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GENOTOXICITY POTENTIAL OF *CHENOPODIUM ALBUM* L. EXTRACTS IN ROOT TIP CELLS OF *ALLIUM SATIVUM* FROM NORTHERN INDIA

RUPINDERPAL KAUR

Department of Botany, Punjabi University, Patiala, Punjab, India.

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Abstract: Aqueous and acetone leaf extracts of *Chenopodium album* L. were tested for genotoxicity. Treatment of *Allium* roots revealed that these extracts were slightly mitodepressive in nature. Leaf extracts of plants prepared in acetone and aqueous showed significant mitodepression in 24 hours. Aqueous extract showed large 26.37% mitodepression against 11.74% in acetone extract. P/M ration exhibited great variation ranging from 1.97 in the aqueous extract and 5.24 in the acetone extract. Total aberrations induced by leaf extracts at A/T phases, aqueous extract produced 1.39± 0.21 followed by acetone extracts 1.04± 0.23.

Keywords: Genotoxicity, *Chenopodium album* L., *Allium sativum*

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Corresponding Author: MS. RUPINDERPAL KAUR

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INTRODUCTION

A number of naturally occurring carcinogens have been known to be present in diet, beverages and in herbal medicines. Loannides et al., (1981), Ames (1983) etc. have listed a number of carcinogenic agents in human diet . Levan (1948) devised Allium test to monitor genotoxicity of various agents, which was later standardized by Grant (1982) and was later modified by Fiskesjo (1988) and Rank (2003). Allium test has been used to evaluate DNA damages, like chromosomal aberrations and disturbances in the mitotic cycle (Leme and Marin-Morales, 2009). The Allium cepa test is considered extremely efficient for analyzing and monitoring in situ genotoxicity of several substances (Silva et al., 2004). It is known that green plants are primary source of antimutagens as well as natural toxic and genotoxic agents (Plewa and Wagner, 1993). Investigations have shown that some plants used as food or in traditional medicine have mutagenic and genotoxic effects in vitro and in vivo assays (Higashimoto et al., 1993). Allium test has been as a bioindicator of genotoxicity for medicinal plants like Tylophora indica (Saggoo et al., 1991), Ricinus communis (George and Geethamma, 1990), Pterocaulon polystachyum (Knoll et al.,2006) etc.

Many of these plants contain compounds which are known to cause diseases or even death in animals and humans (Evans and Osman,1974 etc.). Many constituents act as natural mutagens and carcinogens (Hartwell and Abbott, 1969). The dietary carcinogens identified are safrole, estragole and related compounds (present in sassafras), hydrazine (mushrooms), furocoumarins (family Umbelliferae) solanine, quinines, pyrrolizine, gossypol, sterculic acid, malvalic acid (Ames,1983).The Allium assay is a widely used and popular for screening large number of chemicals and environmental samples. The aim of present work is to determine the genotoxic potential of Chenopodium album L. so as to minimize possible human health risks.

MATERIALS AND METHODS

Plant material

The plants of Chenopodium album L. for investigation were collected from 10 different districts of Northern India. The voucher specimens are deposited in Herbarium, Department of Botany, Punjabi University, Patiala.

Preparation of plant extracts

Extracts were prepared using fresh leaves of the plants. The various kinds of extracts were prepared as follows.

Aqueous extract:

Fresh leaves were crushed and 5gm of these were extracted in 100ml distilled water for 24hrs. Extract was filtered and centrifuged at 6000rpm for 30min. Supernatant was collected and concentrated in a rotary evaporator. The dried remnant was then dissolved in distilled water to make final volume 100ml.

Solvent extract:

For making solvent extract, 5gm of fresh leaves were extracted in Ethanol or Acetone (100ml) for 48 hrs in rotary shaker. The extract of each part was centrifuged and filtered through Whattman filter paper no. 1 and concentrated in rotary evaporator. The concentrate was then dissolved in distilled water to make final volume 100ml.

Genotoxicity assay:

Allium assay was performed to study the cytotoxic potentials of extracts of Chenopodium album, following the procedure proposed by Fiskesjo (1985) and Rank and Nielsen (1993). For this following steps was undertaken.

Test organism and growing conditions:

Healthy and nearly equal sized bulblets of Allium sativum (2n=16), procured from local market, were used as test organism. Outer scales of the bulblets were removed carefully to expose root primordia. Test tubes filled with tap water were taken and bulblets were placed over the test tubes in such a way, that the lower portion of bulblets was dipped in water. Water in the test tubes was changed every 24 hrs. The bulblets were placed in the above condition for 48hrs at 25 1˚C, in an incubator in dark, till the roots attain the size of approximately 2 cm in length.

Treatment of roots:

The germinating bulbs with fresh healthy roots were placed on different test tubes containing, tap water (negative control), sodium azide (1mg/L as positive control), aqueous extract, acetone extract and ethanol extract of leaves. Bulblets were placed in such a way that only roots were immersed in the test liquids. Three bulbs were used for each treatment.

Fixation of roots:

After 24hrs of the treatment of growing roots with test solutions the bulblets were removed from the treatment set up and roots were harvested and fixed in Conroy's fixative (absolute alcohol: chloroform : glacial acetic acid::6:3:1). After 24hr of fixation the roots were shifted in rectified alcohol and stored under refrigerator till use.

Staining and scoring of slides:

The root tips were hydrolysed in a mixture of 2% Acetocarmine (9 parts) and 1N HCL (1 part) at 50 C for 5 min. Hydrolyzed roots were squashed in fresh drop of acetocarmine and sealed with cover slips.

Microscopic examination:

Temporary mitotic slides prepared from each root tip meristem were scanned taking 8-9 observations of cells at random under microscope. Out of total number of cells observed, the dividing cells were counted and categorized into different groups according to their phases of division. The abnormal cells were classified according to nature of aberrations.

Test conditions for *Allium sativum* genotoxicity test:

1.	Test organism	*Allium sativum*
2.	Parameter observed	Micronuclei in interphase and chromosome aberrations in anaphase-telophase root tip cells
3.	Test condition	Temperature 25±1˚C, light cycle 16h/8h (light/dark)
4.	Replicates	3 plants per extract.
5.	Exposition length	24hr pre treatment in control water, 48 hr of exposition with extracts.
6.	Chemical	Ethanol extract, acetone extract, aqueous extract, tap water (+ve extract), sodium azide (-ve control)

Calculations:- The various calculations were made as follows:

$$\text{Mitotic index} = \frac{\text{Total number of dividing RTCs}}{\text{Total number of observed RTCs}} \times 100$$

$$\text{Percent Mitotic phase} = \frac{\text{Number of cells at mitotic phase}}{\text{Total number of dividing cells observed}} \times 100$$

$$\text{Percentage aberration} = \frac{\text{Total number of abnormal RTCs}}{\text{Total number of observed RTCs}} \times 100$$

$$\text{Percentage micronuclei} = \frac{\text{Total number of cells with micronuclei}}{\text{Total number of observed RTCs}} \times 100$$

RESULTS

Allium roots treated with the tap water i.e. the control showed, by and large, normal cell division with very few abnormalities (Table. 1). Treatment of roots of A. sativum with leaf extracts induced a variety of cytological aberrations, as bridges (Figs. 69, 72, 76, 82), laggards (Fig. 77), vagrant chromosomes (Figs. 73, 75, 83), multipolarity (Figs. 70, 78, 80), micronuclei (Fig. 81), etc. Data regarding to cytological aberrations induced in RTCs due to treatment with various plant extracts are represented in table. 2. Among various leaf extracts, the aqueous extracts induced maximum aberrations i.e. 1.39±0.21. Attempt has been made to use Rank's method (1993) to analyze the aberration at anaphase and telophase. As for as total aberrations induced by leaf extracts at A/T phases aqueous extract produced 1.39±0.21, followed by acetone extracts 1.04±.23. The cytotoxic effect of the aqueous extract was, however, not significant.

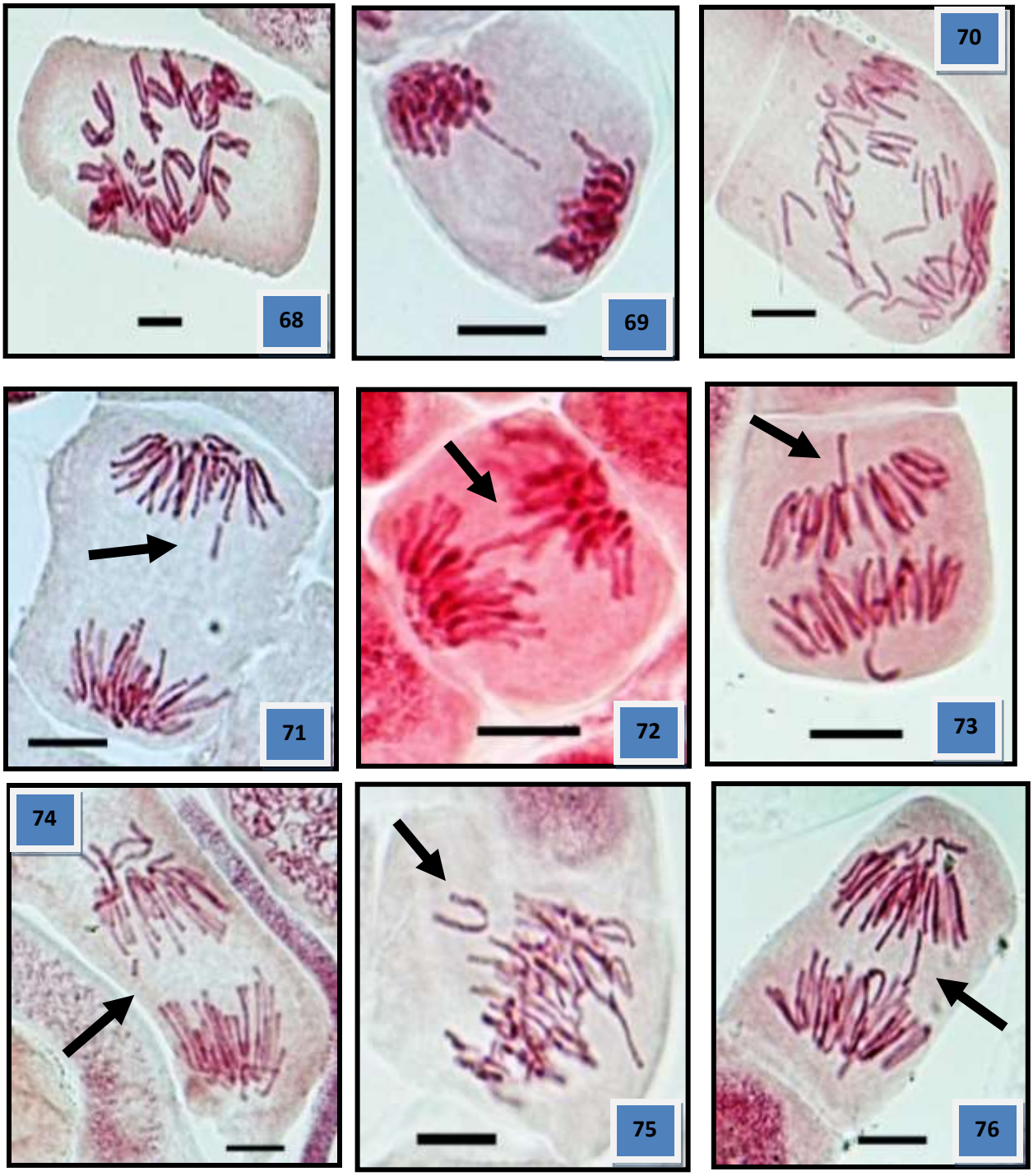
Table 1: Percentage of mitotic phases in RTCs of *Allium sativum* after treatment with different extracts of leaves of *Chenopodium album* L.

S.no	Treatment	Total Cells observed	Total dividing cells	Mitotic index (%±SD)	Mitotic phases (%)				
					Prophase	Metaphase	Anaphase	Telophase	P/M ratio
1.	Tap water	4297	1416	32.95	64.33	16.03	13.48	6.14	4.01
2.	Sodium azide	3876	1153	29.76	68.71	12.22	9.76	9.31	5.62
3.	Leaf extract (acetone)	4099	1192	29.08	78.69	15.01	4.11	2.01	5.24
4.	Leaf extract (aqueous)	4299	1026	24.26	55.65	28.16	11.30	4.87	1.97

Table 2: Total aberrations in RTCs of *Allium cepa* after treatment with Aqueous, Ethanol and Acetone Leaf extract of *Chenopodium album* L.

S.no.	Treatment	Total cells observed	Aberrant cells								Total aberration (%±SD)
			Brg	Lagg	Vag	Pyc	Mp	Mnc	St	Fr	
1.	Tap water	4297	—	—	02	—	—	—	—	—	0.13±.17
2.	Sodium azide	3876	24	14	32	19	17	09	—	38	3.9±2.41
3	Aqueous	4229	02	02	21	—	03	04	01	26	1.39±.21
5.	Acetone	4099	03	01	12	—	06	—	—	21	1.04±.23

Aberrations: Brg- bridges, Lagg-laggard, Vag-vagarant, Pyc-pycnosis, Mp-multipolarity, Mnc-micronuclei, St-stickiness, Fr-fragment.

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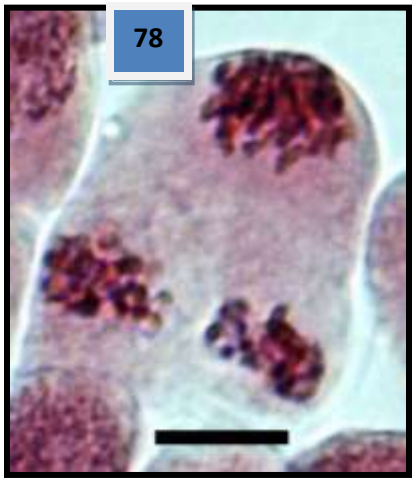
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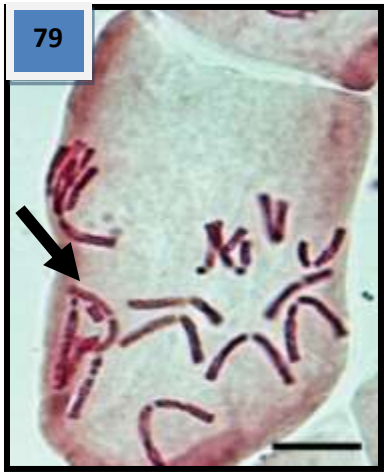
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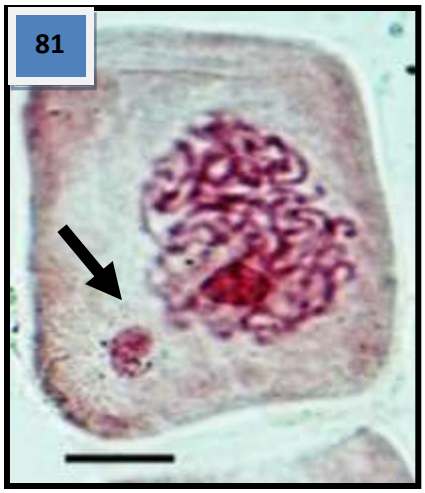
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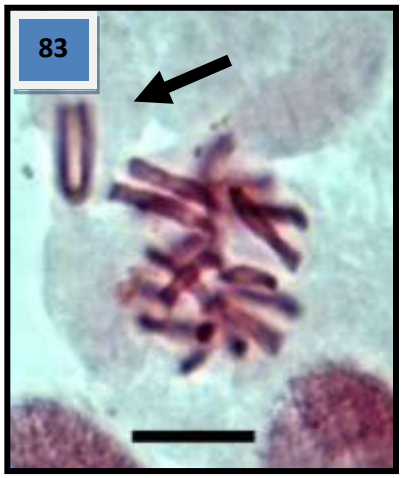
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DISCUSSION

The Allium assay has been used to investigate the genotoxic effect of the various types of leaf extracts of Chenopodium album. Allium roots were exposed for 24 h with various types of leaf extracts. The present study revealed that P/M ratio of treated roots was higher in acetone extract and less in aqueous extract as compared to control (Fig. C). A progressive decrease in mitotic index of Allium sativum root meristem cells was obvious after the treatment with water, ethanol and aqueous extract as compared with their respective control. The induction of mitotic abnormalities was common after treatments with a variety of compounds as previously recorded by Amer and Farah (1983) etc. The inhibition of mitotic index and reduction in frequency of mitotic phases indicate that the treatment interfere with the normal sequence of cell division, thus preventing the number of cells entering prophase stage. A lowered cell division index suggests an inhibitory effect at the interphase stage (Ene-obong, 1995). Epel (1963) and Jain and Sarbhoy (1987) reported that the rate of mitosis was clearly related to mitosis and was closely related to resultant level of ATP. Hoffman –Berling (1954) stated that chromosome move under the influence of ATP. The low mitotic index among treatments and accumulation of cells at prophase with corresponding decrease in other phases would tend to suggest that the treatments interfered with the respiratory pathway resulting in low ATP level. The treatment of Allium roots with various extracts of leaves induced a variety of cytological aberrations ranged between 0.13±.17—1.39±.21 of total cells (Fig. D). It is possible that phenolic compounds present in leaf extract have interfered with cell division. The reduction in the rate of mitotic cell division by allelochemiclas present in other plants has been reported by other workers. Both clastogenic and non-clastogenic aberrations were observed, but are at significant level.). Clastogenic aberrations have been attributed to induction of break at DNA level by treatment with reagents. Production of bridges at anaphase and telophase can be attributed to breakage and reunion of chromatids or subchromatids (Dempong and Maxwell, 1973). The induction of similar aberrations by plants has already been recorded (Saggoo et al., 1991 etc.). Non clastogenic aberrations induced by treatment with plant extracts include stickiness of chromosomes, multipolarity, vagrant chromosomes. These aberrations are also referred as physiological aberrations (Nagpal and Grover, 1994). Aberrations in chromosomal proteins may change the surface nucleoprotein configuration resulting in stickiness and or these may abnormal spindle activity (Kihlman, 1955).

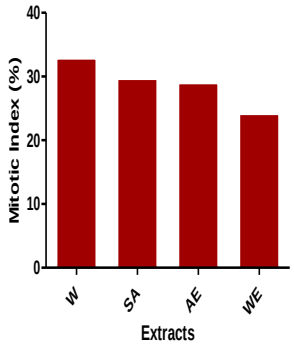

Fig. B Histogram showing effect of different extracts on Mitotic index of RTCs of *Allium sativum*

W- Water (Control), SA- Sodium azide, AE- Acetone extract, W (aqueous) extract

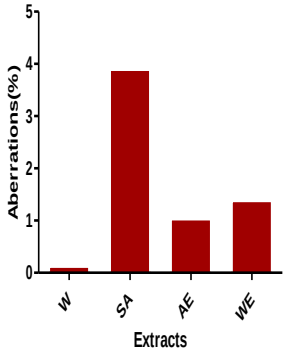

Fig.D Histogram showing total aberration produced by treatment of roots with various leaf extracts.

W- Tap water, SA- Sodium azide, AE-Acetone extract, WE-Water (aqueous) extract

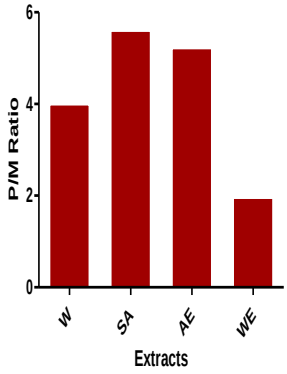

Fig. C Histgram showing effect of different leaf extracts on P/M ratio in RTCs

W- water (control), SA- Sodium azide, AE-Acetone extract, WE- Water (aqueous extract).

CONCLUSION

From the data it is observed that Plant extracts showed obvious effects on Allium roots. The P/M ratio varied from 1.97 in aqueous extract and 5.24 in acetone extract against 4.01 in the control roots. All the extracts showed mild mitodepressive activity. Mitotic index varies from 24.26 in aqueous extract and 29.08 in acetone extract. Aqueous extract showed large 26.37% mitodepression against 11.74% in acetone extract. A very low amount of cytological aberrations are found which insignificant. Since chemical analysis of plant extracts was not studied, it is not possible to pinpoint the causal factor of such a behavior.

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