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Mitodepressive and chromotoxic effects of *Spinacia oleracea* leaves extract on root meristem of *Allium cepa.*

Amit Vaish
Department of Botany, Hindu College, Moradabad, Uttar Pardesh, India

Abstract
Spinacia oleracea is commonly known as spinach and its leaves are consumed as vegetable. The aim of the present study was to evaluate mitodepressive and chromotoxic effects of spinach leaves extract on root meristematic cells of *Allium cepa*. The growing roots of *A. cepa* were treated with five concentrations (100%, 75%, 50%, 25% and 10%) of the spinach leaves extract for 2, 4 and 6 hours respectively. The results indicate that the spinach leaves extract is not only mitodepressive in nature but also induces various types of nuclear and chromosomal abnormalities such as binucleate cells, nuclear disintegration, breaking in chromosomes, scattered metaphases, chromatid separation, stickiness of chromosomes, bridge formation, chromosomal condensation and disturbed polarity etc. Results shows that spinach leaves extract is not only mitodepressive in nature but also shows considerable impact on chromosomal behavior.

Keywords: mitodepressive, chromotoxic, *Spinacia oleracea*, chromosomal abnormalities

Introduction
Spinacia oleracea is commonly known as spinach. It is a green leafy flowering plant of family Amaranthaceae and its leaves are widely consumed as vegetable. It is a rich source of minerals and vitamins. The leaves of this plant are chiefly used as salad, dumplings and curry. Several popular and delicious dishes are prepared from spinach leaves. Till now, several plant extracts have been tested in relations to their mitodepressive and chromotoxic nature such as ginger (Nakamura and Yamomoto, 1982) [9]; garlic, turmeric and asafoetida (Abraham and Kesavan, 1984) [1]; onion (Kaushik and Yadav, 1993) [6]; tomato (Yadav *et al*., 2001) [11]; peel extract from *Citrus limon* and *Citrus sinensis* (Ali and Celik, 2007) [3]; *Hibiscus rosa- sinensis* (Ali, 2010) [2]; *Jatropha gossypiifolia* (Almeida *et.al.*, 2016) [4]; radish (Vaish and Saxena, 2017) [12]; *Euphorbia rigida* (Mert and Burun, 2020) [7] and so on. The present paper throws light on mitodepressive and chromotoxic potentiality of spinach leaves extract on root meristematic cells of *Allium cepa.*

Material and Method
Fresh leaves of spinach were washed thoroughly, chopped and transferred into electric juicer and after filtration, extract was prepared. Different concentrations were prepared by dilution of leaves extract with distilled water.
Allium cepa was taken as test material. The bulbs of the plant were grown in a tray containing sterile moist sand to obtain secondary roots. When secondary roots developed, these were cut from few bulbs, fixed and preserved in 70% alcohol to find out the control value of mitotic index for their meristematic cells. The other bulbs were transferred to the jars containing 100%, 75%, 50%, 25% and 10% of the spinach leaves extract respectively. The treatment of each concentration was given to the roots for the time period of 2, 4 and 6 hours. All the treatments were carried out at 22-25^{0}C. After each treatment, root tips were cut and fixed in Cornoy's fluid (Acetic-Alcohol in the ratio of 1:3) for 24 hours and then transferred to 70% alcohol for preservation. These root tips were hydrolyzed by 1N HCl for 3 minutes and squashed in 2% acetocarmine for cytological studies. The slides were temporarily sealed, examined and micro photographed. Mitotic index was calculated by using the method of Mousa (1982) [8]. Chromosomal abnormalities and their percentage in each concentration and duration were also recorded.

Results and Discussion
The mitotic index of meristematic cells of *A. cepa* root and chromosomal aberrations induced by different concentrations of the aqueous extract of spinach leaves, are given in Table 1. The extract increased the mitotic index of *A. cepa* in 10% and 25% concentrations and even in 50% concentration also, whereas 75% and 100% concentrations of spinach leaves extract showed mitodepressive effect on dividing cells of *A. cepa* roots. When the root tips were treated with 10% concentration for 2 hours, the mitotic index increased to 17.81% from the control value (17.25%). The treatment of the same concentration for 4 and 6 hours, further increased the mitotic index to 18.67% and 19.00% respectively. In 50% concentration, the mitotic index again increased to 19.16% in 2 hours duration whereas in 4 and 6 hour's durations, mitotic index curtailed to 18.96% and 18.52% respectively. The higher concentrations (75% and 100%) were found to be mitodepressive in nature regardless the duration of treatment and concentration of extract. The mitotic index decreased from 17.17% in 75% concentration (2 hours) to 15.27% in 100% concentration when root tips were exposed for 6 hours duration (Table 1).
The spinach leaves extract also induced various types of nuclear and chromosomal aberrations in the dividing cells of roots of *Allium cepa.*

At interphase, binucleate cells and nuclear disintegration were observed as nuclear aberrations. Binucleate cells were found in all the concentrations but in very less numbers. The highest percentage of binucleate cells was recorded as 0.81 in 10% concentration whereas the lowest percentage was 0.47 recorded in 75% concentration. Binucleate cells are produced because of delay or failure of cytokinesis as reported by Ene-Oblong and Amadi (1987) [5]. Sata and Tanaka (1972) [10] studied that the binucleate cells are formed due to the suppression of phragmoplast formation at the early telophase hence neither cell plate nor cell wall appeared. Nuclear disintegration was observed only in 100% concentration showing the percentage of 0.50.

Only condensation of chromosomes was observed at prophase. It was not recorded in lower concentrations but in 50% concentration, the percentage of aberration was 3.63, which increased to 5.88 in 100% concentration.

At metaphase, five types of chromosomal aberrations were studied. Disturbed metaphases (Photo 'b') were scored only in 100% concentration with the percentage value of 11.53. Scattered metaphases (Photo 'c') were recorded only in higher concentrations. In 50% concentration, the percentage of scattered metaphases was 5.55, which increased to 8.33 and 11.53 in 75% and 100% concentrations respectively. Stickiness of chromosomes (Photo'd') was observed only in 75% and 100% concentrations. The highest percentage of stickiness was 15.38 in 100% concentration. Chromatid separation (Photo 'a') was observed in 25%, 50% and 100% concentrations, and its percentage showed an increasing trend from 9.09 in lower concentration to 19.23 in highest concentration. Breaking of chromosome was present only in 100% concentration and its percentage recorded, was 3.84.

At anaphase, bridges, laggards and breaking of chromosomes were observed but they were not frequent. Bridges (Photo 'e') were present in 75% and 100% concentration and highest percentage was 50.0 in 75% concentration. Laggard was observed only in 75% concentration and its percentage was 5.0, whereas breaking of chromosome was present only in 100% concentration. Disturbed polarity was present only in 25%, 75% and 100% concentrations with the percentage of 2.77, 5.0 and 9.09 respectively.

At telophase, no aberration was observed.

It is clear from the experimental observations that spinach leaves extract induced various types of chromosomal abnormalities. It has been pointed out by several workers that chromosomal abnormalities serve as elegant indicator of mutation. Therefore, it is essential that vegetable should be evaluated for possible cellular damage in other systems also.

Table 1: Mitotic index, frequency of aberrations and their percentage as induced by *Spinacia oleracea* leaves extract on root meristematic cells of *A. cepa* (2n = 16).

Concentration	**10%**				**25%**				**50%**				**75%**				**100%**				**Control**		
Duration (In hrs.)	2	4	6	% of abr. (conc. wise)	2	4	6	% of abr. (conc. wise)	2	4	6	% of abr. (conc. wise)	2	4	6	% of abr. (conc. wise)	2	4	6	% of abr. (conc. wise)	2	4	6
Mitotic index (in %)	17.81	18.67	19.00		18.88	19.07	19.11		19.16	18.96	18.52		17.17	16.63	15.81		16.33	15.80	15.27		17.25	17.25	17.25
Types of aberrations																					NO ABERRATION		
B.N.	-	-	B.N.	0.81	-	B.N.	B.N.	0.65	B.N.	B.N.	-	0.65	-	B.N.	B.N.	0.47	B.N.	B.N.	B.N.	0.67			
N.D.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N.D.	N.D.	0.50			
Con. 'P'	-	-	-	-	-	-	-	-	-	Con. 'P'	Con. 'P'	3.63	-	Con. 'P'	Con. 'P'	5.35	-	Con. 'P'	Con. 'P'	5.88			
Br. 'M'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Br. 'M'	3.84			
C.S.	-	-	-	-	C.S.	C.S.	-	9.09	C.S.	C.S.	C.S.	11.11	-	-	-	-	C.S.	C.S.	C.S.	19.23			
D. 'M'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D. 'M'	D. 'M'	11.53			
Sc. 'M'	-	-	-	-	-	-	-	-	Sc. 'M'	-	Sc. 'M'	5.55	Sc. 'M'	Sc. 'M'	-	8.33	-	Sc. 'M'	Sc. 'M'	11.53			
St. 'M'	-	-	-	-	-	-	-	-	-	-	-	-	St. 'M'	St. 'M'	St. 'M'	13.88	St. 'M'	St. 'M'	St. 'M'	15.38			
Br. 'A'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Br. 'A'	4.54			
Bri. 'A'	-	-	-	-	-	-	-	-	-	-	-	-	-	Bri. 'A'	-	5.00	Bri. 'A'	-	-	4.54			
D.P. 'A'	-	-	-	-	D.P. 'A'	-	-	2.77	-	-	-	-	-	D.P. 'A'	-	5.00	-	D.P. 'A'	D.P. 'A'	9.09			
Lag. 'A'	-	-	-	-	-	-	-	-	-	-	-	-	-	Lag. 'A'	-	5.00	-	-	-	-			

Abbreviations: B.N.- Binucleate Cells, N.D.- Nuclear Disintegration, Con. 'P'- Condensation at Prophase, Br. 'M'- Breakage at Metaphase, C.S.- Chromatid Separation, D. 'M'- Disturbed Metaphase, Sc. 'M'- Scattered Metaphase, St. 'M'- Stickiness at Metaphase, Br. 'A'- Breakinge at Anaphase, Bri. 'A'- Bridge at Anaphase, D.P. 'A'- Disturbed Polarity at Anaphase, Lag. 'A'- Laggard at Anaphase.

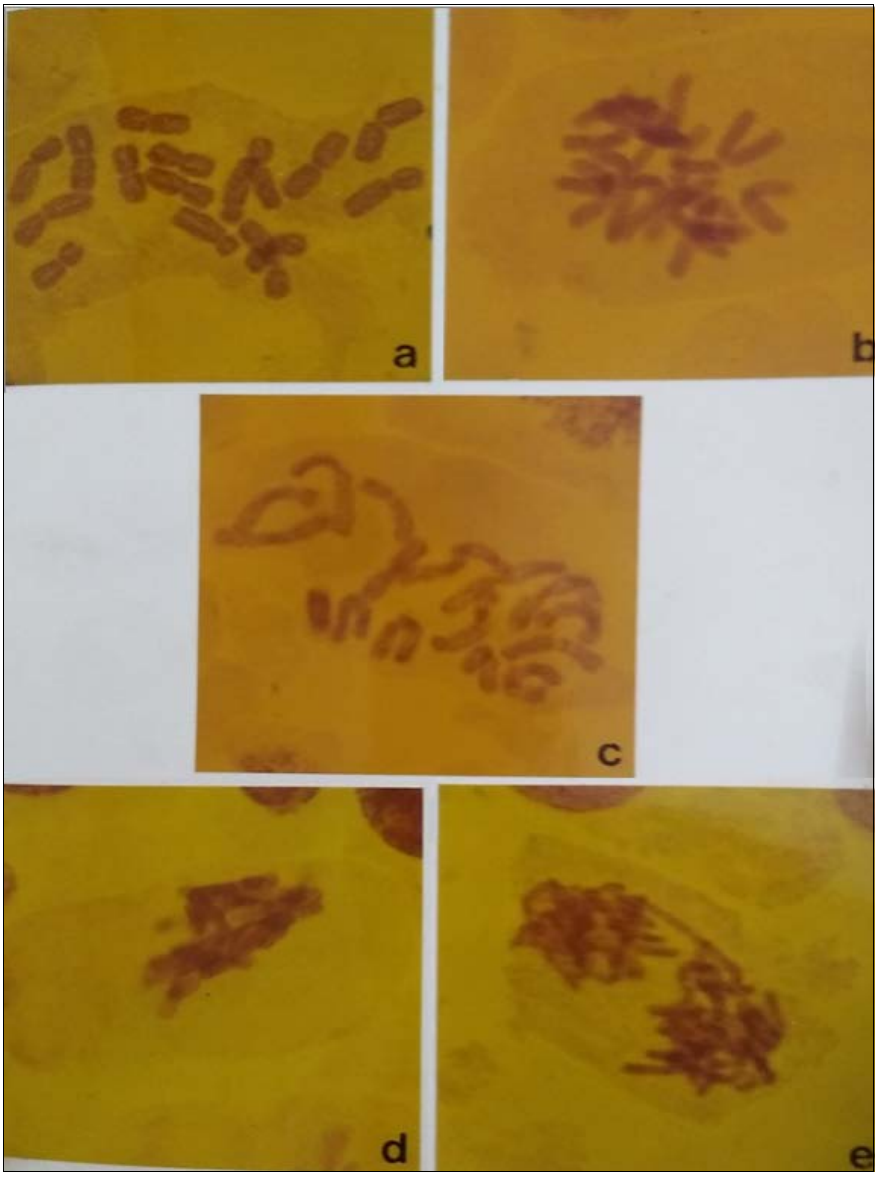

Fig 1: a. Chromatid Separation, b. Disturbed Metaphase, c. Scattered Metaphase, d. Stickiness of Chromosomes, e. Bridge at Anaphase

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