Cytological Effects of Water Extracts of Medicinal Plants

I. Influence of *Ammi majus* extract on root tip of *Vicia faba*

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The use of plant extract instead of synthetic drugs in the treatment of some diseases increased progressively. Many investigators made trials to test the mode of action, toxicity, physiology and cytology of some plant extracts. Among them Marquardt (1950), Ohno (1960), Sarkar (1974), Abraham *et al.* (1976) and Shehab (1979 and 1980).

The water extract of *Ammi majus* (Khella) seeds is used in the treatment of leucoderma and skin diseases (El-Antaki 1923). This plant grows wild in U.A.R. and it belongs to family Umbelliferae, the inflorescence is a compound umbel and the fruit is a schizocarp that splits into two one-seeded mericarps. When boiled with water the seed extract is yellowish brown, possessing a specific odour and a pungent taste. The active component of the extract is xanthotoxin (Balbaa *et al.* 1976).

Material and methods

The test plant was *Vicia faba* (var. Rebaya 40). The plant extract was prepared by boiling a weighed quantity (0.5, 1 and 10 gm) of the dry ripe fruits in 100 ml distilled water for 10, 20 and 30 minutes. The evaporated water was replaced to the original volume. The germinated seeds were treated by dipping the roots in the experimental extracts for three time durations (2, 4 and 6 hrs.) besides the control. In addition a group of seeds were taken off each treatment and placed in distilled water for 24 hrs. to study the effect of recovery.

Results and discussion

The induction of mitotic index decline in all treatments was not correlated with time in both direct and recovery experiments. Generally the 6 hour exposure of *Vicia faba* root tips showed toxicity in 10, 20 and 30 minutes extracts, as can be seen from Table 1.

Mitotic disturbance leading whether to decrease or increase of mitotic index was reported by many authors, Amer and Ali (1969), Badr *et al.* (1972), Nancy and Sandra (1974), Jurgen (1975) and Francis and Macleod (1977).

Table 1 showed also prophase percentage rose over control in most treatments especially after recovery. Metaphase percentage was also higher, these increments in prophase and metaphase percentages were on the expense of ana-telophases.

The results showed that the effect of *Ammi majus* extracts was inhibitory in short durations and lethal in long ones where toxicity occurred in most recovery experiments, this may be explained on the assumption of the high penetrability of the extract and the fast reaction inside the cell. Galal and Abd-Alla (1976) reported similar results in treating root tips with sodium fluoride and diethyl amine.

Table 1. Mitotic index and percentage of mitotic phases of *Vicia faba* as affected by the extract

Treatment	MI%		Mitotic phases					
			Prophase		Metaphase		Ana-telophase	
	D.	R.	D.	R.	D.	R.	D.	R.
Control	8.4	7.5	43.7	30.8	22.1	28	35.9	42.1
10 min. ext.								
0.005% 2h	5.2	5.7	46.5	39	30.8	23.7	22.7	22.2
4h	6.1	6.8	64	41.9	29.5	20.9	6.5	37.2
6h	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
0.01% 2h	4	4.6	54.4	43.1	23.5	34	22	23
4h	3.5	2.8	41.7	50	31.6	33	26.7	17
6h	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
0.1% 2h	4.4	4.8	52	56.4	26.8	22.8	20.9	20
4h	3.5	4.3	41	46.5	33.3	25.6	25.7	27.9
6h	3.2	Toxic	41.4	Toxic	35.8	Toxic	22.8	Toxic
20 min. ext.								
0.005% 2h	5.3	5.3	48.4	58.1	30.5	14.6	20.6	22.3
4h	7	4.5	55.2	44.2	34.4	32.5	10.5	23.2
6h	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
0.01% 2h	4.9	5	71.4	63.2	20.4	23.7	8.2	20.9
4h	3.5	8	54.3	37.4	17.1	48.8	28.6	13.8
6h	2.8	Toxic	41.8	Toxic	45.5	Toxic	12.7	Toxic
0.1% 2h	4	5.2	50	54.3	22.2	26.7	27.8	19
4h	5	3.6	67.8	44.4	15.8	33.3	16.4	22.2
6h	3.1	Toxic	53.7	Toxic	27.2	Toxic	19.1	Toxic
30 min. ext.								
0.005% 2h	5.9	4	83.5	30	6.8	45	10.7	25
4h	5.4	3.2	67.3	43.8	23	34	8.3	21.9
6h	6.9	Toxic	51.3	Toxic	31.2	Toxic	17.5	Toxic
0.01% 2h	3.9	5	71.8	58.9	20.5	14.7	7.7	26.4
4h	3.2	7.1	40.6	49.7	43.1	20.2	16	30
6h	7.2	Toxic	47.8	Toxic	27	Toxic	25.1	Toxic
0.1% 2h	6.4	4.5	66.2	44.4	17.8	22.2	22	33.4
4h	4.9	Toxic	41.9	Toxic	25.8	Toxic	32.3	Toxic
6h	4.2	Toxic	53.1	Toxic	20.7	Toxic	26.2	Toxic
D, direct. R, recovery.								

The mitodepressive effect of the extract may be due to lengthening of mitotic cycle time and or delaying of spindle formation rather than affecting the cell in the G₁, S or G₂ phases where DNA replication takes place. The extract may also affect nuclear membrane disintegration because of the high percentage of irregular prophases and prophase accumulation. This type of prophase abnormality was

Table 2. Total percentage of abnormalities and its distribution in the mitotic phases of *Vicia faba* as induced by *Ammi majus* extract

Treatment	Total percentage of abn.						% of abn. in mitotic phases					
	D.			R.			Prophase			Metaphase		
	No. of div. cells	No. of abn. cells	%	No. of div. cells	No. of abn. cells	%	D.	R.	%	D.	R.	%
10 min. ext. 0.005%	516	111	21.5	667	127	19	55	23.8	33.1	44.4	11.9	31.8
	618	122	19.8	678	353	52.4	7.5	40.7	62	13.8	30.5	45.5
	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	400	170	42.5	460	154	33.5	16	22.6	55.3	77.4	28.7	0
	348	130	37	284	124	43.7	18	23.8	55	61.9	27	14.3
	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
0.1%	440	103	23.4	482	120	24.9	6	9	59	42.4	35	48.6
	351	104	29.6	430	96	22.3	26	34.4	35.6	10.4	38.4	55.2
	324	58	17.9	Toxic	Toxic	Toxic	35	Toxic	53.7	Toxic	11.3	Toxic
20 min. ext. 0.005%	532	106	19.9	530	134	25.3	23	50.7	46.8	28.4	30.2	20.9
	707	154	21.8	448	125	27.9	19.5	22.6	30.5	56.5	50	20.9
	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	490	120	24.5	506	190	36.5	33.3	62.5	58.3	12.5	8.3	25
	350	140	40	803	172	21.4	35.7	11.9	28.6	48.1	35.7	40
	275	70	25.5	Toxic	Toxic	Toxic	21.4	Toxic	42.9	Toxic	35.7	Toxic
0.1%	406	103	25.4	525	70	13.3	48.5	5.5	29.1	86.1	22.3	8.4
	494	118	23.9	360	91	25.3	29.5	46.6	20.5	21.2	50	32.2
	313	55	17.6	Toxic	Toxic	Toxic	35	Toxic	37.5	Toxic	32.5	Toxic
30 min. ext. 0.005%	588	98	16.6	400	120	30	75	48	16.7	28	8.3	23
	542	150	27.7	320	118	36.9	4.6	15.5	53.4	64.5	42	20
	686	238	34.7	Toxic	Toxic	Toxic	37	Toxic	50.2	Toxic	12.8	Toxic
	390	80	20.5	696	180	25.9	37.5	63.3	37.5	6.7	25	30
	320	102	31.9	712	136	19.1	3.7	44.1	5.0	22	46.3	33.8
	716	274	38.3	Toxic	Toxic	Toxic	7.1	Toxic	60	Toxic	32.9	Toxic
0.1%	636	256	40.3	450	82	18.2	55.9	10	32.4	77.9	11.7	12.1
	493	103	20.9	Toxic	Toxic	Toxic	41.7	Toxic	26.2	Toxic	32	Toxic
	420	80	19	Toxic	Toxic	Toxic	37.5	Toxic	25	Toxic	37.5	Toxic

Table 3. Percentage of different types of abnormalities induced in *Vicia faba* by *Ammi majus* extract

Treatment	Types of abnormalities															
	Stickiness		Irr. proph.		Sp. dist.		Bridge		C. met.		Pro. met.		Lag.		Despiraliz.	
	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.
10 min. ext.																
0.005% 2h	37	32.4	23.8	3.6	10.6	18.7	7.6	7.2	10.6	0	2.6	14	5.3	6.2	0	5
4h	30.1	10.2	28.4	46.3	6.8	T.	35	31	0	0	0	6.2	2.8	16.3	1.4	0
6h	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.
0.01% 2h	16.2	12.1	3	21.7	3	26.7	17.4	2.4	7	21.7	0	12.1	12.5	0	6.9	0
4h	12.6	39.7	38	17.5	13	14.3	10.2	0	9.2	20.6	0	12.7	17	0	0	3.2
6h	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.
0.1% 2h	23.8	13.3	30.1	46.3	18	0	7.6	21	10.6	0	2.6	0	5.3	16.3	2	0
4h	25.8	48.3	12.9	24.1	35.5	13.8	6.5	6.9	0	0	3.2	3.4	12.9	3.4	0	0
6h	20.2	40	26.3	56	10	0	21	4	3.7	0	6.2	0	7.3	0	6.3	0
20 min. ext.																
0.005% 2h	43.1	46.3	0	35.8	2.7	4.5	31.1	11	3.1	1.8	6.2	0	9.7	1.4	4.1	0
4h	24	4.04	47.2	0	6.8	13.5	0	29.5	10.5	0	2.6	5.4	5.9	8.5	15	2.7
6h	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.	T.
0.01% 2h	66.7	5.2	25	75	0	4.1	8.3	7.2	0	3.1	3.1	0	0	1	0	1
4h	57.2	0	21.4	45	0	19.7	14.3	0	0	3.8	3.8	0	7.1	3.3	0	7
6h	21.4	T.	21.4	T.	14.2	T.	21.3	T.	14.2	T.	0	T.	0	T.	0	T.
0.1% 2h	24.4	T.	44.1	T.	11.8	T.	0	T.	0	T.	0	T.	8.8	T.	5.9	T.
4h	20.8	26.1	20.8	26.1	4.3	8.7	20.8	4.3	0	0	0	8.3	4.3	0	25	30.4
6h	44.9	T.	24	T.	5.5	T.	10.2	T.	0	T.	0	T.	7	T.	0	T.
30 min. ext.																
0.005% 2h	2	23.4	24.5	42.2	2.04	9.4	6.1	10.9	26.5	12.5	8.2	0	10.2	0	2	15.6
4h	18.7	8.5	32	45.8	8	8.5	17.4	15.2	6.7	34	4	18.6	6.7	3.4	6.7	3.4
6h	10.2	T.	39.8	T.	5.5	T.	23.1	T.	5.5	T.	23.1	T.	6.5	T.	27.8	T.
0.01% 2h	25	6.8	50	58.1	0	1.4	0	20.2	0	2.7	0	1.4	17.5	2.7	7.5	4
4h	0	50.8	41.1	27.7	17.6	3	7.8	4.6	13.7	0	2	0	11.8	4.6	2	0
6h	44.9	T.	1.8	T.	3	27.5	17.4	T.	1.8	T.	4.6	T.	8.3	T.	10	T.
0.1% 2h	68.9	58	27.3	25	1.3	0	0	17	0	0	1.3	0	0	0	0	0
4h	12.9	T.	32.3	T.	29	T.	0	T.	6.5	T.	6.5	T.	9.7	T.	0	T.
6h	37.5	T.	20.8	T.	20.8	T.	4.2	T.	0	T.	0	T.	4.2	T.	4.2	T.

T.=Toxic

reported by Kabarity (1966) who opined that the agents used hindered the individualization of chromatic material.

Concerning the three extracts 10, 20 and 30 minutes boiling, it is clear that

Figs. 1-8. 1, light stickiness in metaphase (2 hr, 0.005%, 10 min). 2, severe sticky metaphase (6 hr, 0.1%, 20 min). 3, irregular prophase (4 hr, 0.005%, 10 min). 4, sticky bridges in telophase (6 hr, 0.1%, 10 min). 5, disturbed anaphase (2 hr, 0.01%, 30 min). 6, C-mitosis (4 hr, 0.005%, 30 min). 7, despiralization in anaphase (6 hr, 0.005%, 30 min). 8, lagging chromosomes (2 hr, 0.01%, 30 min).

the 30 minutes extract was the least in induction of mitotic injury especially in direct treatments as compared to the control (8.4%). This may be ascribed either to degradation of some of the active components affecting cell division by boiling or because of other constituents goes into solution having a neutralizing effect.

Table 2 showed that the total percentage of abnormalities did not follow a regular pattern although recovery caused higher percentage of abnormalities in a number of treatments. It is clear from this table also that prophase and metaphase were affected severely more than ana-telophase especially in the 30 minutes extract where the percentages of abnormalities in these phases were higher and that effect was more pronounced after recovery experiments.

The most dominant abnormalities were stickiness, especially at high concentrations reaching 66.7 and 68.9% as shown in Table 3. It varies from light to severe stickiness (Figs. 1 and 2) respectively. Irregular prophase (Fig. 3) and bridges which are mainly sticky ones at anaphase or telophase (Fig. 4) resulted in appreciable percentages. These sticky bridges resulted from difficulty in anaphase separation due to adhesion or clumping of chromosomes (Evans 1962) or to depolymerization and cross linking of the DNA of the chromosomes (Darlington and LaCour 1945). Other types of abnormalities such as spindle disturbance (Fig. 5) and C-mitosis (Fig. 6), despiralization (Fig. 7) and lagging (Fig. 8) occurred but in low frequencies as can be seen from Table 3. These types were met with in cell division by many investigators such as Sato (1970), Bempong and Maxwell (1973), Hakeem *et al.* (1975) and Shehab (1980).

Summary

The effect of water extract of *Ammi majus* on the mitosis of *Vicia faba* was studied in direct and recovery experiments. The results revealed that the extract caused a delay in the completion of the mitotic cycle. The interphase stages G_1 , S and G_2 were not affected. The prophase and metaphase were the most influenced by the extract. The dominant abnormalities were: Stickiness, irregular prophase, bridges and with low frequency lagging, prophase-metaphase and despiralization.

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