

Cytological Effects of Water Extracts of Medicinal
Plants in Egypt.

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Mitotic Disturbances Induced by Water Extract of
Cymbopogon proximus (Halfa barr) on Vicia faba.

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Introduction

Because of the ever increasing use of water extracts of medicinal plants in curing diseases instead of the synthetic drugs, care must be taken to avoid harmful effect which may arise from wrong or extra usage. The cytological effects of the crude water extracts of medicinal plants may cause undesired heritable changes. Some cytologists tried to investigate the response of dividing cells to plant extracts, among them Keck and Hoffmann (1951), Kato (1957), Tarkowska (1971), Shehab and Adsm (1981,1983), Adam and Rashad (1984, 1985) and Adam and El-Nahas (1988).

In this survey, the mitotic effect of Cymbopogon proximus extract was studied on roots of Vicia faba. Cymbopogon proximus (Halfa barr), is a perennial aromatic grass belonging to family

gramineae. It is widely grown in upper Egypt (Täckholm 1974). The grass extract is used by the inhabitants as carminative, diuretic and urinary antiseptic.

Materials and Methods

The plant extract was prepared by boiling 1,2 and 3 gm of Cymbopogon proximus in 100 ml tap water for 10 minutes. The evaporated water was replaced by tap water to original volume (100 ml). The extract was decanted while hot.

Vicia faba seeds, v. Giza 2, were sown in saw dust. When the roots were 1.5 - 3 cm in length, they were treated with the different concentrations of the extract (1,2 and 3 %), for 4 hours. Tap water was used for the control experiment.

Another group of Vicia faba roots were immersed in 1 % extract for 24 and 48 hours. The 1 % concentration is commonly used for preparation of Cymbopogon proximus beverage (tea spoonful/200 ml water).

Three replicates, three roots/each were used for each treatment. After treatments, the roots were cut and fixed in Carnoy's fixative. Observations were made from permanent leuco basic fuchsin stained preparations. Mitotic index (MI)

was calculated as the number of dividing cells/1000 counted cells. The data were analyzed according to the t-test.

Results and Discussion

The data scored in Table (1) showed that all treatments affected MI negatively, the decline was not significant in short hours of treatment (4 hours). The effect was about significance in roots treated for 24 hours and highly significant after 48 hours treatment with 1% extract. Other plant extracts proved to be mitodepressive such as water extracts of : *Sonchus*, *Chenopodium*, *Crisum* and *Medicago* (Bukolova and Stepanova, 1972), *Lupinus termis* extract (Shehab and Adam, 1981) and *Datura innoxia* and *Hyoscyamus muticus* extract (Shehab et al., 1983).

The results show that the reduced mitotic activity may be ascribed to partial blockage of DNA synthesis, thus minimizing the number of cells entering mitosis rather than hindering spindle formation. Table (1), showed also that analysis of frequency of mitotic phases, points to a preprophasic action. It manifested itself in the decrease of the number of nuclei entering prophase. This effect leads to increase in percentage of metaphases and anaphases over those of the control in some treatments. Kubink (1966), ascribed the increased percentages of phases to be due to prolonged duration of it.

Table (2) indicates that, all treatments with the extract induced significant percentages of abnormal dividing cells, its values increased with increase of concentration and time of treatments. The maximum percentage of abnormal dividing scored was 36.71 % after 48 hour treatment with 1 % extract, and the least was 13.89 % after 4 hour treatment with 1 % extract. It was also obvious that, metaphase stage was the most affected and the least was prophase stage.

These results led us to opine that, Cymbopogon proximus extract has an accumulative effect in induction of chromosomal abnormalities. From Table (3) it is evident that, the most frequent types of irregularities, occurred in the form of sticky chromosomes (Fig. 1) resulting from, liquifaction of the chromatin material. This effect leads to hinderance of normal separation. A maximum value attained (72.39 %) followed 48 hour treatment with 1 % extract. Disturbancy of chromosome orientation, either in metaphase (Fig. 2) or anaphase, was also a common abnormality which may be the result of, affecting the centromere activity or the spindle fibres. Similar results were reported by Adam and Rashad (1985) treating Vicia faba root tip cells with Ammi visnaga water extract. Selim et al. (1981) refered this type of abnormality to be due to, spindle interruptions caused by treatment. Lagging chromosomes in metaphase (Fig. 3) and

anaphase (Fig. 4) and chromosome bridges were also recorded but with lower frequencies. Despiralization, diagonal orientation of chromosomes and chromosome fragmentation were scarcely scored in some treatments (Table 3).

The present results revealed that, Cymbopogon proximus extract resembles other plant extracts in induction of mitotic irregularities specially on increasing concentration and time of treatment, In this respect it resembles the so called mitotic poisons (D'Amato 1954).

Summary

The present investigation concern the influence of the water extract of Cymbopogon proximus (Harka barr) on the mitosis in roots of Vicia faba. All the used concentrations caused mitotic delay, the action of the long durations was significant. The extract also brought about significant percentage of abnormal dividing cells.

The effect of the extract may be determined as preprophasic. The number of pro phases decreased while metaphases and anatelophases increased. The extract induced a number of chromosomal irregularities such as stickiness, disturbancy, lagging chromosomes and bridges.

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Table (1.) : Mitotic Index and frequency of the mitotic stages in Vicia faba roots treated with Cymbopogon proximus extract.

Treatment	Interphase cells			No. of dividing cells	MI \pm S. E.	Mitotic stages				
	Total	No. abn.	% abn.			Prophase %	Metaphase %	Ana- telophase %		
I. Root-treatment for 4 hrs. with :										
1. 3 % ext.	9751	521	5.31	819	77.44 \pm 13.46	43.03	24.99	31.97		
2. 2 % ext.	9235	122	1.31	810	81.26 \pm 4.15	42.13	23.89	33.99		
3. 1 % ext.	9150	56	0.61	780	78.50 \pm 5.66	44.49	27.38	28.13		
Control	9569	68	0.67	1024	96.60 \pm 4.04	45.89	22.25	31.86		
II. Root-treatment with 1 % ext. for :										
1. 24 hrs.	9000	12	0.13	532	55.80 \pm 2.21	37.87	24.04	38.10		
Control	9000	7	0.08	789	80.48 \pm 7.60	45.81	24.43	29.75		
2. 48 hrs.	9000	3	0.03	368	39.27 ^{***} \pm 2.83	28.77	31.14	40.09		
Control	9000	2	0.02	648	67.15 \pm 2.25	40.07	22.93	37.00		

*** Significant to control at 0.01 level of probability (t-test).

Table (2) : Total percentage of abnormalities and its distribution in the mitotic stages of *Vicia faba* treated with *Cymbopogon Proximus* extract.

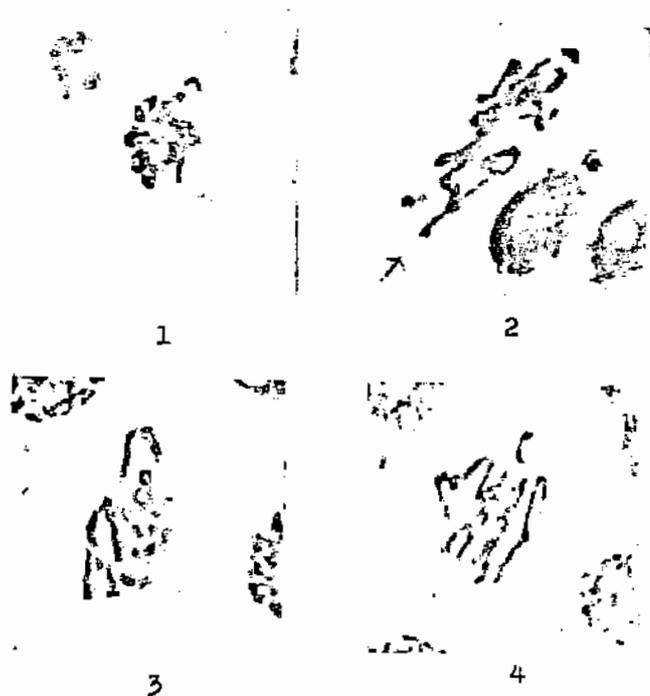
Treatment	Dividing cells			% of abn. in mitotic stages		
	Total	No. abn.	% abn. \pm S.E.	Prophase	Metaphase	Ana-telophase
I. Root-treatment for 4 hrs. with :						
1. 3 % ext.	819	156	20.27 [±] 4.78	1.62	33.38	33.86
2. 2 % ext.	818	151	18.70 [±] 1.93	0.55	32.58	31.42
3. 1 % ext.	780	108	13.85 [±] 1.70	0.55	27.80	21.22
Control	1024	17	1.67 \pm 0.23	0.18	3.58	2.78
II. Root-treatment with 1 % ext. for :						
1. 24 hrs.	532	162	30.62 [±] 2.20	16.24	48.68	33.60
Control	789	36	4.61 \pm 0.50	1.43	7.45	8.06
2. 48 hrs.	368	134	36.71 [±] 2.48	21.32	69.96	23.01
Control	648	31	4.84 \pm 2.00	4.56	4.01	5.69

* Significant to control at 0.05 level of probability (t-test).

** Significant to control at 0.01 level of probability (t-test).

Table (3) : Percentage of the different types of abnormalities relative to the number of abnormal dividing cells in Vicia faba after treatment with Cymbopogon proximus extract.

Treatment	No. of abn. dividing cells	Stick. %	Dist. %	Tag. %	Brid. %	Despiral. %	Diagonal anaph. %	Treg. %
I. Root-treatment for 4 hrs. with :								
1. 3 % ext.	156	69.23	26.03	7.05	7.05	-	-	0.64
2. 2 % ext.	151	29.80	45.03	13.25	9.93	-	-	1.99
3. 1 % ext.	108	57.41	25.93	10.19	6.48	-	-	-
II. Root-treatment with 1 % ext. for :								
1. 24 hrs.	162	54.32	23.46	2.47	3.70	16.10	-	-
2. 48 hrs.	134	72.39	15.67	0.75	0.75	3.73	6.72	-



Figs. 1-4 : Sticky metaphase (1), disturbed metaphase (2) and lagging chromosome in metaphase (3) and anaphase(4) after :

1. Root treatment for 4 hours with :
 - a) 1 % extract (Fig. 4).
 - b) 2 % extract (Figs. 2,3).
2. Root treatment for 48 hours with 1 % extract (Fig. 1).