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CYTOLOGICAL INFLUENCE OF THE HERBICIDE NABU ON THE ROOT MITOSIS OF TRIGONELLA FOENUM-GRAECUM L

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ABSTRACT

This investigation deals with the influence of nabu (sethoxydim) herbicide on the mitosis in roots of fenugreek plant (Trigonella foenum - graecum). The applied concentrations caused mitotic delay. Their action after long durations and high doses was highly significant. Mitotic phases showed a different response to the herbicide effect. However, the herbicide acts mainly during metaphase and has an effect on the centromere and the function of the spindle. Abnormal mitotic figures in all mitotic stages were observed. The major abnormaities were C-metaphase. stickiness, disturbance, lagging chromosomes and bridges.

INTODUCTION

In the last decades the herbicides have used successfully to control the parasitic plants and weeds. However, the application of the herbicides may genetically and cytologically affect crop plants to which they are exposed (Mousa, 1982). Nabu herbicide (+) -2- (1-ethoxyiminbutyl) -5-2- (ethylthio) propyl -3- hydroxycyclohex-2

enone) is proved to be effective in cotrolling weeds of cereals and some legumes plants (Hollaender, 1976). However, in a previous study, El-Ghamery and Abou El-Yousser (1992 b) found that the sethoxydim (nabu) herbicide induced a high percentage of cells with different types of chromosomal aberrations in roots of barley.

In the present investigation, the cytotoxic effect of nabu herbicide on root-mitosis of fenugreek plant (Trigonella foenum-graecum L.) has been studied

MATERIAL AND METHODS

The herbicide was dissolved in distilled water and the following concentrations were used 0.006%, 0.008%, 0.010%, 0.012% and 0.014%.

Seeds were soaked in water for 24 hrs, then germinated on moistened filter paper in petri dishes at room temperature (20 - 25 oC). Treatment of the roots (about 2 cm in length) with the applied concentrations of nabu were carried out for 1,2,4,8,16 and 24 hrs. Treated and control roots were fixed in acetic-alcohol (1:3 V/V) and stained using the Feulgen's squash technique.

Five roots were examined to score the mitotic activity (MI), the percentage of mitotic division and the percentage of the chromosomal irregularities at different stages, of mitosis.

The data of MI were analysed statistically by T-test. Significant (S) and highly significant (HS) differences were decided by

considering the value of "T" at 0.05 and 0.10 propability levels, respectively.

RESULTS AND DISCUSSION

Data shown in Table (1) indicate that the application of different concentrations of nabu to root meristems of Trigonella foenum-graecum resulted in highly significant reductions in mitotic activity (MI). The decrease in the MI value is directly proportional to the concentration of the herbicide and the duration treatment. In this plant, the susceptibility to nabu was comparatively lower than that of barley (El-Ghamery and Abou El-Yousser, 1992 b). The results in Table (1) indicate that the MI values were progressively decrease with the 24 hrs treatment where MI value ranged from 5.9 ± 0.001% to 2.7± 0.001% compared to a control value of 11.20 ± 0.4%. In this concern, this herbicide is similar to other herbicides in causing mitotic depression which was reported by other investigators (Dimitrova, 1987; Dimitrova and Tsikova 1976; El-Sadek and Ashour, 1980; Mousa, 1982; Izumi et al., 1983; Yoshida et al., 1983; Tomuskova and Mydlilova, 1985; Badr and Ibrahim, 1987 and Haliem, 1988).

With regard to the differences in the frequency of mitotic phases, the results show that the herbicide nabu caused an increase in ana-telophase percentage. Such increase was paralleled by a decrease in the percentages of other phases (Table 1).

Table 1: Mitotic index and percentages of the different mitotic stages after treatment of Trigomella foenum- guaecum roots with different concentrations of nabu herbicide.

Time hrs	Conc %	No Counted cells	No dividing cells	Prophase %	Metaphase %	Anaphase %	Telophase %	MI + SE (Singificance)
1	Cont.	2477	290	70.20	13.90	8.20	7.70	11.90 ± 0.400
	0.006	4930	569	73.90	8.80	6.70	10.60	11.80 ± 0.004(-S)
	0.008	5037	567	71.30	9.80	8.50	10.40	11.60 ± 0.005 (-S)
	0.010	4998	475	67.60	11.60	9.50	11.30	8.50 ± 0.006 (-HS)
	0.012	5203	446	66.80	11.00	10.10	12.10	8.40 ± 0.007 (-HS)
	0.014	5176	391	60.70	12.50	14.30	12.50	7.60 ± 0.003 (-HS)
2	Cont	2500	298	70.60	13.30	8.00	7.70	11.90 ± 0.100
	0.006	5097	603	27.30	9.30	7.60	10.80	11.80 ± 0.002(-HS)
	0.008	5153	595	72.60	10.50	8.20	9.60	11.50 ± 0.000 (-HS)
	0.010	4921	385	61.60	14.60	8.10	15.80	7.80 ± 0.001 (-HS)
	0.012	5193	338	61.50	12.50	11.50	13.50	6.50 ± 0.001 (-HS)
	0.014	5238	360	59.80	13.10	14.40	13.30	6.90 ± 0.001 (-HS)
4	Cont	2839	345	71.70	13.80	8.70	6.60	12.10 ± 0.3
	0.006	5157	613	73.70	9.40	6.50	10.50	11.70 ± 0.001 (-HS)
	0.008	5107	512	71.50	10.50	8.20	9.80	10.00 ± 0.002 (-HS)
	0.010	5005	337	60.80	15.60	9.80	14.80	6.70 ± 0.001 (-HS)
	0.012	5263	320	62.80	13.70	10.90	13.80	6.10 ± 0.001 (-HS)
	0.014	5068	298	61.30	12.30	13.10	12.50	5.70 ± 0.003 (-HS)

Time hrs	Conc %	No Counted cells	No dividing cells	Prophase %	Metaphase %	Anaphase %	Telophase %	MI + SE (Singificance)
8	Cont.	2703	354	70.60	13.80	8.10	7.60	13.00 ± 0.500
	0.006	5169	405	71.80	10.60	7.50	11.30	10.11 ± 0.001 (-HS)
	0.008	5032	378	70.40	11.40	8.60	10.50	8.40 ± 0.001 (-HS)
	0.010	5052	252	61.20	17.50	9.60	13.60	5.90 ± 0.001 (-HS)
	0.012	4996	241	60.10	14.50	12.50	13.70	5.40 ± 0.003 (-HS)
	0.014	5163	181	61.80	9.40	11.10	14.80	4.70 ± 0.001 (-HS)
16	Cont	2411	290	71.00	13.80	8.90	6.20	12.00 ± 0.100
	0.006	5332	405	69.60	16.60	7.20	12.60	7.61 ± 0.002 (-HS)
	0.008	5022	378	69.80	11.40	7.20	11.10	7.50 ± 0.001 (-HS)
	0.010	4909	252	55.90	17.50	11.10	15.10	5.10 ± 0.001 (-HS)
	0.012	4995	241	59.80	14.50	15.10	13.30	4.80 ± 0.004 (-HS)
	0.014	4956	181	67.40	9.40	13.30	13.30	3.60 ± 0.001 (-HS)
24	Cont	2550	286	69.90	13.80	9.40	6.90	11.20 ± 0.400
	0.006	5127	300	69.30	11.70	5.70	13.30	5.90 ± 0.001 (-HS)
	0.008	4951	269	67.60	13.00	7.10	12.30	5.40 ± 0.001 (-HS)
	0.010	5075	234	60.80	17.50	8.50	13.20	4.60 ± 0.003 (-HS)
	0.012	4930	176	65.50	13.50	8.50	12.50	3.50 ± 0.001 (-HS)
	0.014	5087	126	66.90	10.90	9.50	12.70	2.70± 0.001(-HS)

These results may indicate that reduction in the MI as a result of decreasing the number of diving cells and percentage of prophase and metaphase. The majority of cells of both stages however, were abnormal. In this concern, T. Foenum-graecum showed different response to nabu compared to Hordeum vulgare (El-Ghamery and Abou El-Yousser, 1992 b).

Almost all treatment with the applied concentrations of the used herbicide induced relatively high percentage of cells with chromosome abnormalities (Table 2). The high number of cells with chromosome abnormalities may be due to the quick absorption of herbicide by the roots (Amer and Farah, 1985). Most cells with chromosomal abnormalities were generally observed at prophase and metaphase. Their percentage was increased as the treatment duration increased for each concentration (Table 2). Also, the total percentage of chromosomal abnormalities increased with the increase of concentration and/or treatment duration (Table 2).

Irregular prophase (Fig. 1), C-metaphase (Fig. 4) and chromosome stickiness (Figs 2&3) were recorded in high frequency with all treatments (Table 3). ON the other hand, bridges (Figs. 6-8), lagging chromosomes (Figs. 9&10) and C-anaphase (Fig. 5) were less frequent types (Table 3). Multipolar anaphase was of rare occurrence (Fig. 11 and Table 3) . These types of abnoumalities were also observed in root tip cells of H. vulgare following treatment with the herbidide used here (El-Ghamery and Abou El-

Table 2: Percentages of chromosomal abnormalities in different mitotic stages and total chromosomal abnormalities recorded in Trigonella foenum-graecum root tips following treatment with different concentrations of nabu for different times

Cocentration %	Treattime (hrs)	No dividing cells	No abnor cells	Abnor %	Abnormal prophase %	Abnormal metaphase %	Abnormal anatelolphase %
0.006	Cont	-	-	-	-	-	-
	1	565	36	6.37	-	5.48	0.88
	2	603	49	8.12	0.83	6.13	1.16
	4	617	64	10.37	2.59	5.83	1.94
	8	523	76	14.53	3.82	7.07	3.63
	16	405	70	17.27	6.66	8.64	1.97
	24	300	61	20.33	9.66	9.66	1.0
0.008	Cont	-	-	-	-	-	-
	1	567	32	5.64	0.70	3.88	1.05
	2	595	51	8.57	0.35	5.04	1.17
	4	512	67	13.08	4.10	6.83	2.14
	8	420	73	17.38	7.14	7.38	2.85
	16	378	78	20.63	9.26	9.26	1.58
	24	269	81	30.11	9.29	9.29	2.97
0.010	Cont	-	-	-	-	-	-
	1	321	29	6.10	0.63	4.84	0.63
	2	236	55	14.28	3.63	9.35	1.29
	4	205	71	21.06	7.71	11.27	2.07
	8	182	81	26.91	11.92	12.29	3.32
	16	141	92	62.50	17.46	15.47	3.57
	24	140	97	41.45	21.79	15.81	3.84
0.012	Cont	-	-	-	-	-	-
	1	412	55	12.33	3.36	7.62	1.34
	2	338	56	16.56	6.80	7.69	2.07
	4	320	65	20.31	8.45	9.06	2.81
	8	271	68	25.09	10.71	11.80	2.58
	16	241	85	35.26	17.01	13.28	4.97
	24	176	72	40.91	21.59	13.63	5.68
0.014	Cont	-	-	-	-	-	-
	1	236	38	9.71	3.83	4.60	1.27
	2	214	53	14.72	6.66	6.66	1.38
	4	176	63	21.79	10.72	7.95	3.11
	8	149	69	28.39	14.40	10.28	3.70
	16	122	63	24.80	21.54	8.28	4.97
	24	97	65	51.58	41.26	6.34	3.97

Yousser, 1992 b) and in Vicia faba and Allium cepa (Dimitrova and Tsikova, 1976; Amer and Ali, 1980; Mousa, 1982; Izumi et al., 1982; Kurinnyl, 1984; Tomuskova and Mydlilova, 1985; Badr and Ibrahim, 1987 and Haliem. 1988.

Chromosome stickiness was observed at propahse and metaphase stages and is considered to result from the failure of chromatid separation (Hussein et al., 1988). This type may be attributed to an effect of this herbicide on nucleoprotein which alters the physicochemical properties of chromosome. El-Ghamery and Abou El-Yousser (1992a) found that this herbicide caused a reduction in nucleic acid contents in root tip cells of T. foenum-graecum.

Multipolar anaaphase was observed in few cells where the chromosomes seggregated irregularity into more than 2 poles. This irregularity may be attributed to the effect of the herbicide on the organization of spindle fibers (Badr and El-Sheikh, 1980) or to the disturbance of the spindle apparatus (Badr et al., 1983; Tomuskova and Mydlilova, 1985 and Amer and Farah, 1985). However, multinucleated interphase cells were not detected in this study.

The cochicine-like effect on chromosome at metaphase was dominant effect of the used herbicide. This effect was detected through the observed partial scattering of chromosomes at this stage. These results indicate that this herbicide is antimitotic agent which is effective in causing suppression or disturbance of the

spindle apparatus.

Chromosomal bridges and lagging chromosome (s) were obserbed following most treatments. The frequency of the former type was higher than that of the latter. Single and multiple bridges were observed. The occurrence of these types are likely to be attributed to chromosome stickiness (Swamura, 1965 and Mansour, 1984) rather the chromosome breakage and reunion of the broken end chromosomes (Garber, 1972), since no chromosome fragmentation was observed in this study.

Finally lagging chromosome was referred to the chromosome which have inactivated centromeres. The most of these laggards fail to participate in the nuclei fromation. Since no micronuclei were observed, this result may indicate that lagging chromosome was dissolved in the cytoplam at the end of mitosis.

In conclusion, it is well known that fragment and bridges lead to structural changes in the chromosomes. Lagging chromosome may result in the loss of genetic material (Schulz-Scheffer, 1980). It is evident from Table (3), however that no fragments were recorded, where the other two types of chromosome aberrations were observed at low percentages. These results thus indicate low mutagenic effect of nabu in Trigonella foenum-graecum

Table 3:Types and percentages of chromosomal abnormalities induced by different concentrations of Trigonella foenum - graecum root tips following treatment for different times

Cocentration %	Treat time (hrs)	prophase		metaphase		Ana - telophasees			
		Irreg %	Stick %	Cmeta %	Stick %	Brig %	lagg %	Cana. %	Multipolar %
0.006	cont.	-	-	-	-	-	-	-	
	1	-	-	4.4	1.1	0.4	0.2	0.2	0.2
	2	0.8	-	4.6	1.5	1.0	-	0.2	-
	4	2.6	-	3.6	2.3	0.8	0.2	0.3	0.7
	8	-	-	2.7	4.4	1.5	0.6	0.6	1.0
	16	-	2.0	4.2	4.4	1.0	0.5	0.5	-
	24	-	4.0	4.7	5.0	1.0	-	-	-
0.008	cont.	-	-	-	-	-	-	-	-
	1	0,7	-	2.2	1.2	1.0	-	-	-
	2	2.8	-	3.1	2.1	0.8	0.2	-	-
	4	4.1	-	3.9	2.9	1.5	0.2	0.4	-
	8	6.8	-	3.3	4.8	1.8	0.4	0.4	-
	16	7.2	2.6	5.6	3.8	1.2	0.2	-	-
	24	12.2	5.5	4.8	4.4	2.6	0.3	-	-
0.010	cont.	-	-	-	-	-	-	-	-
	1	0.6	-	2.3	4.4	0.9	-	-	-
	2	3.6	-	5.2	4.1	1.0	0.2	-	-
	4	7.7	-	6.2	5.0	1.4	0.2	0.3	-
	8	8.6	2.6	6.9	3.3	2.3	0.3	0.3	0.3
	16	11.4	5.9	10.3	5.0	2.7	0.4	0.4	-
	24	10.8	9.5	10.4	4.7	2.5	-	0.8	0.4
0.012	cont.	-	-	-	-	-	-	-	-
	1	3.3	-	4.7	2.9	1.1	0.2	-	-
	2	9.6	-	4.5	3.3	1.2	0.6	0.3	-
	4	5.9	1.0	5.2	3.3	0.6	0.1	0.2	1.0
	8	6.5	4.0	6.2	5.4	1.8	0.7	-	-
	16	9.1	7.7	6.6	6.6	3.7	0.4	0.8	-
	24	12.4	9.1	9.0	3.9	3.9	0.1	0.5	-
0.014	cont.	-	-	-	-	-	-	-	-
	1	3.8	-	2.0	2.5	0.7	-	0.5	-
	2	4.1	2.0	2.9	4.0	1.1	-	0.3	-
	4	7.2	3.4	4.1	3.7	1.7	1.0	0.3	-
	8	8.2	6.1	6.1	4.1	2.4	0.8	0.4	-
	16	11.0	10.42	5.5	2.7	3.3	1.1	0.5	-
	24	19.7	1.4	2.3	3.9	3.1	0.8	0.5	-

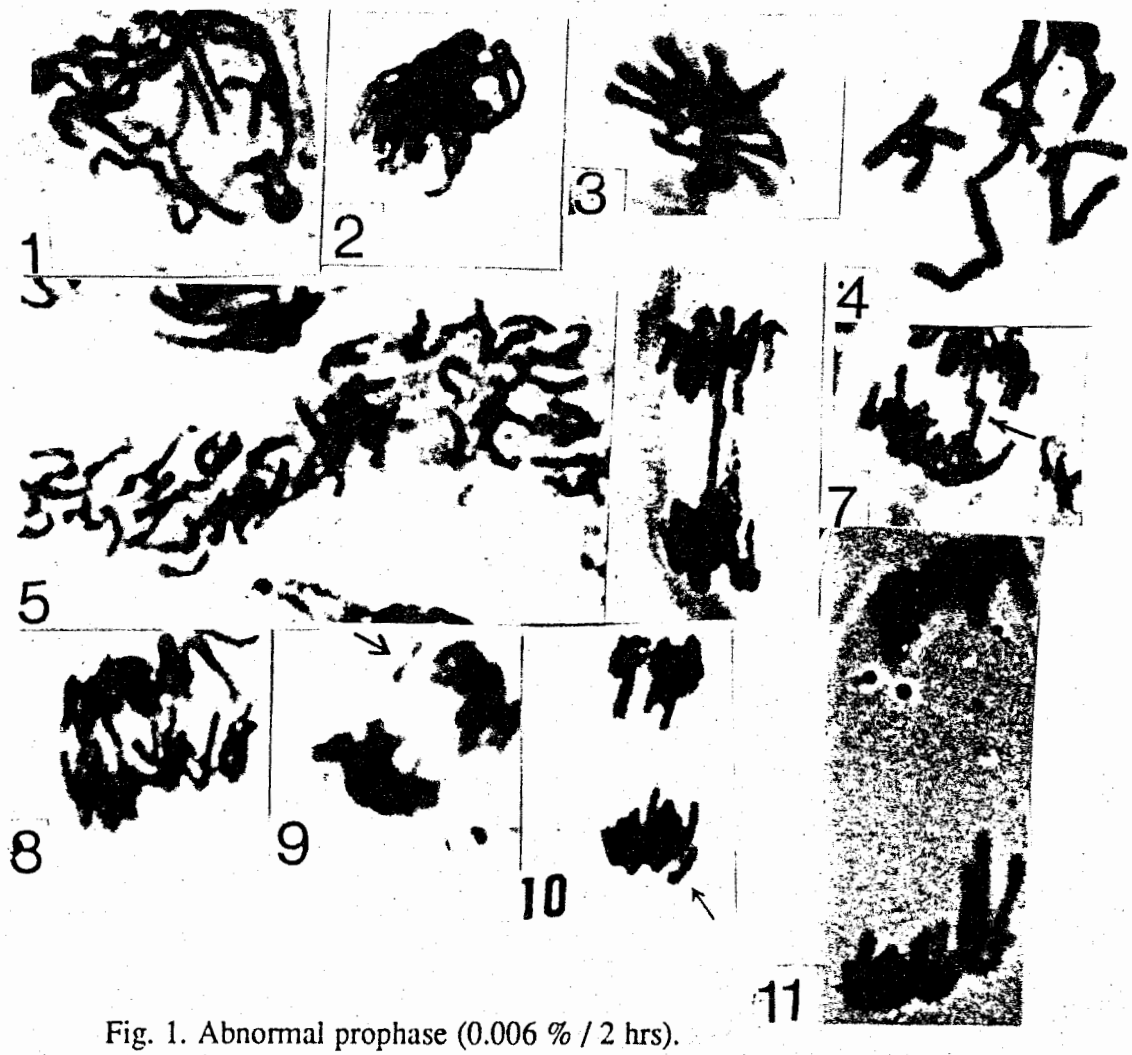

Fig. 1. Abnormal prophase (0.006 % / 2 hrs).
Fig. 2. Sticky prophase (0.014 % / 16 hrs).
Fig. 3. Sticky metaphase (0.014 % / 16 hrs).
Fig.4. C- metaphase (0.014 % / 16 hrs).
Fig. 5. C - anaphase (0.008 % / 16 hrs).
Fig. 6. Chromosomal bridge (0.008 % / 16 hrs).
Fig. 7. Chromosomal bridge (0.008% / 2 hrs).
Fig. 8. Multi- bridges (0.014 % / 42 hrs)
Fig. 9. lagging chromosome at anaphase (0.006 % / 16 hrs).
Fig. 10. Lagging chromosome at anaphase (0.012 % / 8 hrs).
Fig. 11. Multi - polar (0.012 % / 4 hrs).

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التأثير السيتولوجى للمبيد العشبى نابو على الإنقسام الميتوزى فى جذور نبات الحلبة

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إستهدف هذا البحث دراسة تأثير المبيد العشبى نابو على الإنقسام الميتوزى فى جذور نبات الحلبه. أوضحت الدراسة أن جميع التركيزات المستخدمة أدت الى نقص معدل الإنقسام الميتوزى وأن هذا النقص كان عالى المعنوية مع التركيزات العالية وطول المعاملة الطويلة. أدت الدراسة أيضاً الى نقص عدد الخلايا المنقسمة وكذلك أظهرت أطوار الإنقسام الميتوزي استجابة مختلفة للمعاملات.

أوضحت الدراسة أن التأثير الأساسى للمبيد يتركز في الطور الإستوائى حيث يؤثر على السنترومير والمغزل . أحدثت المعاملة صوراً من الشذوذ الكروموسومى في الخلايا المنقسمة وأهمها الطور الإستوائى الكولشيسينى - الكروموسومات اللزجة- بعثرة الكروموسومات- الكروموسومات التائهة- القناطر الكروموسومية.