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A study on cytotoxic and anticytotoxic effects on *Allium cepa* L. root tips treated with Gamma irradiated *Amaranthus dubius* Mart. ex. Thell. aqueous seed extracts

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Abstract

The present investigation appertains about the *Amaranthus dubius* Mart. Ex Thell. Var (Co1) dried seeds exposure under gamma radiation at different doses (50,100,150 & 200 Gray) and the effect of gamma irradiated seed powder on somatic chromosomes of *Allium cepa* L. The seed powder was taken at various concentrations such as 5%, 7% & 9%. For each concentration, the treatment time has been confined to 12hrs, 18hrs and 24hrs respectively. Different types of chromosomal aberrations such as chromosomal fragments, chromosomal bridges, stickiness, binucleate cells and laggards has been observed frequently in the treated root tips of *Allium cepa* L. The onion root tip assay has been used to determine the mitotic index, abnormalities in the chromosomes and hindrances in the mitotic cell cycle. Thus the present work concludes that the acute and chronic effects of gamma radiation on *Amaranthus dubius* seeds consequently targeted the mitosis index and chromosomal aberrations of the *A. cepa* L. cells,depending on potency and time span.

Key words : *Amaranthus dubius*, *Allium cepa*, gamma radiation, mitotic index, chromosomal aberrations.

Amaranthus dubius Mart. Ex. Thell, Amarantaceae family is a short lived perennial crop which is widely distributed in the temperate and tropical regions[11,13]. *A. dubius* and *A. tricolor* seeds containhigh oil content with abundant fat-soluble vitamin E tocotrienol possessinganticancerous, hypocholesterolemic and neuroprotective properties[5,15,18]. In general, physical mutagens are used for plant mutation breeding and among various physical mutagenic source, gamma radiation are fastest, reliable and high-frequency electromagnetic radiation with deep penetrating potential which damagesthe healthy cells[4,10]. The free radicals

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produced through gamma radiation targets the plant cell components leading to altered cellular structures and modified metabolic functions[7,8,17]. The mutagenic effect of gamma radiation influences mitotic indices (MI) of the plant cells. Reduction in mitotic indices may be due to hindrance in G2 phase of the cell cycle. Chromosomal aberrations are genetic damages which is due to various mutagens[6]. The frequency of cell distortion in plants caused by different mutagens and mutagenic doses can be effectively assessed through cytological studies[2]. In the present research work the effect of gamma irradiation with potent gamma source Cobalt-60 at four doses 50,100,150 & 200 Gy on the seeds of *A. dubius* has been used for cytogenetic studies and the treated seeds aqueous extracts on the root tips of *Allium cepa* L. somatic chromosomes at three different concentrations and duration has been analysed along with control extracts. Through *A. cepa* assay study, the scoring of cells and data analysis for mitotic index and chromosomal aberrations was carried out to assess the effect of gamma radiation on seeds which caused various chromosomal abnormalities and reduction in mitotic indices in cells based on time intervals, concentrations and dosage.

Collection and processing of seeds :

Seeds of *Amaranthus dubius* were purchased from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu. The seeds were exposed to gamma rays at different doses such as 50 Gy, 100 Gy, 150 Gy, and 200 Gy in the Indian Council of Agricultural Research (ICAR), Bengaluru, Karnataka, India. Seeds treated at different doses were grounded into powder, sieved, stored in air tight containers and used for cytological studies (Fig. 1).

Allium cepa assay :

Healthy, equal-sized *Allium cepa* bulbs were selected as a study material. It is one of the best biological models for cytotoxicity studies. The loose and dried outer scales were removed and for the fast emergence of root tips, the root areas were scraped. Each onion bulb was placed on the top of the test tube filled with distilled water. To avoid the fungal attack, the distilled water in the test tubes was changed periodically[12] (Fig. 2).

Initiation of onion roots :

The onion root tips were allowed to grow for 2 to 3 days till the root reached the length of about one inch. The assay was performed under constant room temperature and kept away from direct sunlight (Fig. 3).

Fixative :

The onion bulbs with root tips of about one inch in length were washed under running tap water to remove the dirt adhering to the root tips. The root tips were removed from the root primordial part using a sharp blade and stored in Farmer's fluid (Glacial acetic acid: Ethanol) at a 1:3 ratio for 24 hours. After 24 hours, the tips were preserved in 70% ethanol for further use.

Preparation of seed extract :

The seeds of *Amaranthus dubius* were purchased from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu.

The dried irradiated and non-irradiated seeds were grounded with the aid of mortar and pestle to obtain seed powder and stored in sterilized airtight containers for further use. The aqueous extracts were prepared by adding *Amaranthus dubius* seed powder in distilled water, for a respective concentration of the seed extract (5%, 7%, and 9%). This seed extract was used as the test solution for treatments in roots of onion bulbs[12].

Fig. 1. Gamma Chamber 5000

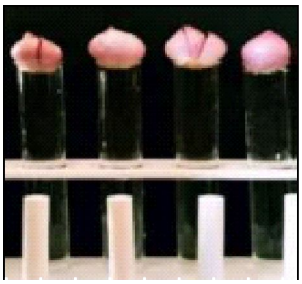

Fig. 2. Bulbs placed in Distilled water

Fig. 3. Bulbs with One-inch root length

Fig. 4. Dried leaves of *Amaranthus dubius*

Fig. 5. *Amaranthus dubius* leaf powder

LIST OF CHROMOSOMAL ABERRATIONS

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18

Explanation of Figures (Fig 1-18)	Conc.	Hours	Doses (in Gy)
Cell with 16 chromosomes (control)	-	-	-
Anaphase with succession and precessions	7%	12	150
Chromosomal bridges	7%	24	50
Stickiness during late anaphase and delayed movement of 2 chromosome	5%	18	150
Long cell exhibiting stickiness	9%	12	100
Sticky metaphase	5%	18	200
Anaphase with irregular clumping of chromosomes	9%	24	150
Multiple chromosomal bridges	9%	12	200
Binucleate cell	5%	18	150
Stickiness during anaphase	5%	12	200
Laggard	9%	12	50
Single chromosomal bridge at anaphase	7%	18	50
Chromosomal bridges at anaphase	5%	18	50
C-metaphase exhibiting partial stickiness	9%	24	150
Metaphase with chromosomes in horizontal and vertical fashion	7%	24	200
Cells with varying amount of cytoplasm	5%	18	100
Promiscuous movement of chromosomes	9%	12	50
Fragmentation with more than 16 chromosomes	5%	24	150

Squash method[1] *:*

Somatic chromosome studies were done in *Allium cepa* root tips treated with the aqueous extracts of *Amaranthus dubius* seed powder of concentrations 5%, 7%, 9% and maintained for an appropriate time of 12 hours, 18 hours & 24 hours. The treated and control root tips of onion were cut and used for squash preparation. The non-irradiated aqueous seed extract was also experimented with to have a comparative study over various concentrations of stock solution (Fig. 4,5). The root tips were hydrolysed with 1N Hydrochloric acid for one minute and then washed with distilled water. The root tips were stained with acetocarmine and gently heated for about one minute. The tips were allowed to remain in the stain for 20 minutes in a watch glass. Now,

Table-1. Effect of gamma-irradiated aqueous seed extracts of *Amaranthus dubius* on *Allium cepa* root cells in 5%, 7% & 9% concentrations at 12 hours duration.

Treatment	No. of dividing cells	Mitotic index (%)	Chromosomal aberrations observed					Total No. of aber-rated cells	Aberr-ation percen-tage
			Stic-kiness	Chromo-somal bridges	Lagga-rds	Chromo-somal frag-ments	Binuc-leate cells		
(5% conc)									
Control	201	67	-	-	-	-	-	-	-
50 Gray	192	64	13	-	-	5	11	29	10
100 Gray	185	62	10	-	2	5	17	34	11
150 Gray	169	56	6	8	13	15	25	67	22
200 Gray	148	49	9	7	21	17	44	98	33
(7% conc)									
Control	179	59	-	-	-	-	-	-	-
50 Gray	159	53	10	6	11	12	21	60	20
100 Gray	151	50	11	2	11	19	22	65	22
150 Gray	120	40	13	9	21	18	24	85	28
200 Gray	105	35	16	8	28	24	36	112	37
(9% conc)									
Control	143	47	-	-	-	-	-	-	-
50 Gray	107	35	10	12	18	16	23	79	26
100 Gray	97	32	14	15	11	20	26	86	29
150 Gray	89	29	17	12	25	23	18	95	31
200 Gray	77	26	18	16	30	27	29	120	40

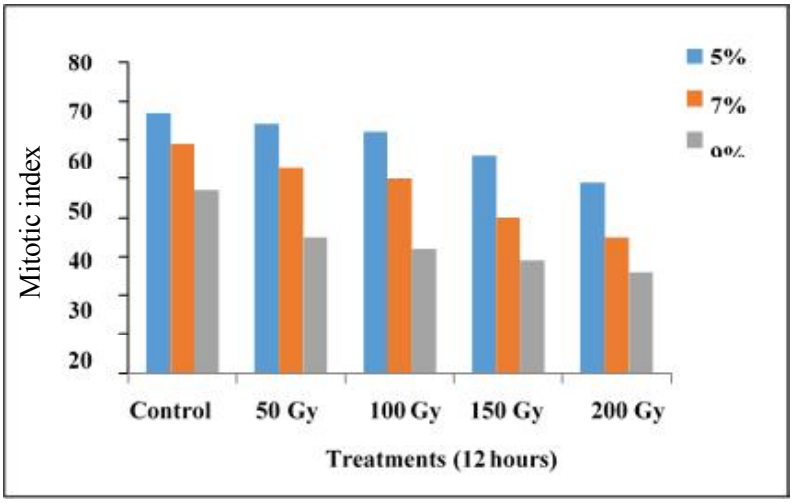

Fig. 6. Effect of gamma irradiated aqueous seed extracts of *Amaranthus dubius* mitotic index on *Allium cepa* root cells

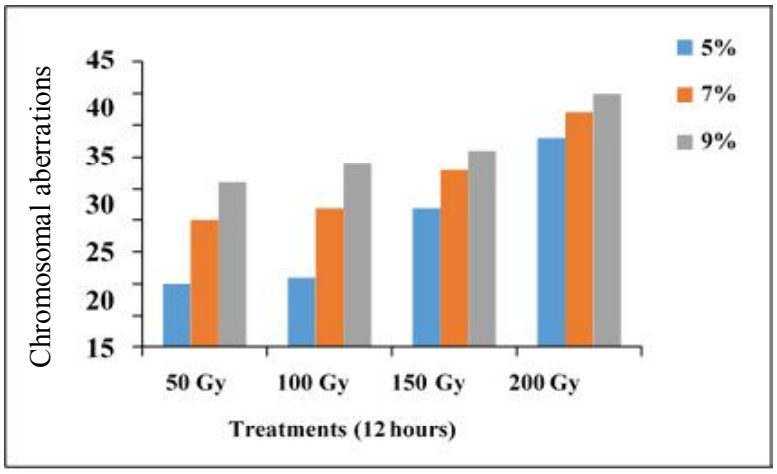

Fig. 7. Effect of gamma irradiated aqueous seed extracts of *Amaranthus dubius* chromosomal aberrations on *Allium cepa* root cells

Table-2. Effect of gamma-irradiated aqueous seed extracts of *Amaranthus dubius* on *Allium cepa* root cells in 5%, 7% & 9% concentrations at 18 hours duration

Treatment	No. of dividing cells	Mitotic index (%)	Chromosomal aberrations observed					Total No. of aberrated cells	Aberration percentage
			Stickiness	Chromosomal bridges	Laggards	Chromosomal fragments	Binucleate cells		
(5% conc)									
Control	195	65	-	-	-	-	-	-	-
50 Gray	183	61	6	8	11	14	29	68	23
100 Gray	173	57	7	9	18	20	28	82	27
150 Gray	145	48	10	14	20	18	35	97	32
200 Gray	126	42	13	16	27	28	44	128	43
(7% conc)									
Control	137	46	-	-	-	-	-	-	-
50 Gray	129	43	10	13	21	27	25	96	32
100 Gray	120	40	13	16	22	31	20	102	34
150 Gray	112	37	18	23	19	24	31	115	38
200 Gray	101	34	21	22	26	37	28	134	45
(9% conc)									
Control	101	34	-	-	-	-	-	-	-
50 Gray	96	32	17	19	26	30	16	108	36
100 Gray	92	30	31	27	23	28	13	122	41
150 Gray	74	24	27	31	24	30	26	138	46
200 Gray	52	17	29	32	35	41	22	159	53

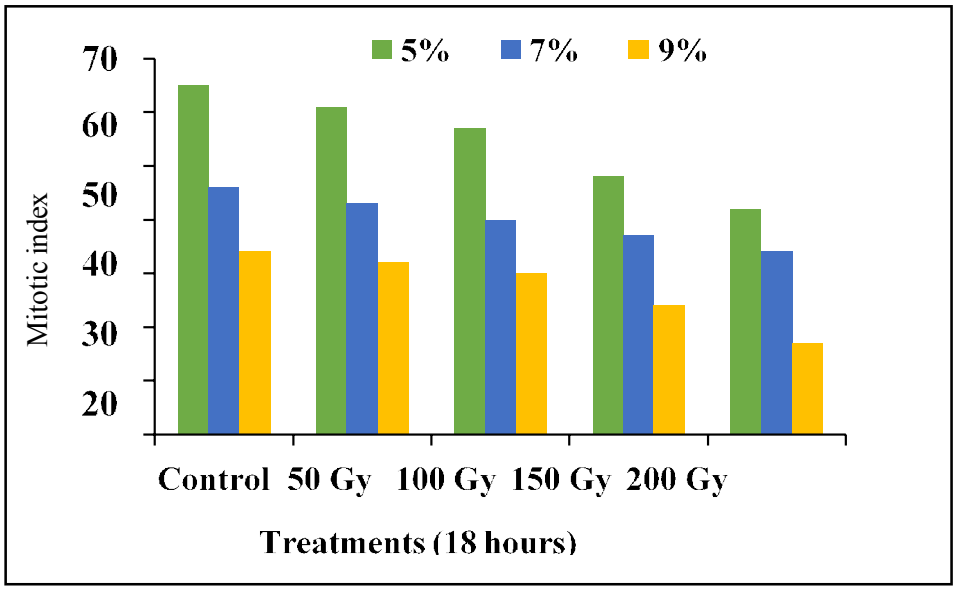

Fig. 8. Effect of gamma irradiated aqueous seed extracts of *Amaranthus dubius* mitotic index on *Allium cepa* root cells

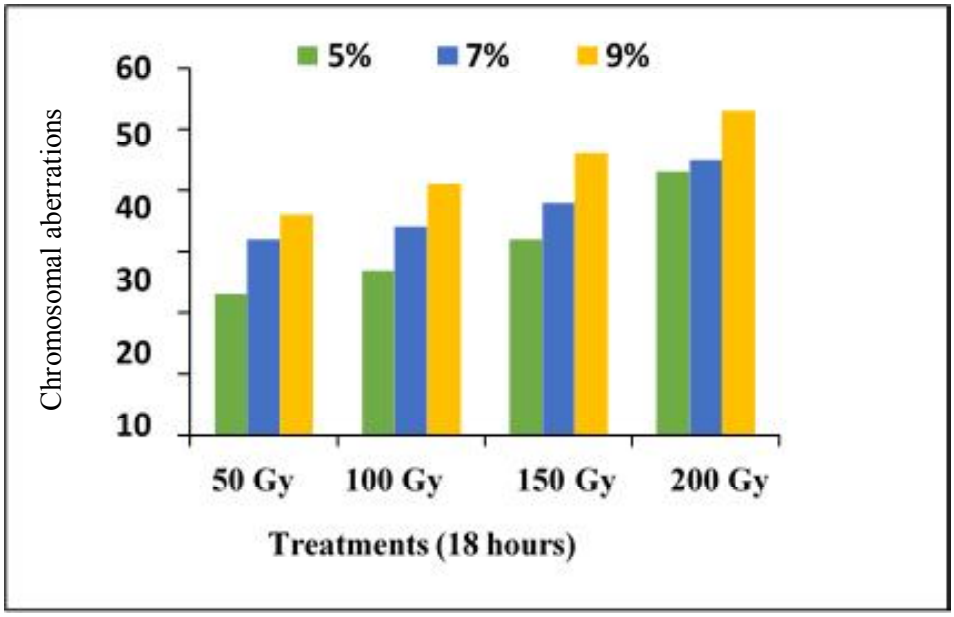

Fig. 9. Effect of gamma irradiated aqueous seed extracts of *Amaranthus dubius* chromosomal aberrations on *Allium cepa* root cells

Table-3. Effect of gamma-irradiated aqueous seed extracts of *Amaranthus dubius* on *Allium cepa* root cells in 5%, 7% & 9% concentrations at 24 hours duration

Treatment	No. of dividing cells	Mitotic index (%)	Chromosomal aberrations observed					Total No. of aberrated cells	Aberration percentage
			Stickiness	Chromosomal bridges	Laggards	Chromosomal fragments	Binucleate cells		
(5% conc)									
Control	181	60	-	-	-	-	-	-	-
50 Gray	164	55	11	19	20	25	31	106	35
100 Gray	144	48	12	16	21	33	32	114	38
150 Gray	129	43	25	29	34	32	42	162	54
200 Gray	111	37	27	24	35	36	46	168	56
(7% conc)									
Control	125	42	-	-	-	-	-	-	-
50 Gray	120	40	17	22	25	33	28	125	42
100 Gray	119	39	18	23	28	32	29	130	43
150 Gray	94	31	28	25	35	40	39	167	56
200 Gray	84	28	27	33	37	41	38	176	59
(9% conc)									
Control	91	30	-	-	-	-	-	-	-
50 Gray	81	27	27	29	39	43	26	164	55
100 Gray	77	26	31	35	38	40	30	174	58
150 Gray	58	19	34	36	40	42	32	184	61
200 Gray	42	14	41	37	39	43	28	188	63

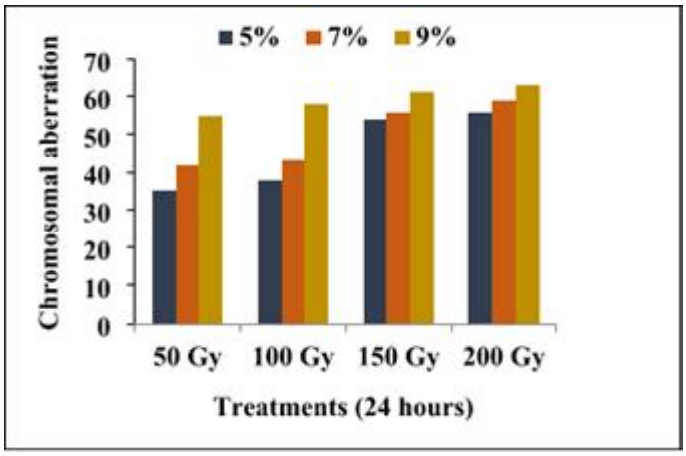

Fig. 10. Effect of gamma irradiated aqueous seed extracts of *Amaranthus dubius* mitotic index on *Allium cepa* root cells

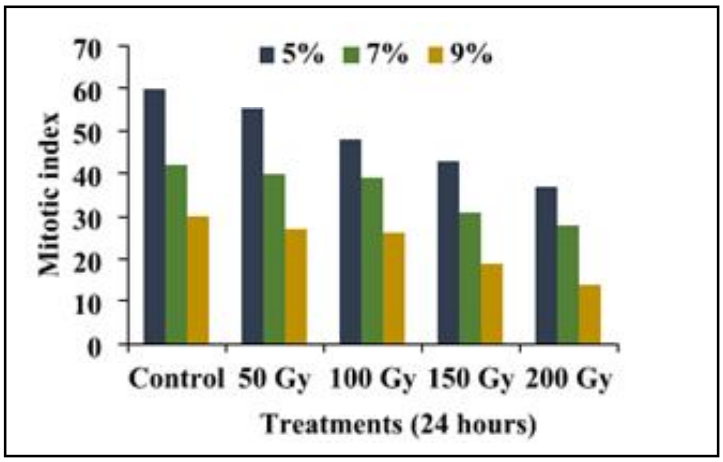

Fig. 11. Effect of gamma irradiated aqueous seed extracts of *Amaranthus dubius* chromosomal aberrations on *Allium cepa* root cells

a root tip is taken and placed on a clean glass slide, a drop of 45% glacial acetic acid is added and a cover slip is placed on it. It is then gently tapped for equal spreading of cells. The prepared slides were viewed under the microscope for mitotic stages and chromosomal aberrations.

Scoring of cells and Data analysis[12] *:*

Approximately, cells per treatment and control were analysed to score the frequency of mitotic index (MI) and chromosomal aberrations. The prepared slides were viewed under a monocular light microscope (Magnus HSA) using the 45X objective. Photographs of some representative stages were taken.

Mitotic index is the ratio between the number of dividing cells and the total number of cells

$$\text{Mitotic index (MI)} = \frac{\text{No. of dividing cells}}{\text{Total No. of cells}} \text{ X } 100$$

The percentage of chromosomal aberrations is calculated by dividing the number of aberrated cells by the total number of cells.

$$\text{Aberration percentage (\%)} = \frac{\text{No. of aberrated cell}}{\text{Total No. of cells}} \text{ X } 100$$

Mitotic indices (MI):

The aqueous seed extracts of *Amaranthus dubius* of gamma-irradiated and non-irradiated root tips mitotic indices were analysed based on the time intervals and concentrations. Maximum mitotic index percentage was observed in non-irradiated root tips compared to gamma-irradiated root tips & a gradual decrease in the mitotic indices in gamma-irradiated root tips based on the treatments. After 12 hours of treatment, maximum mitotic indices were observed followed by, a gradual decrease in 18 hours and 24 hours of gamma-irradiated and non-irradiated root tips. Root tips at 5% concentration showed the highest mitotic indices at 12 hours, 18 hours, and 24 hours in

gamma-irradiated and non-irradiated treatments. Progressive decline in 7% concentration, a gradual decrease in mitotic indices of gamma-irradiated and non-irradiated treatments at 12 hours, 18 hours, and 24 hours respectively. Minimum mitotic index was observed in 9% concentration at 18 hours and 24 hours in gamma-irradiated and non-irradiated root tips (Figs. 6,8,10). According to Kumar *et al*.,[9] observed usual mitotic division in non-irradiated roots tips of *Allium cepa* and decreased dividing cells percentage after gamma treatment. Eroğlu *et al*.,[3] remarked that increasing gamma doses (50, 100, 150, 200, 250, and 300 Gy) suppressed the mitotic index in root tip meristem of *Hordeum vulgare* L. Therefore in the present study, a dose-dependent increase in the mitotic indices was observed in gamma-irradiated and non-irradiated root tips in all three concentrations (5%, 7%, and 9%) and all three durations (12 hours, 18 hours, and 24 hours).Low gamma doses enhanced mitotic indices percentage in the root tips. In controversy; high gamma doses reduced mitotic indices percentage.

Chromosomal aberrations (CA):

The aqueous seed extracts of *Amaranthus dubius* exposed to gamma irradiations (50 Gy, 100 Gy, 150 Gy, 200 Gy) and non-irradiated root tips showed different types of chromosomal abnormalities at 12 hours, 18 hours, and 24 hours. Chromosomal aberrations were observed in 5%, 7% and 9% concentrations. Based upon the time duration, common types of chromosomal abnormalities like chromosomal fragments, laggards, chromosomal bridges, stickiness, c-metaphase, ghost cells, and binucleate cells were observed at high concentrations. At 12 hours duration, aqueous seed extracts of non-irradiated and gamma-irradiated root tips showed ghost cells, chromosomal fragments, elongated cells, and binucleate cells. In 18 hours and 24 hours duration, the gamma-irradiated root tips showed chromosomal bridges, stickiness, c-metaphase, and laggard more frequently. The chromosomal aberration percentage was found to be high at 7% and 9% concentrations at 12 hours, 18 hours, and 24 hours duration. Minimum aberration percentage was observed in 5% concentration in 12, 18, and 24 hours. The highest aberration percentages were observed in 200 Gy and 150 Gy followed by, a gradual decrease in 50 Gy and 100 Gy of gamma-irradiated root tips. Vaijapurkar *et al*.,[16] stated that when onions were exposed to low gamma doses 50 cGy (0.5 Gy) to 2000 cGy (20 Gy), chromosomal abnormalities in the form of decreased cell division within the cell were observed, and increased micronuclei percentage were observed between 200 cGy (2 Gy) to 400 cGy (4Gy) (Table 1, 2 & 3) (Figs. 7,9,11). According to Ramesh and Verma[14] the mitotic index and chromosomal aberration percentage in *Phlox drummondii* after gamma treatment produced maximum dividing cells in 5kR, minimum in 25kR. In controversy, maximum chromosomal aberrations in 25kR and minimum in 5kR were observed. Therefore in the present study, a dose-dependent increase in the aberrations was observed in gamma-irradiated root tips in all three concentrations 5%, 7%, and 9%, and all three durations 12 hours, 18 hours, and 24 hours.Low gamma doses minimized the chromosomal aberration percentage in the root tips. In contrast; high gamma doses increased the chromosomal aberration percentage (Figs 6-11).

The present study confirms that the

different cytological changes due to gamma-irradiated (50, 100, 150, and 200 Gy) aqueous seed extracts of *Amaranthus dubius* at three different concentrations (5%, 7%, and 9%) at 12 hours, 18 hours, and 24 hours duration on *Allium cepa* root tip cells showed chromosomal abnormalities in the dividing somatic root tip cells. Commonly seen chromosomal abnormalities includes binucleate cells, chromosomal fragments, chromosomal bridges, laggards, stickiness, and ghost cells. The binucleate cells laggards and ghost cells were frequently observed in the dividing cells. Chromosomal fragments, chromosomal bridges, and stickiness were abundantly observed in the higher concentrations (7% and 9%) in all three durations (12, 18, and 24 hours). At 7% and 9% concentrations at 12 hours, 18 hours, and 24 hours showed the highest chromosomal aberration percentage. A moderate aberration percentage was observed in 5% concentration at 12 hours, 18 hours, and 24 hours durations. Thus the study concludes that a progressive mitotic indices was calculated in the onion root tips at limited time span and doses and aberration percentage intensified when the concentration in all three duration and level of doses increased.

We would like to thank TamilNadu Agricultural University (TNAU), Coimbatore, Tamil Nadu for providing the seeds and our special thanks to Indian Council of Agricultural Research (ICAR), Bengaluru, Karnataka for providing the gamma radiation facility.

Conflict of interest

The authors declare no conflict of interest.

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