



Chromosomal Changes in *Allium cepa* induced by
Ruta graveolens water extract

A.I. El-Nahas*, A.S. Shehab** and H. Hegazi*

* Botany Department, Faculty of Education

** Botany Department, Faculty of Girls

Ain Shams University, Cairo, Egypt.

Ruta graveolens belongs to family Rutaceae (the citrus fruit family) (Heywood, 1978). It has been used for centuries as a medicinal plant. It was an ancient herbal remedy for different diseases, and it decreases capillary fragility (Balbaa, 1976).

The aim of this work was to elucidate the effect of *Ruta* water extract on *Allium cepa* mitosis.

Material and Methods

a) Chromosome analysis:

A weighed quantity (1,3,5 & 10 gm) of *Ruta* dried leaves were boiled in 100 ml of tap water for 15 minutes. The evaporated water was replaced to maintain the original volume (100 ml).

The extract was filtered immediately while hot. Root tips of *Allium cepa* were treated with different concentrations 1,3,5 and 10% of plant water extract for different durations (4, 8, 12, 24 hours). After each interval the roots were cut, fixed in Carnoy's fixative (1:3) acetic: alcohol and were stained by the Feulgen squash method. For control tap water was used.

b) Nucleic acids amount:

Four concentrations (1, 3, 5 & 10%) of Ruta were used to elucidate their effects on nucleic acid contents after treating Allium cepa roots for 8 hours. The method applied for total DNA and RNA determinations is that of Schmidt & Thanhauser (1945) with slight modifications described by Morse & Carter (1949).

Results and Discussion

As a result of treatment with different concentrations of Ruta water extract and with different times of exposure, an immediate depression in mitotic index was observed. The mitotic percentage was intensively depressed in most treatments or completely inhibited in 10% after 12 h. (table 1). Toxicity appeared following 24 h. treatment in all concentrations and the root became softened and lost their turgor. Thus, this time interval was excluded. The statistical analysis revealed that MI was significantly affected by Ruta water extract. Depression in the MI had a negative correlation with concentration. Mitodepressive activity was recorded by many investigators after treating Vicia roots either with Cannabis, Mallah and Kabarity (1982), Carum carvy, Farah (1987) and or Gomphocarpus sinaicus, Adam and El-Nahas (1988).

Some investigators attempted to relate depression in the mitotic index with blockage or inhibition of DNA Synthesis and therefore the cell would remain at G₁, (Kihlman 1966 and Schneiderman et al., 1971).

So the inhibition of MI in our results were not related to DNA content since a fluctuation in the percentage of DNA were observed. While RNA percentage increased in most concentrations except in 1% (Table 5).

The drop of MI could be due to arrest of one of the stages preventing the continuation of normal division. This naturally lead to accumulation of some mitotic stages.

This was in accordance with our results since accumulation of prophase stage on the expense of other phases were recorded.

Concerning the total percentage of abnormalities the extract has a significant effect. The highest percentage of abnormalities was in prophase stage. So, the extract may act as prophase poison.

Ruta induced a wide range of mitotic abnormalities (Tables 2 & 3). Prophase abnormalities are the most conspicuous type, stickiness and irregularities being

the most frequent ones, (Table 4 and Figs. 1&2). Such abnormalities were reported by a number of authors among these, Shehab (1980) and Shalaby et al., (1985).

Metaphase stage was also affected. Disturbed metaphase (Figs. 3 & 4) appeared in all concentrations. Stickiness (Fig. 5) was more prominent in 3% and C-metaphase appearing only in cells treated with 1%. The infrequency of cells showing C-metaphase indicates that Ruta extract does not act specifically on inhibiting the spindle fibers completely, thus the extract is less effective on spindle fibers than colchicine.

Bridges were the most prominent feature at ana-telophases (Figs. 6-9). These bridges might be due to stickiness or as a result of breakage and reunion.

Similar results were obtained by Yadov and Rathoe (1984) and Halvonkar and Patil (1986). Multipolar anaphases were only induced by 1% after 8 hrs. (Figs. 8 & 10).

Binucleate and multinucleated cells were a dominant abnormality that appeared at interphase in most of the treatments. (Fig. 11). It is suggested that the Ruta extract could act by preventing the small golgi vesicles which contain cell wall precursors from translocating during telophase by interzonal microtubules to the

equatorial region of mitotic apparatus where they fuse with each other giving rise to the new cell wall. Lopez-saez et al., (1982) are in favour of this suggestion.

Thus we may conclude that Ruta water extract induce mitodepressive effect as well as a number of chromosomal aberrations. Moreover, it has a clastogenic effect.

SUMMARY

The roots of A. cepa were subjected to different concentrations of the water extract of the Egyptian medicinal plant "Ruta graveolens". The water extract showed a mitodepressive effect. An accumulation of prophase was also noticed. The extract affected the nucleic acid contents.

Different types of abnormalities occurred such as, disturbance, stickiness, C-metaphase, bridges, multipolar, micronuclei, binucleate and multinucleated cells.

Table (I) : Percentage of mitotic index and phases as affected by

Ruta graveolens water extract on Allium cepa

Conc. and time in hrs.	Treat. of cells examined	tot.No. of cells	tot.No. of cells divided	M.I. value \pm S.E.	Pro.		Meta		Ana-telo	
					No.	%	No.	%	No.	%
Cont :	1	1565	103	6.60 \pm 0.15	36	34.95	25	24.27	42	40.78
1%	4	1169	47	4.02 \pm 0.15*	25	52.08	9	18.75	14	29.17
	8	1097	27	2.46 \pm 0.15**	19	70.37	3	11.11	5	18.52
	12	1169	34	2.91 \pm 0.18**	15	41.66	14	38.89	7	19.44
	4	1158	45	3.89 \pm 0.25*	29	65.91	11	25	4	9.09
5%	8	1135	25	2.20 \pm 0.15**	15	60	8	32	2	8
	12	1206	16	1.33 \pm 0.17**	7	46.67	7	46.67	1	6.67
	4	1121	39	3.48 \pm 0.17*	35	86.84	4	10.53	1	2.63
	8	1124	27	2.40 \pm 0.32**	22	81.48	4	14.81	1	3.70
10%	12	1107	25	2.26 \pm 0.12**	19	76	4	16	2	8
	4	1328	44	3.31 \pm 0.33*	34	77.27	6	13.64	4	9.09
	8	1075	18	1.67 \pm 0.22**	15	83.33	1	5.56	2	11.11

* Significant from control at 0.05 level (t-test).

** Highly significant from control at 0.01 level (t-test).

Table (2) Total percentage of abnormalities and percentage of abnormalities

in the different mitotic phases after treatment with *Rufa garxolana* extract.

Conc. and time in hrs	Treatment	total percent. of Ab.	percent. of abnormal interphase	Proph.			Meta			Ana-telo.		
				No. n.	No. Ab.	Ab %	No. n.	No. Ab.	Ab %	No. n.	No. Ab.	Ab %
12	4	44.90 ± 5.09 ¹	0.18	20	6	27.27	0	9	40.91	7	7	31.82
	8	33.33 ± 4.32 ¹	0.00	16	3	33.33	0	3	33.33	2	3	33.33
	12	77.77 ± 5.55 ^{**}	0.53	5	10	35.71	0	14	50	3	4	14.29
32	4	75 ± 4.60 ^{**}	0.63	10	19	57.50	0	11	33.33	1	3	9.09
	8	81 ± 4.50 ^{**}	0.45	4	11	52.30	0	8	38.09	0	2	9.52
	12	80 ± 6.16 ^{**}	0.76	3	4	33.33	0	7	58.33	0	1	8.33
52	4	73.68 ± 3.24 ¹	0.46	9	24	85.71	0	4	14.28	1	0	0
	8	62.96 ± 3.55 ^{**}	0.46	9	13	76.47	0	4	23.53	1	0	0
	12	68.00 ± 5.65 ^{**}	0.83	7	12	70.59	0	4	23.53	1	1	5.89
102	4	72.73 ± 5.01 ^{**}	0.47	10	24	75	0	6	18.75	2	2	6.25
	8	66.67 ± 3.83 ^{**}	0.96	5	10	83.33	0	1	8.33	1	1	8.33

¹ Significant at 0.05 level. (t-test) ^{**} Highly significant at 0.01 level (t-test).

Table (3) : Percentage of different types of abnormalities occurring in the mitosis of *Allium cepa* roots as affected by treatment with *Rufa* water extract.

Conc. Treatment and time in hrs	Percentage of each type of abnormalities in all mitotic phases.					
	Irrig.	St.	dist.	bridge	C-m.	
12	4	18.18	9.09	31.82	31.82	9.09
	8	33.33	-	55.55	11.11	0
	12	10.71	25	35.71	14.29	14.29
32	4	31.51	15.15	27.27	6.06	0
	8	33.33	42.86	14.29	9.52	0
	12	25	16.67	50	8.33	0
52	4	57.14	28.57	14.29	0	0
	8	64.71	17.65	17.65	0	0
	12	64.71	5.00	23.53	5.80	0
102	4	46.00	31.25	18.75	3.13	0
	8	41.67	41.67	8.33	8.33	0

Women's Coll. Ann. Rev. Vol. 17 (1992)

Table (4): Distribution of types of abnormalities

in each mitotic phases.

Women's Coll. Ann. Rev. Vol. 17 (1992)

Conc. treat. and Time in hrs.	Pro.			meta.			Anaplo.			Inter.			
	St.	irrig	dist.	St.	E-w.	dist.	multi-polar	bridge	St.	micro	multi.	bin.	
1Z	4	33.33	66.66	77.78	0	22.22	0	0	100	0	50	0	50
	8	0	100	100	0	0	33.33	33.33	33.33	0	0	0	0
	12	70	30	71.43	0	28.57	0	0	100	0	0	50	50
3Z	4	10.50	89.50	81.02	18.18	0	0	0	66.67	33.33	0	47.86	57.14
	8	36.36	63.63	37.50	62.50	0	0	0	100	0	0	20	80
	12	25	75	85.70	14.28	0	0	0	100	0	0	77.77	22.22
5Z	4	33.33	66.66	100	0	0	0	0	0	0	0	80	20
	8	15.38	84.60	75	25	0	0	0	0	0	0	80	20
	12	8.33	91.66	100	0	0	0	0	100	0	0	66.67	33.33
1	4	37.50	62.50	100	0	0	0	0	50	50	0	66.67	33.33
	8	50	50	100	0	0	0	0	100	0	0	60	40

Table (5): Effect of Ruta extract on nucleic acid content (DNA & RNA) after treating Allium cepa root tips with different concentrations for 8 hours.

Conc	DNA		RNA	
	Value	%	Value	%
Control	0.774	100	135	100
1Z	0.72	93.02	130.5	96.67
3Z	1.08	139.5	144	106.67
5Z	0.774	100	148.5	110
10Z	0.95	123.36	137.5	101.85

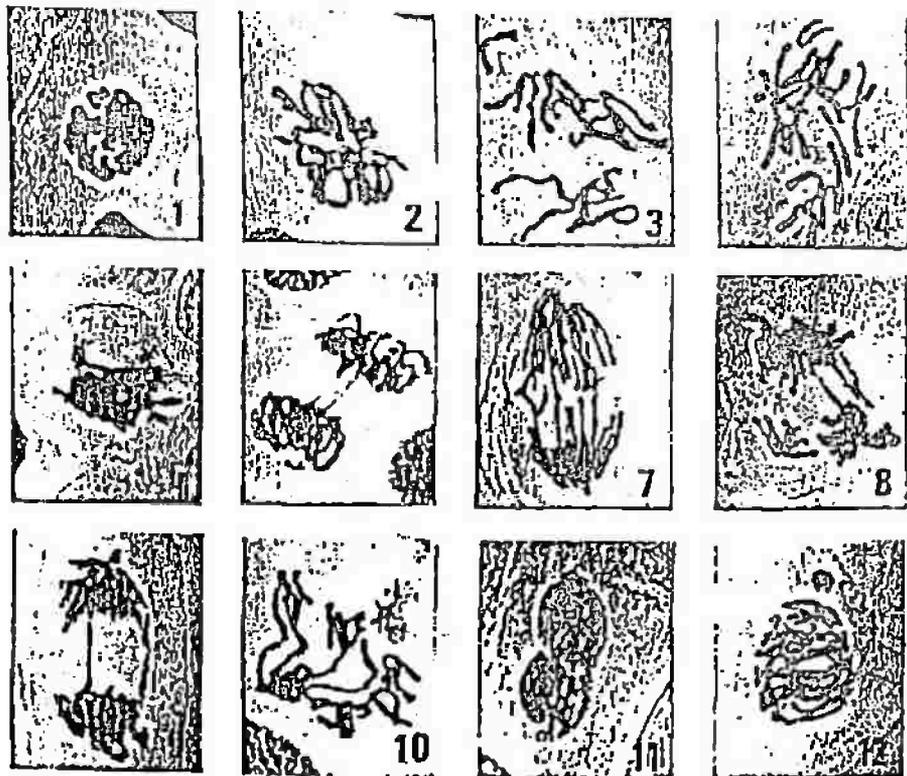


Fig 1 : Sticky disturbed prophase after treating with 5% for 12h.

Fig 2 : Irregular prophase after treating with 10% for 8h.

Figs 3,4 : Disturbed metaphase after treating with 3% for 12h. and 5% for 4h. respectively.

Fig 5 : Sticky metaphase after treating with 5% for 8h.

Fig 6 : Sticky telophase with chromatin bridge after treating with 3% for 4h.

Fig 7 : Anaphase with chromosomal bridge after treating with 1% for 4h.

Fig 8 : Tetrapolar anaphase with broken bridge after treating with 1% for 8h.

Fig 9 : Sticky anaphase with multichromatin bridges after treating with 10% for 4h.

Fig 10: multipolar anaphase after treating with 1% for 8h.

Fig 11: Binucleate cell after treating with 1% for 12h.

Fig 12: Micronucleate cell at interphase after treating with 1% for 4h.

REFERENCES

- Adam, Z.M. and El-Nahas, A. (1988): Cytological effect of water extract of medicinal plants in Egypt. IX. Mitotic irregularities in Allium cepa root meristem cells heated with Gomphocarpus sinicus (haryal) extract. Ann. rev. Science section, Ain Shams Univ. Women's College no 14, 219-230.
- Balbaa, S.I., Hllat, S.H. and Zaki, A.Y. (1976): Medicinal plant constituents. General Organization for Univ. and school books, Second Edition.
- Farah, O.R. (1987): Mitotic effect of water extract of Carum corvi on Vicia faba roots. Bull. NRC. Egypt, 12, 111.
- Halvankar, G.B. and V.P. Patil (1986): Effects of agrochemicals on mitotic chromosome behaviour in tetraploid and hexaploid wheats. Indian J. Genet. Plant Breed. 44(3): 438-446.
- Heywood, V.H. (1978): Flowering plants of world. P. 77-78. In Oxford University Press. Oxford London Melbourne.
- Kihlman, B.A. (1966): Deoxyribonucleotide synthesis and chromosome breakage. Chromosome today 1:108-117.
- Lopez-Saez, J., R. Mingo and A. Gonzalz-Fernandez (1982): ATP level and Caffeine efficiency on cytokinesis inhibition in plants. EUR J. Cell Biol. 27(2):185-190.
- Motallah, G. and A. Kabarity, (1982): Effect of cannabis (hashish) on mitosis of Allium cepa L. root tips. II. Dissolution of the chromatic material in the interphase nuclei of Allium cepa L. cells after long exposure times with cannabis. Cytologia, 47:565-573.

Morse, J.L. and Carter, C.F. (1949): The synthesis of nucleic acid in cultures of Escherchia coli strain B and B/R. *J. Bacteriology*, 58,317.

Schmidt, G. and Thanhauser, S.J. (1945): A method for the determination of deoxyribonucleic acid, ribonucleic acid and phosphoprotein in animal tissues. *J. Biol. Chem.*, 161-83.

Schneiderman, M.H.W.C., Dewey and D.P.Highfield (1971): Inhibition of DNA synthesis in synchronized Chinese hamster cells treated in G₁ with cycloheximide. *Exp. Cell Res.* 67:147-155.

Shalaby, A.aA., Adam, Z.M. and Abdel-Gawad, M.M. (1985): Some mitotic and metabolic Aspects of salinity and Growth-regulators effects in root tips of broad-bean seedlings. *Desert. Inst. Bull., A.R.E., Vol. 36, No. 2, pp. 431-444.*

Shehab, A.S. (1980): Cytological effects of medicinal plants in quatar II. Mitotic effects of water extracts of Teucreum pilosum on Allium cepa *Cytologia.* 45,57-64.

Yadav S.K. and J.S. Rathoe (1984): Cytological effects of the aqueous fruit extract of Emblica officinales on Allium sativum root tips. *Egypt. J. Genet. Cytol.*, 13:181-188.