

## Cytogenetic Activities of the Fungicides Nimrod and Rubigan-4 on Root-tip Cells of *Vicia faba* L.

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**ABSTRACT.** The two fungicides Nimrod and Rubigan-4, were tested for genotoxicity using root-tip cells of *Vicia faba*. In the first experiment lateral roots were treated with different concentrations of the fungicides for different intervals and observations were taken about mitotic index and percentage of mitotic phases. Both fungicides acted as mitostatic agents, which reduced the mitotic index in general. They also altered the percentage of mitotic phases. A recovery experiment was conducted using the treatment that gave the lowest mitotic index in each fungicide. It appeared that the fungicide Rubigan-4 had a more permanent effect on reducing the mitotic index, that stayed even after 96 hours of recovery. Nimrod increased mitotic index after 48 and 96 hours significantly. Both fungicides showed a permanent effect on spindle formation ending up with low frequency of ana/telophase. In the third experiment, DNA content of root tip cells was estimated calorimetrically using the diphenylamine procedure. No significant difference was noticed between control and the roots treated with Nimrod. Rubigan-4 showed significant increase in DNA content especially when used at high concentrations, indicating that the fungicide arrested cells in interphase stage, (S) period ending up with low mitotic index and high DNA content.

### Introduction

The number of chemicals used by man is enormous. More than 500 new compounds appear every year other than thousands already available. Some of these chemicals are pharmaceutical, alkylating agents, food additives and pesticides (Moutschen 1985). Fungicides represent an important group of pesticides and are commonly used to control plant diseases caused by fungi. Many workers reported on the mutagenetic

potentiality of these chemicals. Genetical side effects of fungicides include alteration of mitotic index, course of mitoses and DNA content: Kiraly *et al.* (1985), Samashekar (1986), and Ziogas and Davidse (1987).

In a previous investigation, the authors reported on the effect of the two fungicides: Nimrod and Rubigan-4 on mitotic index, mitotic phases and induction of chromosomal aberrations (Shahin and El-Amoodi 1986). In the present investigation, the authors aimed to study the effect of the same two fungicides on mitotic index and mitotic phases after plant recovery and on DNA content of root tips of *Vicia faba*.

### Material and Methods

The two fungicides: Nimrod (2, amino ethyl, 5 butyl, 4N methyl sulphonate, 6 methyl pyrimidine) and Rubigan-4 [(2-chlorophenyl) (4-chlorophenyl) 5-pyrimidine methanol] were tested for genotoxicity in this investigation. *Vicia faba* Var. Giza II was used as the biological test system. Seeds were obtained from the Egyptian Ministry of Agriculture. The standard method of Kihlman (1975) for germinating and treating roots with chemicals was used. In the first experiment, lateral root tips were treated with the two fungicides at five concentrations: 50, 250, 450, 650, and 850 ppm. Samples of roots were taken after 2, 4, 6 and 24 hours from each concentration to calculate mitotic index and percentage of each mitotic phase according to Badr *et al.* (1983). A second experiment was conducted to study the effect of each fungicide after plant recovery. From experiment 1, the concentration that gave the lowest mitotic index in each fungicide was chosen. Lateral roots were treated with the fungicides and left to recover in fresh water for 24, 48 and 96 hours. The same observations were taken as in experiment 1. In the last two experiments, the difference between values of different treatments and control were compared with critical values for test involving a difference of two proportions (Bras and Bras 1978). In the third experiment, DNA content of 500 milligrams root tips of each sample in experiment 1 was estimated. Nucleic acids were extracted according to the method of Ogur and Rosen (1950), using same steps suggested by Markham (1955) and Yeoman and Macleod (1977) for plant tissues. DNA was estimated using the diphenylamine procedure described by Balamesh (1987). The optical density of the solution was measured using Beckman spectrophotometer at 540 nanometer (nm) and DNA amount was calculated from a standard curve of thymus DNA. For statistical analysis *t*-test was applied (Bras and Bras 1978).

### Results and Discussion

It is clear from Table 1 that the fungicide Nimrod decreased mitotic index in most treatments. The reduction of mitotic index was significant in some treatments and highly significant in others. The fungicide showed also a high significant increase in mitotic index when used at 850 ppm for four hours interval. Reduction of mitotic index may be explained as a result of one or more of the following factors:

- a) Lengthening of DNA synthesis period (s) by the fungicide and preventing cells from entering  $G_2$  period.
- b) Affecting  $G_2$  period and reducing cells entering prophase.
- c) Occurrence of chromosomal aberrations that reduce dividing cells.
- d) Reduction of DNA synthesis.

TABLE 1. Mitotic index in root tip cells of *Vicia faba* treated with the fungicides Nimrod and Rubigan-4 at different concentrations for different intervals.

Type of treatment (ppm)	Mitotic index %			
	2 hr	4 hr	6 hr	24 hr
Control	7.40	4.61	8.30	10.03
N 50	5.19*	4.03	1.09**	6.27**
R 50	1.50**	2.81*	1.17**	6.69**
N 250	5.19*	1.30**	1.41**	5.26**
R 250	9.12	10.07**	0.49**	0.30**
N 450	4.07**	4.39	7.76	9.41
R 450	3.00**	1.95**	4.03**	9.23
N 650	3.73**	3.72	7.12	8.58
R 650	6.11	6.33	7.50	8.37
N 850	4.66**	9.14**	6.32	7.06*
R 850	5.26**	4.86	8.41	7.92

N : Nimrod, and R : Rubigan-4.

\* Significant at 5% level.

\*\* Significant at 1% level.

The results in Table 2 showed that the fungicide, in general, decreased number of cells in prophase. These results indicated the effect of the fungicide on ( $S$ ) and ( $G_2$ ) periods and reducing cells entering prophase. Similar results were obtained by Shah *et al.* (1970) using the antifungus phleomycin on *Vicia faba*. Reduction of mitotic index was also reported by using different fungicides in plants: Behera *et al.* (1982) and Sahu *et al.* (1983).

Increasing mitotic index by the fungicide Nimrod when used at 850 ppm for four hours can be interpreted as a result of increasing number of cells in prophase in the same treatment (Table 2). The fungicide in this treatment may have affected  $G_2$  period and enhanced cells entering mitosis or it may have affected cells in prophase, delayed nuclear membrane breakdown and reduced cells entering metaphase or both. Reduction of metaphase stage in the same treatment (Table 3) indicated the last effect. Similar results were obtained the last effect. Similar results were obtained by Pandita *et al.* (1981) using the fungicide *bavistin* on *Allium cepa*. The fungicide Nimrod also reduced metaphase stage when used at 50 ppm for four hours (Table 3). In this case it was on the expense of ana/telophase percentage (Table 4). However, the fungicide Nimrod increased metaphase stage significantly in several treatments. These results may be explained by poisoning the spindle by the fungicide and arresting metaphase. Similar results were obtained by Kozera and Klein (1975) and Al-najar and Soliman (1980) using different fungicides. Spindle is thought to be formed

TABLE 2. Percentage of mitotic cells in prophase of root tips of *Vicia faba* treated with Nimrod and Rubigan-4 at different concentrations and different intervals.

Type of treatment (ppm)	% Mitotic cells in prophase			
	2 hr	4 hr	6 hr	24 hr
Control	45.83	40.35	51.50	62.11
N 50	28.57*	20.37*	83.33	38.67*
R 50	63.16	30.95	38.89*	17.33**
N 250	42.50	21.88	52.00	40.16**
R 250	49.19	80.18**	11.76**	25.00*
N 450	34.48	24.29	43.36	41.88**
R 450	69.57**	76.19**	25.52**	46.81
N 650	22.50*	34.59	30.34**	49.56
R 650	62.86*	81.43**	66.23	38.37**
N 850	46.15	61.17*	52.69	44.91*
R 850	57.58	70.59**	56.04	37.50**

N : Nimrod, and R : Rubigan-4.

\* Significant at 5% level.

\*\* Significant at 1% level.

of RNA and protein, Mazia (1959). RNA's are synthesized and accumulated in three steps: one immediately post telophase, one during the *S* period and one pre-prophase period, Woodard *et al.* (1961). Rates of protein synthesis are high during interphase and mitosis with the highest degree at  $G_2$  period and prophase, Van't Hof (1963). The effect of Nimrod may be due to its effect on RNA or on protein synthesis.

TABLE 3. Percentage of mitotic cells in metaphase of root tips *Vicia faba* treated with Nimrod and Rubigan-4 at different concentrations and different intervals.

Type of treatment (ppm)	% of mitotic cells in metaphase			
	2 hr	4 hr	6 hr	24 hr
Control	21.88	50.88	36.08	28.23
N 50	41.42*	25.93**	16.67	32.00
R 50	31.58**	54.76	33.33	29.33
N 250	43.75**	65.63	32.00	36.22
R 250	47.58**	18.02**	52.94	0.00
N 450	39.66**	48.57	33.63	34.19
R 450	28.99	14.29**	51.06	51.06**
N 650	27.50	54.29	46.07	33.63
R 650	34.29	18.57**	32.74	38.37**
N 850	30.77	28.16**	25.81	44.91*
R 850	40.91**	25.49**	42.86	62.50**

N : Nimrod, and R : Rubigan-4.

\* Significant at 5% level.

\*\* Significant at 1% level.

TABLE 4. Percentage of mitotic cells in ana/telophase of root tips of *Vicia faba* treated with Nimrod and Rubigan-4 at different concentrations and different intervals.

Type of treatment (ppm)	% of mitotic cells in ana / telophase			
	2 hr	4 hr	6 hr	24 hr
Control	32.29	8.77	12.37	9.68
N 50	30.00	33.70**	0.00	29.33**
R 50	5.26	14.29	27.28	53.33**
N 250	13.75**	12.50	18.00	22.95*
R 250	3.23**	1.80*	35.29*	75.00**
N 450	25.86	27.14**	23.00*	23.93**
R 450	1.45**	9.52	23.40	2.13*
N 650	50.00	10.17	23.59*	16.81
R 650	2.86**	0.00*	1.30**	1.16*
N 850	23.08	10.68	21.51	10.20
R 850	1.52**	3.92	1.10**	0.00**

N : Nimrod, and R : Rubigan-4.

\* Significant at 5% level.

\*\* Significant at 1% level.

Table 5 represents the effect of Nimrod on mitotic activity after recovery. One effect of the fungicide was the significant increase in mitotic index after 48 and 96 hours recovery. Another effect was the notable reduction of ana/telophase percentage after 96 hours recovery. These effects may be interpreted as a result of one or more of the following factors:

- Effect of the fungicide on lengthened  $S$  period was temporary;
- Effect on  $G_2$  period was also temporary and disappeared after 96 hours of recovery causing increase in dividing cells;
- The fungicide altered the period of mitotic phases, the shortest was ana/telophase interval.

Table 6 represents the DNA content of root-tip cells of *Vicia faba* treated with the fungicides. No significant difference was noticed between control and the roots treated with Nimrod. These results eliminate our previously mentioned assumption that the fungicide reduced mitotic index due to reduction of DNA synthesis.

With respect to Rubigan-4, it is clear from Table 1 that the fungicide reduced mitotic index in most treatments. In this respect the fungicide resembles Nimrod, in affecting cells in  $S$  or  $G_2$  period and reducing the number of cells entering mitosis. The results in Table 6, that represent amount of DNA in root tip cells treated with Rubigan-4, showed more significant increase in DNA content in treated roots with high concentrations than control. These results indicate the effect of fungicide on delaying cells in  $S$  period or  $G_2$  period and increasing number of cells in interphase ending up with low mitotic index and high content of DNA. Ismail and Ahmed (1984) obtained almost similar results in wheat.

TABLE 5. Mitotic index, percentage of mitotic phases in root tips of *Vicia faba* treated with Nimrod and Rubigan-4 for after plant recovery.

Period of recovery	Type of treatment	% mitotic cells			
		Mitotic index	Prophase	Metaphase	Ana/telephase
2 hr	C	7.04	32.98	43.62	23.40
	N 250	6.44	29.27	34.15	36.59
	R 450	7.87	28.97	39.25	31.78
48 hr	C	3.08	37.84	35.14	27.03
	N 250	8.55**	50.51	21.78	27.72
	R 450	4.43	44.78	29.85	25.37
96 hr	C	6.99	50.00	21.43	28.57
	N 250	11.72**	60.00	23.70	16.31*
	R 450	2.59*	38.24	58.83**	2.94**

C : Control, N : Nimrod, and R : Rubigan-4.

\* Significant at 5% level.

\*\* Significant at 1% level.

TABLE 6. DNA content of root-tip cells of *Vicia faba* treated with Nimrod and Rubigan-4 at different concentrations and different intervals.

Type of treatment (ppm)	Amount of DNA $\mu\text{g}/\text{mlg}$ fresh weight			
	2 hr	4 hr	6 hr	24 hr
Control	48.99	35.19	41.72	39.71
N 50	18.73	30.30	27.44	63.47
R 50	40.40	56.94	37.51	45.71
N 250	34.85	52.65	37.51	39.14
R 250	49.62	46.21	45.83	50.76
N 450	41.92	36.21	50.11	56.90
R 450	86.62	94.44*	94.96*	75.11*
N 650	66.16	65.96	67.68	74.44
R 650	75.88	86.87*	104.67**	72.98
N 850	65.82	81.82	74.44	98.99
R 850	76.77	83.71*	94.32*	76.0*

N : Nimrod, and R : Rubigan-4.

\* Significant at 5% level.

\*\* Significant at 1% level.

The fungicide Rubigan-4 caused significant increased in mitotic index when used at 250 ppm for four hours. The increase in this statement may be a result of the increase of prophase stage (Table 2). Increasing prophase stage also appeared at all concentrations when roots were treated for short intervals (2 or 4 hours). The fungicide may resemble Nimrod in this respect and delays nuclei membrane breakdown ending up with high percentage of prophase stage and low percentage of metaphase stage. When roots were treated for longer intervals (6 and 24 hours), the fungicide showed opposite effect (Tables 2 and 3). The fungicide altered percentage of ana/

telephase also (Table 4). In general, it decreased percentage of these two phases. These results are in agreement with the results of Dekergommeau *et al.* (1983) using the fungicides "Folpet" and "Captan" in *Vicia faba*. These results may be due to the effect of Rubigan-4 on poisoning and inhibition of spindle formation.

Table 5 represents results of experiment 2. It shows that the fungicide Rubigan-4 had a more permanent effect than Nimrod on reducing mitotic index that appeared even after 96 hours of recovery. The fungicide resembles Nimrod in reducing percentage of ana/telephase after recovery. It may be due to its effect on spindle or on altering intervals of mitotic phases. The similarity of the effects of Nimrod and Rubigan-4 is not strong since the two fungicides belong to the same group "pyrimidinc".

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## النشاط الوراثي الخلوي لمبيدي الفطريات نمرود وروبيجان - ٤ في خلايا قمم جذور الفول البلدي

سعاد أبو بكر شاهين و خديجة حسين العمودي

قسم النبات ، كلية التربية للبنات ، جدة ، المملكة العربية السعودية

المستخلص . تم اختيار مبيدي الفطريات نمرود وروبيجان - ٤ للسمية الوراثية من خلال دراسة تأثيرهما على معدل الانقسام الميتوزي ونسبة حدوث كل دور من ادوار الانقسام باستخدامها بتركيزات مختلفة وتعريض الجذور الجانبية للنبات لها لفترات مختلفة . وقد اوضحت النتائج أن كلا المبيدين يعملان كمثبط للانقسام الميتوزي وبغيران من نسبة حدوث كل دور من ادوار الانقسام .

وفي تجربة ثانية ، تم تعريض الجذور للتركز الذي أعطى أقل نسبة للانقسام الميتوزي من كل مبيد ، وتم انعاش النبات بعد ذلك لفترة ٢٤ ، ٤٨ ، ٩٦ ساعة . وأوضحت النتائج أن المبيد روبيجان - ٤ له تأثير أكثر ثباتاً في خفض معدل الانقسام الميتوزي والذي استمر ٩٦ ساعة بعد إزالة المبيد . أما المبيد نمرود فقد أظهر زيادة في معدل الانقسام بعد ٤٨ ، ٩٦ ساعة ، كما أظهر المبيدان تأثيراً ثابتاً على تكوين المغزل مما أدى في النهاية إلى قلة تكوين الدور الانفصالي والنهائي .

وفي التجربة الثالثة ، قدرت نسبة الحمض النووي د . ن . ا . بعد معاملة الجذور بكل من المبيدين بتركيزات مختلفة وعلى فترات مختلفة . وتم الكشف عن سكر الديوكسي ريبوز باستخدام طريقة الداى فينيل أمين ، وقدر الحمض النووي من المنحنى القياسي لـ د . ن . ا . المستخلص من الغدة التيموسية . وأظهرت النتائج عدم وجود فروق معنوية بين المقارنة والجذور المعاملة بالمبيد نمرود ، أما المعاملة بتركيزات عالية من المبيد روبيجان - ٤ ، فقد ظهرت فروق معنوية بينها وبين المقارنة ، وهذا يوضح أن المبيد قد أعاق الخلايا في الدور البيني بعد فترة تمثيل د . ن . ا . وذلك أدى إلى ظهور نسبة منخفضة من الانقسام الميتوزي وكمية كبيرة من د . ن . ا .