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Cytogenetic Effects of Radiation from Projector on Meristematic Cells of *Allium Cepa* (Onions) Root

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ABSTRACT: The objective of this study is to evaluate the cytogenetic consequences of exposing root tips of *Allium cepa* (onion) to varying distances and durations of radiation from the projector and treatment with sodium azide and distilled water using standard methods. The sodium azide and distilled water served as positive and negative controls respectively. Results obtained in this study revealed that radiations from the projector induced eleven chromosomal aberrations which included; binucleate cells, sticky chromosomes, vacuolated cells, star metaphase, bride chromosome, vagrant chromosome, faculty polarity, C-mitosis, spindle fibre disturbance, ghost cells, and fragmented chromosomes. This suggests that radiation from the projector poses danger to genetic systems. The higher mitotic index of irradiated onion root tip cells compared to negative control groups indicates that radiation from the projector exhibited a promontory effect on cell division. The findings in this study revealed that exposing cells to radiation beyond 20cm from projector reduced its potencies to induce aberrations as well as distortion of mitotic cell division cycles irrespective of the duration of exposure. This suggests that the genotoxic effects of radiations from a projector depend more on distance than the duration of exposure.

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Radiation according to Usikalu *et al.* (2018) is the transfer of energy in form of rays, particles, or waves capable of penetrating various materials. Chandel *et al.* (2019) reported that a ray will be tagged menacing if it has the potential to penetrate living tissue and link up with molecules in it. The wavelength and frequency of a ray according to Kumar *et al.* (2020) determine the extent of damage it can do to living systems. Xavier *et al.* (2021) grouped the sources of electromagnetic radiations in the environment into natural or man-made. According to Usikalu *et al.* (2018), sunlight and radioactive substances coming from the earth's crust are natural sources of radiation while Bolsunovsky *et al.* (2018) and Usikalu *et al.* (2014) implicated nuclear energy, X-rays, diagnostic and therapeutic radiochemicals as the major artificial sources of radiations into the environment. Radiations coming from projectors according to Nizhelska *et al.* (2020) and Otitoju *et al.* (2012) belong to the very low-frequency range of the electromagnetic spectrum. Projectors have been useful for visual presentations in classrooms or corporate meeting rooms. Duration of presentations and distances maintained from the projector varies among presenters and operators. The possible risks associated with radiations coming from the machine on genetic systems have not gained the required attention Aweda *et al.* (2010). The dangers associated with projector radiation should be investigated to compare its pros and cons (Gupta *et al.*, 2020). Therefore, it is pertinent to study the effect of radiation from the projector on biological systems using the *Allium cepa* bioassay. *Allium cepa* bioassay provides feasible, affordable, and infallible method of assessing the hazardous extent of an agent (Akinboro *et al*., 2021; Alege, 2016). *Allium cepa* test system

exhibits excellent agreement with other test systems including mammals (Srivastava and Singh, 2020). Fiskesjö (1985) supported this by stating that data from the *Allium cepa* test agreed perfectly well with mammalian test systems. Adroyic *et al.* (2021) reported that chromosomes of onion cells lend themselves well to the assessment of environmental pollutants because they possess few and comparatively large chromosomes, which allow easy identification of chromosome damages. Kumar *et al.* (2020) reported cytogenetic consequences of 900 MHz and 1800 MHz radiations from the electromagnetic field using *Allium cepa* root meristematic cells. Xavier *et al.* (2021) recommended the use of *Allium cepa* meristematic cells for monitoring the level of damage caused by ionization and non-ionization radiations. Abu *et al.* (2015) affirmed the suitability of the *Allium cepa* test for genotoxic assessment of a wide range of chemical and physical agents. According to Bonciu *et al.* (2018) data from the mitotic index, percentages of cells in each mitotic phase as well as different chromosomal abnormalities induced by chemical or physical agents on *Allium cepa* meristems are valuable cytological parameters for assessing their genotoxic abilities. Kumar *et al.* (2020) reported that studies on cytotoxic and DNA damaging effects of electromagnetic field radiations on genetic systems have not received the required attention. Given this, the study aimed to evaluate the cytogenetic consequences of exposing root tips of *Allium cepa* (Onion) to varying distances and durations of radiation from the projector.

MATERIALS AND METHODS

Establishment of Onion roots: Fresh onion bulbs ranging in diameter from 5.5 to 7.5cm were dissected transversely. The upper portions were thrown away while the lower parts containing the reduced stems were allowed to touch the water in beakers for root development. To maintain uniform genotypes, the dissected onions were allowed to grow into several plants (sets). Onion bulbs that produced not less than five small onion plants (sets) were selected while those with root tips measuring up to 1cm in length were considered for further study.

Irradiation of Root Tips: Roots of the onion bulbs were then uninterruptedly exposed to radiation from the projector (DELL 121 Os) at distances and durations given in table 1 according to the methods outlined by Chandel *et al.* (2019) with slight modifications. The irradiations of onion roots were done in a dark room to avoid exposing the roots to radiations from other sources.

Table 1: Distances and Durations of Exposure of *Allium cepa* root tip cells to radiations from the projector

S/n	Treatments
1	Sodium Azide
2	Distilled Water
3	10cm/1hr
4	10cm/30mins
5	20cm/1hr
6	20cm/30mins
7	30cm/1hr
8	30cm/30mins
9	40cm/1hr
10	40cm/30mins
11	50cm/1hr
12	50cm/30mins

For this study, root tips treated with sodium azide (a standard mutagen) and distilled water served as positive and negative controls respectively. The treated roots were harvested into vials between 7:30 am to 9:30 am Central African Time (CAT). The methods of Akinyele (2007) modified by Alege (2016) were adopted for the cytogenetic study.

Fixation: After harvesting the onion roots, each root was transferred into the specimen vial containing the fixative. The fixative used in this study comprised of a mixture of glacial acetic acid and absolute ethanol in the ratio of 1:3 respectively. The samples were then kept in the freezer for a minimum of 24 hours. Fixing the samples in this condition was meant to preserve them in their natural condition.

Hydrolysis: The root tips were then removed from the fixatives after 24 hours, washed in distilled water for 3 minutes before hydrolysis. Hydrolysis of the fixed root tips was carried out using 10% hydrochloric acid solution in the water bath at 60^{o}C for 10 minutes. Hydrolysis was necessary to soften the root tips for easy squashing.

Staining and Squashing: Hydrolyzed roots were washed with distilled water and each root tip was then placed on a separate filter paper to drain excess water from them. The meristematic regions (milky-white region) were then cut off using a sharp clean razor blade and transferred onto clean glass slides. Two drops of aceto-orcein stain were then added to the meristematic tips on the slides. These slides were then covered with coverslips. Excess stain was drained from the slide by placing the slide between a folded filter paper and pressing firmly and gently. To squash the cells into a thin layer, the coverslips were tapped with the broader end of a cylindrical search needle. When necessary, the slides were quickly passed over a spirit lamb to improve the staining of chromosomes. Five slides were prepared for each treatment for chromosome observation.

Chromosome Observation: The prepared slides were then observed under the light microscope at 4X, 10X, 40X, and 100X objectives. Photomicrographs of normal mitotic division stages and aberrant cells were taken at the 100X objective.

Data Analysis: Twenty counts each were taken from each of the ten prepared slides per treatment. These counts included the total number of dividing cells, number of cells at interphase, number of cells at prophase, number of cells at metaphase, number of cells at anaphase, number of cells at telophase, and number of aberrant cells classified according to the types of aberrations. Data obtained for these attributes were subjected to Analysis of Variance (ANOVA) and significant means were separated using Duncan Multiple Range Test (DMRT) using Post hoc test with SPSS version 23. The Mitotic Index (MI) and Percentage of Aberrant Cells (PAC) for the treated cells and controls were calculated using the formulae outlined by Malode *et al.* (2012) and presented in bar charts.

$$MI = \frac{Total\ number\ of\ dividing\ cell}{Total\ numberof\ cells\ examined}\ X\ 100$$

$$\text{Cells}\ (PAC) = \frac{Total\ number\ of\ abnormal\ cells}{Total\ numberof\ cells\ examined}\ X\ 100$$

Where MI = Mitotic Index; PAC = Percentage of Aberrant

The numbers of prophase, metaphase, anaphase, and telophase were summed to represent the total number of dividing cells.

RESULTS AND DISCUSSION

Table 2 and plate I represent the effects of radiation from the projector on the mitotic cells of *Allium cepa* root tip cells. As shown in Table 2, five out of the six cytological stages showed significant differences among the treatments. The attributes with significant differences included the number of interphases, number of prophases, number of anaphases, number of telophases, and the total number of cells, while the number of metaphases did not show any statistically significant differences. It was also observed from the table that samples treated with sodium azide recorded a significantly high total number of cells (129.40) while the least was recorded in root tips treated with distilled water (102.70). Root tips exposed to radiation from the projector at 40cm/1hr (81.50) and 50cm/30min (80.50) produced a significantly higher number of interphase cells compared to other treatments including the positive (74.40) and negative controls (70.20) considered in this study. The positive control produced a significantly higher number of prophase (42.30) compared to the negative control (29.80) and other treatments considered in the study. The negative control produced a higher number of anaphase cells (1.50) compared to the positive control (0.90) and other treatments. Exposing root tip cells to radiations from projector at a distance of 20cm for 30min produced telophase cells (1.40) that are significantly higher than other treatments. This significant alteration in mitotic stages is in agreement with the report of Fabrigar and Porquis (2019) that X-ray significantly altered all the stages of cell division in *Allium cepa* irradiated root meristems. The fact that the number of cells at metaphase did not vary significantly vary with radiation treatment suggests the presence of regulating mechanism at the metaphase stage during the cell division cycle. The non-significant response of metaphase cells to aqueous extract of *Ampelocissus latifolia* was attributed by Chaudhuri *et al.* (2015) to hindrances created in cell cycle mobility in the treated onion root meristematic cells. This according to Ray *et al.* (2013) and Barman *et al.* (2020) could operate through metaphase arrest as the cell cycle progresses.

Table 3 and plate II show the result of chromosomal aberrations induced by radiation from the projector on *Allium cepa* root tip cells. It was observed that eleven chromosomal aberrations were induced in *Allium cepa* root tip cells exposed to radiation from the projector at different distances and duration. The eleven chromosomal aberrations recorded in this study are binucleate cells, vacuolated cells, sticky chromosomes, bridge chromosomes, star metaphase, faulty polarity, ghost cells, C- mitosis, fragmented cells, spindle disturbance, and vagrant chromosomes. Binucleate cells, vacuolated cells, and sticky chromosomes were recorded in all the treatments except root tips grown with distilled water. Among the radiation-treated root tips, ghost cell was only recorded in onion root tips exposed to radiation at 30cm for 30mins. From the same table 3, it was observed that treating onion root tips with sodium azide (positive control) significantly induced binucleate cells (3.70), ghost cells (0.50), and spindle disturbance (0.60). Onion roots exposed to radiation from the projector at a distance of 10cm for 1hr significantly induced sticky chromosomes (3.10) and faulty polarized cells (3.20) while roots exposed for 30mins at a distance of 20cm, 30cm for 30mins, 40cm for 1hr and 40cm for 30mins significantly induced vacuolated cells (8.80), C-mitosis (0.03), vacuolated cells (0.60) and bridged chromosomes (1.00). Roots exposed to radiation from the projector at distances and durations of 30cm for 1hr and 50cm for 30mins both significantly induced star metaphase

(0.70). The highest significant (P<0.05) total number of aberrant cells was recorded in onions root cells exposed to projector radiation positioned at 10cm for 1hr (16.10) while the least number of aberrant cells was recorded in roots exposed to projector radiation for 1hr at 50cm (4.50). The binucleate cells, vacuolated cells, sticky chromosomes, bridge chromosomes, star metaphase, faulty polarity, ghost cells, C- mitosis, fragmental cells, spindle disturbance, and variant chromosomes reported in this study is in congruence with the vacuolated cells, sticky chromosomes, anaphase bridges, vagrant chromosomes, faulty polarity, C-mitosis, binucleate cell and ghost cells reported by Adroyic *et al*. (2021) on radiation from radon decay process. Tkalec *et al.* (2009) reported that radiation from Electromagnetic fields (EMFs) at frequencies of 400 and 900 MHz induced aberrations such as vagrant chromosomes, lagging chromosomes, sticky chromosomes, and disturbed anaphases. This finding also conforms to the chromosome fragmentation, bridged chromosomes, sticky chromosomes, vagrant chromosomes, vacuolated cells, multipolar anaphase, and unequal distribution of chromatin reported by Çavuşoğlu *et al.* (2022) on *Allium cepa* meristematic cells subjected to ultraviolet light.

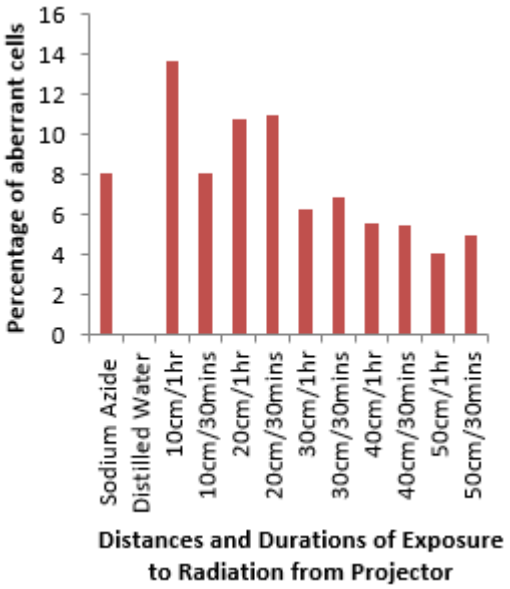

Fig 1: Percentage Aberrations observed in Positive (Sodium Azide), Negative (Distilled water), and Radiation Treated *Allium cepa* Root Tip Cells

This finding corroborates the report of Aweda *et al.* (2010) and Chandel *et al.* (2019) that exposing *Allium cepa* root meristem to non-ionizing radiations like radiation from microwave and radiofrequency produced somatic and genetic consequences. In this study, binucleate cells, vacuolated cells, and sticky chromosomes were recorded across the radiation treated root tip cells irrespective of distance and duration of exposure which suggests that radiation from projector within the distance of 50cm is capable of inducing the three chromosomal aberrations. On the contrary, the fact that ghost cell was only recorded in onion root tips exposed to radiation at a distance of 30cm for 30mins suggests that radiations from the projector hardly induce the aberration.

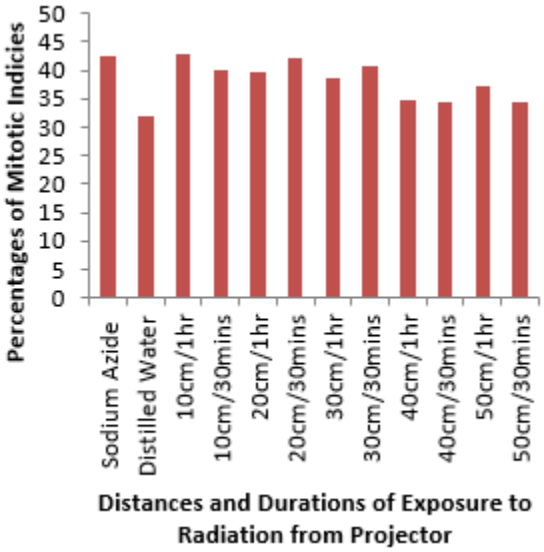

Fig 2: Mitotic Indices for the Sodium Azide (positive control), Distilled water (negative control), and Radiation Treated *Allium cepa* Root Tip Cells

Figure 1 shows results of the percentage of aberrant cells produces in onions root tips treated with sodium azide (positive control), distilled water (negative control), and projector radiation. It was observed that roots grown in distilled water did not produce aberrations while onion roots exposed to radiation from the projector at distances between 10cm and 20cm irrespective of the duration of exposure (i.e. 10cm for 1hr (13.64), 10cm for 30mins (8.11), 20cm for 1hr (10.81) and 20cm for 30min with 10.93) produced percentage aberrations that are higher than the value obtained for sodium azide (positive control) (8.04). It was observed that onion roots exposed to radiation from the projector beyond 20cm showed a progressive decrease in the percentage of aberrant cells. Also, onion roots exposed to projector radiation at distances longer than 20cm irrespective of duration of exposure (i.e., 30cm/1hr (6.27), 30cm/30mins (6.83), 40cm/1hr (5.58), 40cm/30mins (5.43), 50cm/1hr (4.06), 50cm/30mins with 4.94) produced percentage of aberrant cells lower than sodium azide (positive control) with 8.04).

Table 2: Effects of Radiation from the Projector on Mitotic Stages of *Allium cepa* root tip Cells

Treatments	TNC	NOI	NOP	NOM	NOA	NOT
Sodium Azide (PC)	129.40^{a}	74.40^{ab}	42.30^{a}	0.70	0.90^{ab}	0.50^{abc}
Distilled Water (NC)	102.70^{d}	70.20^{ab}	29.80^{b}	0.80	1.50^{a}	0.70^{abc}
10cm/1hr	118.50^{abcd}	64.50^{b}	32.00^{ab}	0.60	1.00^{ab}	1.20^{abc}
10cm/30mims	109.70^{cd}	66.10^{b}	32.40^{ab}	1.00	0.60^{ab}	0.90^{abc}
20cm/1hr	107.30^{d}	64.60^{b}	28.50^{b}	1.00	0.70^{ab}	0.90^{abc}
20cm/30mins	107.00^{d}	61.90^{b}	30.20^{b}	1.10	0.70^{ab}	1.40^{a}
30cm/1hr	108.40^{d}	66.40^{b}	34.30^{ab}	0.30	0.50^{ab}	0.01^{c}
30cm/30mins	105.40^{d}	62.30^{b}	35.10^{ab}	0.30	0.30^{b}	0.20^{bc}
40cm/1hr	125.40^{ab}	81.50^{a}	35.30^{b}	0.70	0.40^{b}	0.50^{abc}
40cm/30mins	105.00^{d}	68.00^{b}	29.80^{ab}	0.30	0.30^{ab}	0.10^{c}
50cm/1hr	110.80^{bcd}	69.40^{ab}	35.80^{ab}	0.50	0.50^{ab}	0.10^{c}
50cm/30mins	123.40^{abc}	80.50^{a}	34.40^{ab}	1.00	0.70^{ab}	0.30^{bc}
LSD Value	17.19	13.31	11.45	NS	1.08	1.05

❖ *Means with the same alphabets in the same column are not significantly different at p<0.05*

Key: TNC- Total number of cells; NOI- Number of cells at interphase stage; NOP- Number of cells at prophase stage; NOM- Number of cells at metaphase stage; NOA- Number of cells at anaphase stage; NOT- Number of cells at telophase stage; NS- Not Significant

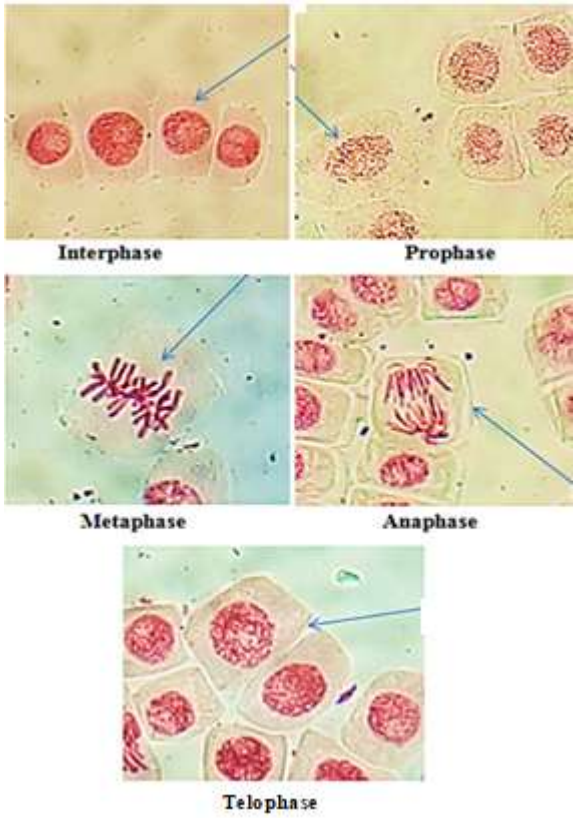

Plate I: *Allium cepa* root tip cells showing normal stages of mitotic division for onions root tips treated with the projector radiation

The observed relationship between the percentage of aberrant cells and distance of exposure to radiation to projector radiation suggests that the mutagenic potential of projector radiation is dependent on the distance from the projector. This finding is further strengthened by the sharp and progressive decrease in the percentage of aberrant cells induced by the radiation beyond the distance of 20cm and a higher percentage of aberrant cells induced below the distance of 20cm compared to values obtained for sodium azide (standard mutagen). Thus, the cytogenetic consequence of radiation from the projector is affected by the distance from the projector than the duration of exposure. These findings further buttressed the earlier recommendations of Usikalu *et al.* (2018) that users of interactive screens and projectors should not be too close to them during presentations because of the hazardous nature of radiations coming from them. Figure 2 represents mitotic indices for onions root tips treated with sodium azide (positive control), distilled water (negative control), and radiation from the projector. It was observed from the figure that sodium azide (positive control) and radiation-treated onion roots had higher mitotic indices compared to distilled water (negative control) (31.94). The highest mitotic index was recorded in roots exposed to radiation at 10cm for 1hr (42.95) while apart from roots grown in distilled water only roots exposed to projector radiation at distances longer than 30cm (i.e., 40cm/1hr (34.91), 40cm/30mins (34.48) and 50cm/30mins with 34.44) produced mitotic indices that are less than 35. The higher Mitotic Index (MI) of onion root tip cells exposed to projector radiation in comparison with the negative control (distilled water) indicates that the radiation exhibited a promontory effect on cell division by interfering with replication processes of cellular DNA thereby enhancing the proliferation of cells during mitotic cell division. Kumar *et al.* (2020) also reported an increase in the mitotic index of *Allium cepa* root tip cells subjected to radiation from the electromagnetic field at frequencies of 900 MHz and 1800 MHz. This increase in the mitotic index of treated meristematic cells compared to control observed in this study contradicts the finding of Çavuşoğlu *et al.* (2022) who reported a significant decrease in mitotic index values of *Allium cepa* root tip cells exposed to ultraviolet light at a frequency of 254 nm compared

to the control. The increase in the mitotic index according to Jayasree *et al.* (2014) often emanates from the promontory effect of the agents on the cell cycle leading to enhanced DNA synthesis. This study revealed that radiation from the projector within a distance of 50cm is capable of inducing chromosome abnormalities. Although exposing cells to radiation beyond 20cm from projector reduced its potencies to induce aberrations as well as distortion of mitotic cell division cycles irrespective of the duration of exposure.

Table 3: Chromosomal aberrations induced by radiations from the projector on *Allium cepa* root tip cells

Treatments	NBN	NVL	NSC	NBC	NSM	NFP	NGC	NCM	NFG	NSD	NVC	TAC
Sodium Azide (PC)	3.70^{a}	2.40^{b}	1.50^{bc}	0.50^{ab}	0.20^{b}	0.30^{b}	0.50^{a}	0.00^{b}	0.40^{bc}	0.60^{a}	0.30^{ab}	10.40^{bc}
Distilled Water (NC)	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{d}
10cm/1hr	0.60^{ab}	7.50^{a}	3.10^{a}	0.20^{b}	0.00^{b}	3.20^{a}	0.00^{b}	0.20^{b}	1.30^{ab}	0.00^{b}	0.00^{b}	16.10^{a}
10cm/30mins	0.40^{ab}	4.30^{ab}	1.90^{ab}	0.00^{b}	0.00^{b}	0.80^{b}	0.00^{b}	0.00^{b}	1.50^{a}	0.00^{b}	0.00^{b}	8.90^{bc}
20cm/1hr	0.60^{ab}	7.30^{a}	2.20^{ab}	0.00^{b}	0.00^{b}	0.90^{b}	0.00^{b}	0.00^{b}	0.60^{abc}	0.00^{b}	0.00^{b}	11.60^{bc}
20cm/30mins	0.20^{ab}	8.80^{a}	1.50^{bc}	0.10^{b}	0.00^{b}	0.90^{b}	0.00^{b}	0.00^{b}	0.20^{c}	0.00^{b}	0.00^{b}	11.70^{ab}
30cm/1hr	3.30^{ab}	1.60^{b}	1.00^{bc}	0.10^{b}	0.70^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.10^{c}	0.00^{b}	0.00^{b}	6.80^{ab}
30cm/30mins	1.40^{ab}	2.30^{b}	1.30^{bc}	0.70^{ab}	0.00^{b}	0.70^{b}	0.20^{b}	0.30^{a}	0.10^{c}	0.10^{ab}	0.10^{b}	7.20^{bc}
40cm/1hr	0.40^{ab}	3.90^{ab}	0.60^{bc}	0.30^{ab}	0.00^{b}	0.60^{b}	0.00^{b}	0.00^{b}	0.10^{c}	0.50^{ab}	0.60^{a}	7.00^{bc}
40cm/30mins	1.10^{ab}	1.70^{b}	1.20^{bc}	1.00^{a}	0.40^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.20^{c}	0.10^{ab}	0.00^{b}	5.70^{bc}
50cm/1hr	0.40^{ab}	1.30^{b}	1.30^{bc}	0.40^{ab}	0.00^{b}	0.60^{b}	0.00^{b}	0.00^{b}	0.40^{bc}	0.10^{b}	0.00^{b}	4.50^{cd}
50cm/30mins	1.50^{ab}	1.70^{b}	0.90^{bc}	0.20^{b}	0.70^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.90^{abc}	0.10^{ab}	0.10^{b}	6.10^{bc}
LSD Value	0.31	0.50	1.65	0.07	0.30	0.13	0.21	0.09	0.50	0.50	0.04	0.62

❖ Means with the same alphabets in the same column are not significantly different at $p<0.05$

KEY; PC – Positive control; NC – Negative control; NBN – Number with binucleated cells; NVL – Number of cells with vacuolated cells; NSC – Number of cells with sticky chromosomes; NBC – Number of cells with bridged chromosomes; NSM – Number of cells with star metaphase; NFP –Number of cells with faulty polarity; NGC – Number with ghost cells; NCM – Number of cells with C-mitosis; NFC – Number of fragmental cells; NSD – Number of cells spindle disturbance; NVC – Number of cells with vagrant chromosomes; TAC – Total Number of Aberrant Cells; LSD – Least Significant Difference

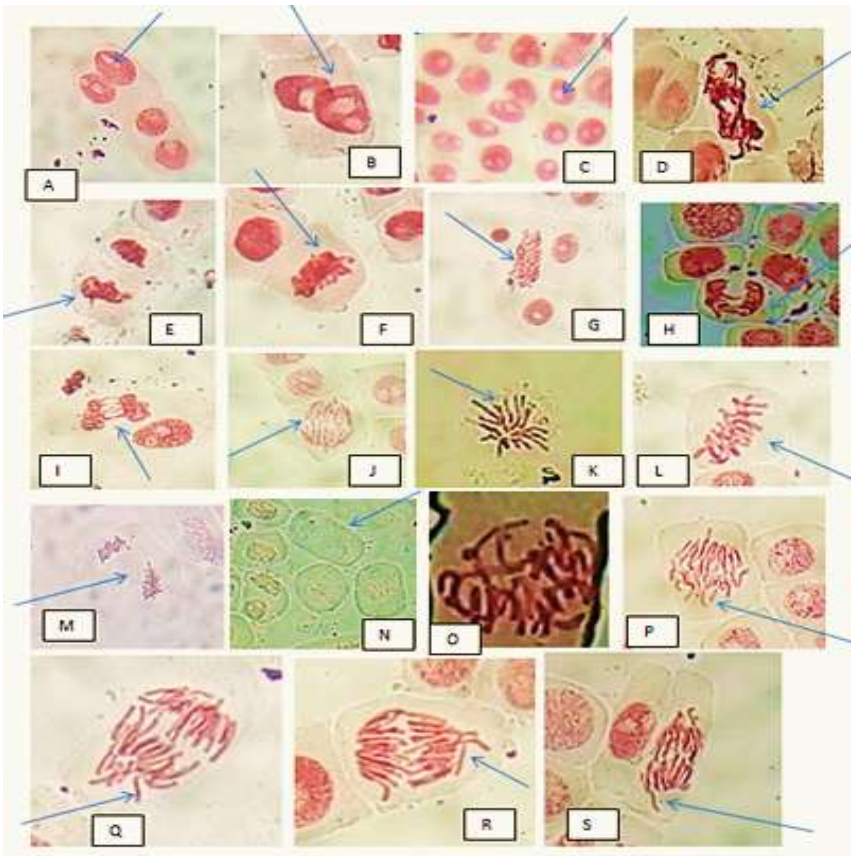

Plate 2: *Allium cepa* meristems showing aberrant cells induced by the projector radiation

KEY: A-B: Binucleate cells, C: Vacuolated cells, D: Sticky/bridged chromosomes, E-G: Sticky chromosomes, H-J: Bridged chromosomes, K: Star metaphase, L-M: Faculty polarity, N: Ghost cells, O: C-mitosis, P: Fragmented chromosomes, Q-S: Vagrant chromosomes.

Conclusion: This study revealed that radiation from projector within the distance of 50cm had genotoxic effects on meristematic cells of *Allium cepa* roots. Specifically, exposing *Allium cepa* cells to radiation within a distance of 20cm from projector significantly induce chromosome aberrations as well as distort mitotic cell division cycles irrespective of the duration of exposure. This suggests that the cytogenetic effects of radiations from a projector depend more on distance than the duration of exposure.

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