

# COMPARATIVE CYTOLOGICAL EFFECTS OF WATER EXTRACTS OF SOME EGYPTIAN PLANTS ON ALLIUM CEPA AND VICIA FABA

By

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## INTRODUCTION

In this investigation a comparative cytological effects of some Egyptian plant extracts which are used as a popular medicinal plants and as a source of some medicinal drugs was studied on roots of *Allium cepa* and *Vicia faba*.

These plants are *Artemisia herba-alba*, *Artemisia abrotahum*, *Achillea fragrantissima* and a mixture of the previous three plants was also tested.

## MATERIAL AND METHODS

*Preparation of the plant extract :*

A weighed quantity (3, 5 and 9 gm) of dry *Artemisia herba-alba*,

*A. abrotahum*, and *Achillea fragrantissima* (stem, leaves and flowers) was boiled in 100 ml. tap water 5 mn. The evaporated water was replaced by tap water to the original volume (100 ml), then the extract was filtered.

A mixture of the three plants was also used. An equal weight of the three plants were mixed, then a weighed quantity of the mixture 3,5, and 9 gm. (Sub-lethal concentrations) was boiled in 100 ml. tap water for 5 mn. then filtered.

*Vicia faba* seedlings (var Rebaya 40) with roots 2-4 cm. in length were hanged in jars containing enough extract for 1 and 2 hours for each concentration. The concentration. The concentrations used were 3, 5 and 9% for each extract used. Tap water was used for the control experiments.

*Allium cepa* roots 2-4 cm. in length were dipped in jars containing the different concentrations of the used extracts for 1 and 2 hours.

After each time interval the roots were cut, fixed in Carnoy's fixative (1 : 3 acetic alcohol) for 24 hours then stored under refrigeration in 70% alcohol.

Permanent root tip squash preparations stained with leucobasic fuchsin were examined.

Another group of *Vicia faba* seedlings and *Allium cepa* bulbs were taken off the water extract solutions after every time intervals (1 and 2 hours) of each concentration, washed with tap water and dipped in tap water for 24, 48 and 72 hours. The recovered roots were cut, fixed, stained and examined as previously mentioned.

### RESULTS

Inhibition of mitotic index was observed in *Allium cepa* and *Vicia faba* roots after treatment with the different concentrations of the three used extracts and their mixture. Inhibition of mitotic index was more obvious in treated *A. cepa* roots, Tables (1 - 4).

Toxicity appeared after 48 or 72 hours recovery in treated *Vicia* roots with all the used extracts. While in treated *A. cepa* roots, toxicity appeared after 24 hours recovery in the treated roots with *Artemisia herba-alba* extract in the higher concentration only. In the lower concentrations of the used extracts *A. cepa* roots regained nearly their normal condition after recovery, Tables (1 - 4).

Accumulation of prophases with respect to the other phases was observed in most of the treatments of all the used extracts in both *A. cepa* and *Vicia faba* roots after direct treatment.

After recovery, accumulation of prophases was noticed in treated *Vicia* with all the used extracts. Also it was noticed in treated *A. cepa* roots with some treatments of *A. abrotanum* after 24h recovery and nearly all treatments of the mixture extract, tables (1 - 4).

Accumulation of metaphases was observed in treated *A. cepa* roots with *A. herba-alba*, *A. abrotanum* and *Achillea* extracts in most of the treatments after the different recovery periods, tables (1 - 4).

Increase in the total percentage of abnormalities was observed in the two treated plants with all the extracts used. This increase of abnormalities was not related to concentration or time of treatment or type of treatment (direct or recovery), tables 5 - 8).

Abnormal prophases, was observed only in treated *Vicia* with *Artemisia - herb-alba* extract.

Stickiness was a common abnormality in nearly all treatments of the used extracts in either direct or recovery tests, Fig. 1, 2 and 3.

Bridges were also a common abnormality. It was found in nearly most of the treatments of direct and recovery tests in both *A. cepa* and *Vicia faba* with all the extracts used. Most of the observed bridges were sticky bridges, Fig. (4).

The most dominant abnormality was the disturbed type. It was noticed in the two tested plants after treatment with all the extracts used after both direct and recovery treatments. Fig. (5) show prophasemetaphase stage in which the chromosomes retain their prophase position. Fig. (6) shows c. metaphase and Fig. (7) show disturbed anaphase. Polyploidy was noticed in small percentage in all the direct treatments of *Achillea* extract on *Allium cepa* only

Laggards, and breaks, Fig. (8, 9 and 10) were a less dominant abnormalities. Breaks was noticed in treated *Vicia* roots with *Artemisia herba. alba* and *A. abrotahum* extracts in some of the direct and recovery treatments. It was noticed also in treated *Allium cepa* with *A. abrotahum* extract only.

Despiralization was observed in most of the direct treatments of treated *Vicia* with the *Achillea* extract only, Fig. (11).

Micro-nuclei, Fig. (12) and binucleate cells were noticed in a small percentage in nearly all treatments and all the recovery periods of all the extracts used on the two used plants.

### DISCUSSION

*Artemisia herba-alba*, *A. abrotahum*, *Achillea* and the mixture extract may be considered as a strong anti-mitotic agents on *Allium cepa* since they reduced the mitotic index after 2 hours treatment. Their effect was inhibitory (temporary) and not lethal (permenant). This result was similar to that reported by Kaul (1972) after treatment with ISDA.

*Vicia faba* proved to be a resistant plant in all direct treatments. This resisting action of *Vicia* roots collapsed and showed mito-depressive effect

Toxicity appeared in treated *A. cepa* roots with the higher concentration of *Artemisia herba-alba* extract after 24 or 48 hours recovery. While in the other three extracts *Artemisia abrotahum*,

*Achillea* and the mixture extract depression of mitotic index was obvious in this concentration (9%). This means that the higher concentration of the former extract has a lethal effect (irreversible).

Accumulation of prophases which was observed in treated *A. cepa* and *Vicia faba* roots with the four extracts used indicate a delay in the spindle formation. While accumulation of metaphases observed after the different recovery periods may be the result of a disturbance of the normal function of the spindle mechanism.

Abnormal prophases were recorded in treated *Vicia* with *A. herba-alba* extract only. This abnormality was explained by kabarity (1966) on the assumption that the agents used hindered the process of individualiation of chromatin threads to normal chromosomes.

Stickiness was a common abnormality in the two treated plants *Allium cepa* and specially *Vicia faba* in nearly all treatments of the used extracts in either direct or recovery tests.

Some of the other chemicals which produced stickiness were 8-ethoxy coffeine (Kihlman 1955), potassium cyanide (Lily 1958) and asafoetida (Narasimba Das et al. 1968). Sudharson and Reddy (1971) noticed high percentage of stickiness in 0.5% 72 hours soaking period of *Lathyrus sativus* extract.

Most of the observed bridges were sticky bridges. Seshachar and Nambiar (1955) using carbon tetrachloride attributed the occurrence of sticky bridges in anaphases of *Allium* roots to be due to decrease in viscosity of the matrix causing the distal ends of chromosomes to fail to separate.

The «prophase metaphase» type induced in this study was similar to those observed by other authors as a result of treatment with various chemical agents viz. colchicine (Barber and Callen 1943), ethylene glycol (D'Amato 1948) and sulphanilimide (Amer 1960).

Disturbed metaphases and anaphases were a common abnormality by all the used extracts in the two plants *Allium cepa* and *Vicia faba*. These abnormalities may be the result of inhibition of spindle mechanism by these extracts.

Laggards, breaks and fragments occurred in this study led to the formation of micronuclei.

Despiralization was noted in treated *Vicia* with the *Achillea* extract after direct treatment only. Despiralization was observed only

in the anaphase stage, thus the *Achillea* extract may cause a partial dissolution of the matrix substance of the chromosomes.

A survey of this discussion of the present results may lead us to report that the used extracts are non carcinogenic agents as far as the present study shows.

### SUMMARY

The cytological effects of water extracts of some Egyptian plants of popular medicinal use were studied on root mitosis of *Allium cepa* and *Vicia faba*. These plants are *Artemisia herba-alba*, *Artemisia abrotanum*, *Achillea fragrantissima* and a mixture of the previous three plants was also studied.

The four used extracts showed a mito-depressive effect on *Allium cepa* roots after direct treatment which was temporary and regained its normal course after recovery experiments. While *Vicia faba* roots resisted the action of these extracts but this resistance collapsed after recovery experiments.

These extracts affected also the percentage of mitotic phases in the two plants used.

The different mitotic abnormalities induced by the used extracts were ; abnormal prophase, stickiness, bridges, spindle disturbance prophase metaphase, C-metaphase, multipolarity and polyploidy, laggard breaks, despiralization, micronuclei and binucleate cells.

### REFERENCES

1. Amer, S. 1960. Vergleichend Untersuchungen über die Wirkung stathmokinitischer stoff. Ph.D. Thesis. Naturwissenschaft lichen Fakultat Universital Muachen.
2. Barber, H. and H. Callen 1943. The effect of cold and colchicine on mitosis in the newl. Proc. Roy. Soc. London, 131, 258 - 271.
3. D'Amato, F. 1948. The effect of colchicine and ehtyl glycol an sticky chromosomes in *Allium cepa*. Hereditas, 34, 83 - 103.
4. Kabarity A. 1966. The effect of cettain mutagenis substances upon prophases Beitre Biol Pflanzen 42, 371 - 326.
5. Kaul, B.L. 1972 Studies on the antimitotic and cytological effects of some amides. 1. isobutyl, 2 - trans, 4 - trans, decadienamide cytologia 37, 531 - 539.

6. Kihlman, B.A. 1955 Studies on the effect of oxygen on chromosome zreakage induced by 8-ethyxy caffeine Exp. cell res. 8 : 404 - 407.
7. Lily, L. J. 1958 Effects of cyanide and ionizing radiation on roots of *Vicia faba* Exp. cell res. 14 : 259 - 67
8. Narasimba Das, T. Sudharson Raj, A., and Ramana Roa, B.V. 1968, Cytological studies in *Vicia faba* treated with asafoetida.
9. Seshachar, B.R. and Nambiar, P. K. 1955. Effects of Carbon tetra chloride on mitosis. Nature, Lond. Lond. 176, 796
10. Sudharson, R.J, and Subba Reddy S. 1971. Cytological studies in *Vicia faba L.* treated by leaf extract of two varieties of lathyrus sativus. Cytologia 36,. 4 : 702 - 714.

TABLE I  
Mitotic index and percentage of phases in *Allium cepa* and *Vicia faba* roots treated with  
*Artemisia hebraea* extract after direct and recovery experiments

Concentration	M.I.	% Prophase						% Metaphase						% Anatelophase					
		Direct		Recovery		Direct		Recovery		Direct		Recovery		Direct		Recovery			
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h			
<i>Allium cepa</i>																			
Control	47	47	47	47	41	41	41	41	41	20.8	20.8	20.8	20.8	20.8	20.8	20.8	38.2		
3% 1h	53	—	27	45	—	—	31	28.0	27.0	28.0	27.0	—	39.0	39.0	—	—	30.0		
2h	25	47	35	48.2	—	20.0	26.8	25.9	25.9	25.9	25.9	—	30.0	29.8	—	50.0	43.4		
5% 1h	50	52	58	43	59.0	20.4	26.3	14.6	14.6	33.3	49.8	31.2	40.8	31.2	26.4	46.3	29.8		
2h	20	25	22	42	25.0	24.7	21.5	20	37.5	39.1	40.8	20.0	40.8	20.0	37.5	36.2	37.7		
9% 1h	2	Toxic	Toxic	Toxic	49.0	Toxic	Toxic	Toxic	21.0	Toxic	Toxic	Toxic	Toxic	Toxic	30.0	Toxic	Toxic		
2h	20	Toxic	Toxic	Toxic	57.1	Toxic	Toxic	Toxic	14.3	Toxic	Toxic	Toxic	Toxic	Toxic	28.6	Toxic	Toxic		
<i>Vicia faba</i>																			
Control	54	54	54	54	20.1	20.1	20.1	20.1	20.1	32.1	32.1	32.1	32.1	32.1	32.1	32.1	47.8		
3% 1h	50	42	46	Toxic	48.0	20.4	35.7	Toxic	32.0	34.1	33.9	Toxic	33.9	Toxic	20.0	45.5	30.4		
2h	33	52	21	37	67.0	32.5	56.3	44.4	16.5	33.0	23.8	26.8	23.8	16.5	34.5	19.9	28.8		
5% 1h	40	38	41	Toxic	58.8	29.2	53.0	Toxic	22.9	29.2	23.5	Toxic	23.5	Toxic	18.3	41.6	23.5		
2h	43	22	10	30	61.6	34.6	5.9	39.0	21.8	32.7	94.1	31.0	94.1	16.6	32.7	0	30.0		
9% 1h	68	22	76	3	63.6	72.6	49.5	51.9	19.9	19.7	25.0	29.5	25.0	16.8	7.7	25.5	18.6		
2h	49	9	68	Toxic	60.0	57.4	52.2	Toxic	29.0	12.7	20.1	Toxic	20.1	Toxic	11.0	29.9	27.7		

TABLE 2

Mitotic index and percentage of phases in *Allium cepa* and *Vicia faba* roots treated with *Artemisia abrotanum* extract after direct and recovery experiments

Concentration	M.I.	% Prophase						% Metaphase						% Anatelephase					
		Recovery			Direct			Recovery			Direct			Recovery			Direct		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
<i>Allium cepa</i>																			
Control	47	47	47	41	41	41	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8
3% 1h	17	42	40	52	41.2	46.3	53	38.3	22.8	25.3	13.5	33.8	36.0	28.4	33.5	27.9			
3% 2h	25	56	47	58	36.4	26.9	5.3	24.8	33	33.1	43.0	40.1	30.6	40.0	4.0	35.1			
5% 1h	30	37	37	40	30.0	34.4	30.6	20.0	20.0	23.8	40.0	43.5	50.0	41.8	30.0	36.5			
5% 2h	28	32	40	40	55.0	66.0	34.5	30.0	23.0	12.0	34.6	40.0	22.0	22.0	30.9	30.0			
9% 1h	65	30	35	38	58.9	62.2	44.2	44.0	18.5	14.6	11.2	29.6	22.6	33.2	44.6	26.4			
9% 2h	17	12	Toxic	Toxic	60.0	60.0	Toxic	Toxic	10.0	13.0	Toxic	Toxic	30.0	27.0	Toxic	Toxic			
<i>Vicia faba</i>																			
Control	54	54	54	54	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1
3% 1h	52	22	Toxic	Toxic	57.1	55.6	Toxic	Toxic	27.6	22.7	Toxic	Toxic	15.3	15.3	21.7	Toxic			
3% 2h	52	4	Toxic	Toxic	58.9	90.4	Toxic	Toxic	26.5	0	Toxic	Toxic	14.6	14.6	9.6	Toxic			
5% 1h	41	15	35	Toxic	76.3	69.0	45.1	Toxic	11.2	20.5	20.1	Toxic	12.5	10.7	34.8	Toxic			
5% 2h	64	7	72	4	71.5	73.5	48.5	62.5	20.5	26.5	12.9	37.5	8.0	0.0	38.6	0.0			
9% 1h	38	22	80	20	67.0	26.7	49.0	61.6	19.0	36.5	23.0	19.6	14.0	36.8	28.0	18.8			
9% 2h	35	6	Toxic	Toxic	61.0	38.9	Toxic	Toxic	26	46.4	Toxic	Toxic	13.0	14.7	Toxic	Toxic			

TABLE 3

Mitotic index and percentage of phases in *Allium cepa* and *Vicia faba* roots treated with *Achillea* extract after direct and recovery experiments

Concentration	M.I.	% Prophase			% Metaphase			% Anatelephase									
		Direct	Recovery	Direct	Recovery	Direct	Recovery	Direct	Recovery								
Time of treatment		2h	48h	72h	24h	48h	72h	24h	48h	72h							
<i>Allium cepa</i>																	
Control		47	47	47	41	40.0	40	20.8	20.8	20.8	39.0	39.0	39.0				
3% 1h		38	—	41	62.6	32.5	—	28.0	13.8	33.4	—	45.1	23.6	33.6	0	26.6	
2h		37	1	47	48.4	60.0	23.1	22.2	22.2	40.0	43.6	29.6	29.4	0	33.3	47.6	
5% 1h		30	—	27	70.9	—	26.3	21.2	8.7	—	41.3	31.8	20.5	—	32.4	47.1	
2h		40	—	48	49.7	—	21.4	22.2	29.0	—	40.0	35.0	21.3	—	38.6	42.8	
9% 1h		9	—	29	33.3	—	20.9	40.5	17.1	—	46.7	27.3	49.6	—	33.1	31.4	
2h		16	38	29	65.6	31.1	11.5	20.4	21.8	32.8	42.8	24.8	12.5	36.6	45.9	46.7	
<i>Vicia faba</i>																	
Control		54	54	54	20.1	20.1	20.1	20.1	32.1	32.1	32.1	32.1	47.8	47.8	47.8	47.8	
3% 1h		71	69	42	Toxic	40.8	47.6	45.3	Toxic	38.9	29.1	22.9	Toxic	20.3	23.2	31.0	Toxic
2h		52	53	Toxic	Toxic	40.0	43.4	Toxic	Toxic	37.8	28.3	Toxic	Toxic	22.0	28.0	Toxic	Toxic
5% 1h		49	22	43	Toxic	45.8	40.6	38.8	Toxic	26.0	31.3	29.1	Toxic	27.4	28.7	32.1	Toxic
2h		57	35	55	Toxic	39.9	36.5	39.0	Toxic	35.5	24.6	29.0	Toxic	24.6	28.8	32.0	Toxic
9% 1h		58	42	Toxic	Toxic	38.0	57.7	Toxic	Toxic	27.5	21.2	Toxic	Toxic	34.0	21.2	Toxic	Toxic
2h		63	47	Toxic	Toxic	45.2	58.7	Toxic	Toxic	26.6	21.5	Toxic	Toxic	27.4	20.0	Toxic	Toxic



TABLE 5

Total percentage of abnormalities in treated *Allium cepa* and *Vicia faba* roots with *Artemisia herba-alba* extract after direct and recovery experiments.

Concentration	Time of treatment	Direct	24h	Recovery 48h	72h
<i>Allium cepa</i>					
	Control	0	0	0	0
3%	1h	6.1	—	—	10.3
	2h	22.2	—	12.1	26.3
5%	1h	12	29.6	21.7	15.6
	2h	0	8.8	16.3	4.6
9%	1h	0	Toxic	Toxic	Toxic
	2h	14.3	Toxic	Toxic	Toxic
<i>Vicia faba</i>					
	Control	0	0	0	0
3%	1h	8.2	4.5	8.2	Toxic
	2h	2.0	11.6	13.1	10.6
5%	1h	12.3	8.3	6.7	Toxic
	2h	12.2	7.7	7.7	22.5
9%	1h	13.5	2.6	8.6	8.3
	2h	25.1	20.8	9.3	Toxic

TABLE 6

Total percentage of abnormalities in treated *Allium cepa* and *Vicia faba* roots with *Artemisia abrotanum* extract after direct and recovery experiments.

Concentration	Time of treatment	Direct	24h	Recovery 48h	72h
<i>Allium cepa</i>					
	Control	0	0	0	0
3%	1h	0	8.4	0	19.1
	2h	0	19.2	9.3	0
5%	1h	19.3	33.3	21.7	10.1
	2h	27.9	20.8	0	11.0
9%	1h	0	8.3	0	8.5
	2h	18.9	12.8	Toxic	Toxic
<i>Vicia faba</i>					
	Control	0	0	0	0
3%	1h	11.2	3.1	Toxic	Toxic
	2h	9.8	0	Toxic	Toxic
5%	1h	13.7	3.9	11.4	Toxic
	2h	14.1	28.6	1.5	16.7
9%	1h	16.1	11.9	15.6	21.1
	2h	15.1	29.6	Toxic	Toxic

TABLE 7

Total percentage of abnormalities in treated *Allium cepa* and *Vicia faba* roots with Achillea extract after direct and recovery experiments.

Concentration	Time of treatment	Direct	24h	Recovery 48h	72h
<i>Allium cepa</i>					
	Control	0	0	0	0
3%	1h	11.2	17.5	—	24.5
	2h	7.4	0	7.7	22.1
5%	1h	0	—	15.9	10.3
	2h	9	—	14.2	0
9%	1h	0	—	3.2	9.9
	2h	8.9	20.3	20.9	9.1
<i>Vicia faba</i>					
	Control	0	0	0	0
3%	1h	4.9	7.7	0	Toxic
	2h	12.4	6.3	Toxic	Toxic
5%	1h	16.6	4.1	2.3	Toxic
	2h	11	5.6	2.6	Toxic
9%	1h	11.1	4.4	Toxic	Toxic
	2h	8.9	4.8	Toxic	Toxic

TABLE 8

Total percentage of abnormalities in treated *Allium cepa* and *Vicia faba* roots with mixture after direct and recovery experiments.

Concentration	Time of treatment	Direct	24h	Recovery 48h	72h
<i>Allium cepa</i>					
	Control	0	0	0	0
3%	1h	34.7	—	6.6	13.1
	2h	11.4	Toxic	Toxic	Toxic
5%	1h	21.2	—	19.6	12.6
	2h	11	—	17.9	8.3
9%	1h	24.1	100	9.6	3.1
	2h	—	—	33.3	12.3
<i>Vicia faba</i>					
	Control	0	0	0	0
3%	1h	7.3	0	Toxic	Toxic
	2h	2.4	6.7	0	Toxic
5%	1h	13.4	2.9	14.3	Toxic
	2h	15.3	Toxic	Toxic	Toxic
9%	1h	7.7	13.8	0	Toxic
	2h	16.3	0	100	Toxic