The Effect of Nuvacron on the Mitotic Behaviour of *Vicia faba* L.

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Accepted December 27, 1986

Due to the mutagenic effects of the pesticides they are not released in U.S.A. till several tests that proves its safe use. Brusick (1980) added that genetic toxicology has been applied to screen the chemicals with mutagenic properties. Therefore, evaluation of the imported ones is needed. The Etenol and Fouadin imported drugs were found to be mutagenic by Younis (1977, 1983).

Kihlman (1966), suggested that the use of plant root tips in the detection of chromosome-breaking chemicals is reliable and rapid. Brusick (1980) added that it is one of the major tests to identify the mutagenic products.

Nuvacron, the Ciba product is a systemic insecticide widely used to protect vegetable, fruit and field crops which penetrates into all plant tissues to ensure insecticidal activity escaped the direct action (Ciba 1972). Nuvacron is an organo-phosphorus compound having 4 methyl groups causing toxic metabolites (Lindquist and Bull 1976). Ethylating agents give large alteration which weaken both 7-N-ethyl and 9-N-sugar bonds of guanine so hydrolysis takes place (Bautz and Freese 1960). They cause cross-linking and may react with DNA strands (Fishbein *et al.* 1970). Cytological studies on the effect of insecticides on *Vicia faba* and cotton were undertaken by Amer and Farah (1974) and on Barley by Singh *et al.* (1978), Banda and Sharma (1980) and others.

Therefore, this work was carried out to study the cytological effects of Nuvacron on *Vicia faba* since it was introduced to Egypt and little is known on its effects on the hereditary system.

Materials and methods

Vicia faba L., variety Giza 2 seeds were used in the present study. Six fresh concentrations of Nuvacron of 1, 2, 4, 6, 8, 10 ml/ltr. Distilled water was used in preparing different solutions in addition to the control.

1. The dry seeds were immersed in the required concentration for 24 hours followed by washing the seeds with distilled water before germination in Petri-dishes on 20°C. The secondary roots of 10-15 mm were fixed in 3:1 Carnoy solution and stored in 70% ethanol in the refrigerator.
2. The second experiment was carried out by using untreated seeds after germination for 6 days. The seedlings having lateral roots of 10-15 mm were treated with aqueous solution of 2 or 4 ml Nuvacron/ltr, in addition to the control treatment for 24 hours. Thereafter, the root-tips were washed and fixed in 3:1 solution and stored. The aceto-carmine dye was used for cytological examination.

Results and discussion

The mitotic index, mitotic inhibition and the percentages of aberrant mitotic anaphases and telophases of *Vicia faba* induced by the different concentrations of the Nuvacron insecticide

ticide for the two experiments are given in Table 1.

The results show a gradual reduction in the mitotic index by the increase in the concentration of the Nuvacron while the increase in the concentration increased significantly the mitotic inhibition and the mitotic aberrant anaphase and telophase percentages in both the seedlings of *Vicia faba* and the dry seed experiments. This shows the damage of the Nuvacron insecticide

Table 1. Mitotic indices and frequencies of aberrant anaphases and telophases with their respective standard errors in *Vicia faba* root tips and seeds treated with Nuvacron

Concentration/ltr	No. of cells scored	Mitotic index (%)	Mitotic inhibition (%)	No. of anaphases and telophases scored	Aberrant anaphases and telophases (%)
Control	1490	14.90 $\pm$ 0.92	—	105	1.90 $\pm$ 1.33
1a	1736	12.38 $\pm$ 0.79	16.91*	195	23.59* $\pm$ 3.04
2a	1901	12.05 $\pm$ 0.75	19.13*	164	31.10* $\pm$ 3.61
4a	2400	11.17 $\pm$ 0.64	25.03*	179	41.90* $\pm$ 3.72
6a	1145	9.96 $\pm$ 0.89	33.15*	47	48.94* $\pm$ 7.29
8a	1225	7.84 $\pm$ 0.77	47.38*	31	51.61* $\pm$ 8.98
10a	1831	7.27 $\pm$ 0.61	51.21*	24	58.33* $\pm$ 10.06
2b	2035	13.56 $\pm$ 0.76	8.99	137	24.82* $\pm$ 3.69
4b	1490	12.89 $\pm$ 0.87	13.49	104	28.85* $\pm$ 4.44

* Significant at 0.05 level.

a, treated seeds; b, treated root-tips.

Fig. 1. The effect of the Nuvacron on *Vicia faba* seeds.

ticide on the mitotic division, the seedling growth and the significant aberrant cells show its efficiency in inducing mutations in *Vicia faba* L. even the concentration was lower than that recommended to be used as an insecticide.

Comparing the same concentrations of 2 and 4 ml/ltr. H₂O of the same Nuvacron insecticide on the treated dry seeds and growing root-tips, the results show that the mitotic inhibition and the aberrant anaphases and telophases were higher in the dry seeds than that of the growing seedling root-tips. This may be due to the accumulation of the insecticide inside the dry seeds before starting the growth while the lower damage to the growing seedlings may be due to the repair mechanism that occurs during the mitotic division and the effect of the chemical differs

from one stage to another. Adding these factors to each other may show the cause of the less damage that occurred to the growing root-tips.

However, Figure 1 shows that the inhibition and the aberrant mitotic anaphases and telophases due to the Nuvacron was increased by increasing the concentration of the used insecticide.

Table 2 shows the *t*-test between the effect of the different Nuvacron concentrations on

inducing inhibition and mitotic aberrant anaphases and telophases in the treated dry seeds and growing root-tips of *Vicia faba*. The results show that increasing the concentration induced significantly increase in the inhibition and the mitotic aberrant anaphases and telophases with

Table 2. *t*-test between the different concentration of Nuvacron inducing different mitotic inhibition and aberrations in *Vicia faba* treated dry-seeds and seedling root tips

Conc./ltr	Mitotic inhibition							
	Treated dry-seeds						Treated roots	
	1	2	4	6	8	10	2	4
1	—	0.30	1.19	2.03*	4.12**	5.12**		
2		—	0.89	1.80	3.92**	4.94**	1.41	0.73
4			—	1.10	3.33**	4.41**	0.73	1.54
6				—	1.80	2.49*		
8					—	0.58		
10						—		

Conc./ltr.	Aberrant anaphases and telophases							
	1	2	4	6	8	10	2	4
1	—	1.59	3.81**	3.21**	2.96**	3.31**		
2		—	2.09*	2.12*	2.12*	1.92*	0.24	0.39
4			—	0.86	1.00	1.53*	0.39	2.25*
6				—	0.23	0.76		
8					—	0.50		
10						—		

*,** Significance at 0.05 and 0.01, respectively.

Table 3. The frequency and percentages of the aberrant cells induced by Nuvacron in *Vicia faba*

Stage of division	Type of aberration	Total dividings	Aberr. cell no.	%	Total of aberr.	No.	%
Metaphase	Tetraploids		3	0.28	Bridges	14.21	62.74
	Disturbed chr.		13	1.23	Dist. chr.	3.03	13.38
	Lagging chr.		4	0.38	Fragments	2.93	12.94
	Ring chr.		1	0.10	Laggings	1.99	8.79
	Totals		21	1.99	Tetraploids	0.28	1.24
					Rings	0.20	0.91
Anaphase	Distributed chr.		19	1.80	Tatols	22.65	100.00
	Lagging "		17	11.61			
	Bridges		147	13.93			
	Fragments		31	2.93			
	Totals		214	20.28			
Telophase	Bridges		3	0.28			
	Ring chr.		1	0.10			
	Totals		4	0.38			
Total of all stages		1055	239	22.65			

respect to the treated dry-seeds while those of the growing root tips were less affected which matched with the results of Table 1.

On the other hand, Table 3 shows the frequency distribution and the percentages induced by the Nuvacron in each mitotic division stage. The Table showed also the type of aberration

induced in each stage. The results show that 214 aberrant cells out of the total of 239 aberrations observed in all the stages were those at the mitotic anaphase stage representing 89.54% of the total aberrations. The aberrations of the metaphase stage were only 21 cells representing 8.79% while those of the telophase stage were only 4 cases representing 1.67% of the total

Fig. 2. The types of aberrations induced in *Vicia faba* by Nuvacron. A, diploid cell. B, tetraploid. C, ring chromosome. D, lagging chromosome. E and F, chromosome bridges.

aberrations induced. The present results support the previous conclusion of the effect of the chemical which may differ from one stage to the other.

With respect to the total aberration types observed in the different stages, the results show that 62.7% of the aberrations were bridges, 13.38% were disturbed chromosomes, 12.94% were fragments, 8.79% were lagging chromosomes, 1.24% were tetraploids and 0.91% were rings.

The presence of the tetraploids may show that the Nuvacron affected the spindle fibers in addition to its effect on the DNA causing the breaks, bridges, fragments, lagging chromosomes and rings totaling 85.38% of chromosomal aberrations.

Figure 2 shows the different types of chromosome aberrations induced in *Vicia faba* by Nuvacron including the chromosome bridges, fragments, ring and lagging chromosome.

The present results show clearly the deleterious effect of the Nuvacron on the chromosome of *Vicia faba*. Therefore, nuvacron is like other insecticides and should be used carefully.

Abstract

Due to the mutagenic effects of several pesticides, drugs, and chemicals used daily by man, several studies started to investigate the mutagenic effects of the new released ones. Nuvacron, the Ciba product is a systemic insecticide used against cotton leafworm, potato tuber moth and other insects. It is an organo-phosphorus compound having 4 methyl groups and highly toxic. *Vicia faba* dry seeds were treated with 6 fresh diluted solutions while growing seedlings were treated with only 2 concentrations of Nuvacron in addition to the control one. The root-tips were fixed and cytological studies were carried out. The results showed gradual reduction in the mitotic index by increasing the concentration and significant gradual increase in the inhibition and the induced aberration percentages by the increase in the concentration. The treated seedling root-tips were less affected due to the repair mechanism. The Nuvacron induced about 63% chromosome bridges, 13% fragments, 9% lagging chromosomes and 1% rings of the total induced aberrations. The presence of induced tetraploids and disturbed chromosomes may be due to the effect on the spindle fibers in addition to the effect on DNA.

References

- Amer, S. M. and Farah, O. R. 1974. Cytological effects of pesticides VI. Effect of the insecticide Rogor on the mitosis of *Vicia faba* and *Gossypium barbadense*. *Cytologia* **39**: 507-514.
- Banda, B. B. and Sharma, C. B. S. R. 1980. Cytogenetic hazards from agricultural chemicals. 3 Monitoring the cytogenetic activity of "Trichlorofon" and "Dichlorovoson" on *Hordeum vulgare*. *Mutat. Res.* **78**: 341-345.
- Bautz, F. and Freese, E. 1960. On the mutagenic effect of alkylating agents. *Proc. Natl. Acad. Sci. U. S. A.* **46**: 1585-1594.
- Brusick, D. J. 1980. *Principles of Genetic Toxicology*. Plenum Press, N. Y.
- Ciba-Geigy 1972. Nuvacron Product, Profile No. A C 6. 31. Basel, Switzerland.
- Fishbein, L., Flamm, W. G. and Falk, H. L. 1970. *Chemical Mutagens. Environmental Effects on Biological Systems*. Academic Press, New York.
- Kihlman, B. A. 1966. *Action of Chemicals on Dividing Cells*, 260 pp.; Prentice-Hall Inc., Englewood, Cliffs, N. J.
- Lindquist, D. A. and Bull, C. L. 1967. Fate of 3-hydroxy-N-methyl-cis-crotonamide dimethyl phosphate in cotton plants. *J. Agric. and Food Chem.* **15**(2): 267-272.
- Singh, B. D., Singn, R. B., Singh, R. M., Sharma, N. D. R. K. and Singh, J. 1977. Cytogenetic aberrations and morphological changes induced by insecticide treatments of barley seeds. *Indian J. Exp. Biol.* **15**: 688-691.
- Younis, S. E. A. 1977. The mutagenic effects of the Etrenol drug on *B. subtilis*. *Alex. J. Agric. Res.* **25**(2): 297-301.
- 1983. The mutagenic effects of the Fouadin drug on *Bacillus subtilis*. *Assiut J. Agric. Sci.* **14**(2): 65-76.