water. II. Seeds were soaked for 24 hours in four different concentrations of Treflan solution and water as control.

The two treatments induced a significant increase in the percentage of abnormal mitoses, but did not affect the mitotic index.

The types of the induced abnormalities were: disturbed pro-meta-, and ana-telophases, lagging and sticky chromosomes. Fragments, bridges and micronuclei were observed in a low percentage.

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Table (I): Mitotic index in Vicia faba root-tip meristems after treatment with "Treflan" solutions.

Type of treatment	Count. cells No.	Mitosis No.	Mitotic index	
			Mean	S.E.
I. Irrigation :				
1. Control	5658	563	99.20 ±	9.42
2. Treflan 390 ppm	5796	693	119.51 ±	4.43
II. Seed-soak treatment :				
1. Control	5782	581	100.49 ±	11.02
2. Treflan 390 ppm	5899	706	119.03 ±	9.73
" 195 ppm	6035	774	128.13 ±	14.50
" 97.50 ppm	6269	768	121.78 ±	12.98
" 48.75 ppm	5834	459	78.57 ±	8.35

Table (II): Percentage of abnormal cells in the different mitotic stages in the root-tip meristems of Vicia faba with Treflan solutions.

Type of treatment	% of abn. div. cells		% of abn. proph. cells		% of abn. meta-cells		% of abn. anu-telo-cells		% of abn. inter. cells	
	mean	± S.E.	mean	± S.E.	mean	± S.E.	mean	± S.E.	mean	± S.E.
I. Irrigation :										
1. Control	0.78	± 0.34	---	---	2.35	± 2.35	1.76	± 1.18	---	---
2. Treflan 390 ppm	5.57	± 0.76*	1.58	± 0.65	17.26	± 3.23*	9.63	± 3.35	---	---
II. Seed soaked treatment :										
Control	2.12	± 0.42	1.53	± 0.43	2.18	± 0.76	3.64	± 1.80	0.18	± 0.06
Treflan 390 ppm	8.09	± 1.16*	3.78	± 0.84	13.23	± 3.50*	13.17	± 2.25*	0.50	± 0.11
" 195 ppm	7.33	± 0.80*	2.72	± 1.35	11.68	± 1.53**	11.77	± 1.86**	3.26	± 0.08
" 97.50 ppm	5.29	± 1.10*	1.80	± 0.66	7.39	± 1.02**	10.16	± 2.36	0.32	± 0.08
" 48.75 ppm	3.08	± 0.60	2.11	± 0.56	3.07	± 1.38	4.71	± 1.26	0.30	± 0.20

* Significant at 0.05 level (t-test).

** " at 0.01 " (t-test).

Table (III): Percentage of different types of the abnormalities* occurring in the mitosis of Vicia faba roots after treatment with Treflan solutions.

Type of treatment	No. of No. of		% of the different types of abnormalities relative to No. of abn. mitosis					
	all	abn.	Dist.	Lag.	Frag.	Stick.	Brid.	Micro.
I. Irrigation :								
Treflan 390 ppm	693	38	63.16	10.53	2.63	15.79	13.16	--
II. Seed-soak-treatment :								
Treflan 390 ppm	1509	117	61.54	17.09	1.71	21.37	1.71	0.85
" 195 ppm	1433	106	58.49	27.36	--	8.49	2.83	2.83
" 97.5 ppm	3080	170	51.18	25.29	1.77	18.24	2.35	1.77
" 48.75 ppm	2480	79	39.24	27.85	3.80	25.32	2.53	6.33

(287)

* In the analysis of the different types of abnormalities, a cell containing more than one type of abnormalities was recorded under those types in the same time (e.g. a cell with stickiness and fragments).



Fig. (2)



Fig. (3)



Fig. (4)

Figs. (2-4): Disturbed metaphase (2), light sticky metaphase (3), and telophase with bridge (4), after irrigated (2,4) and seed soaked treatment (3).