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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/17638
DOI URL: http://dx.doi.org/10.21474/IJAR01/17638

ISSN NO. 2320-5407

RESEARCH ARTICLE

CYTOTOXIC AND GENOTOXIC EFFECTS OF UV IRRADIATION ON ROOT MERISTEM CELLS OF ALLIUM CEPA L

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Manuscript Info

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Manuscript History
Received: 28 July 2023
Final Accepted: 31 August 2023
Published: September 2023

Key words:-
Ultraviolet Radiation, Allium Cepa Roots, Mitotic Index, Chromosomal Aberrations

Abstract

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The widespread use of ultraviolet radiation as a disinfectant necessitates an investigation into its possible harmful effects in living organisms. In the present study the effect of exposure to UVC radiation was studied using the Allium cepa assay. To determine the effect of UVC radiation, roots of four sets of A. cepa bulbs were irradiated with 254 nm UV radiation for durations of half, one, two and four hours, respectively. The cytotoxicity of UVC radiation was assessed by comparing the mitotic index of root meristem cells of exposed roots and unirradiated roots (control). Frequency of chromosomal aberrations in the dividing cells of the root meristem was taken as a measure of the genotoxicity of UVC radiation. The roots subjected to UVC exposure showed several types of chromosomal aberrations including sticky chromosomes, c-metaphase, vagrant chromosomes, irregular anaphase, multipolar anaphase, chromosomal bridges and laggards. The study clearly shows that exposure to UVC radiation for even a brief period of half an hour is cytotoxic and genotoxic.

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Introduction:-

Solar radiation is comprised of mainly ultraviolet, visible light and infrared radiation. The wavelength of UV radiation in the electromagnetic spectrum lies between 100 to 400 nm. On the basis of wavelength, UV radiation is categorised into long-wave UVA (315-400 nm), medium-wave UVB (280-315 nm) and short-wave UVC (200-280 nm) (Hsu et al., 2021). Sunlight that reaches earth’s surface consists of 5% UV radiation mainly UVA (95%) and UVB (5%); UVC and most of UVB are prevented from reaching the troposphere by the ozone layer of the stratosphere (IARC Monograph, 2012). Though UVA penetrates the ozone layer, due to its low energy content it is the least hazardous (Rahimzadeh et al., 2011; Nashnoush & Rodgers, 2020). However, due to the thinning of the ozone layer a higher amount of UV radiation can pass through and organisms are at great risk of exposure to UVB and UVC which can be extremely detrimental to metabolic and genetic processes (Hollośy, 2002; Correa et al., 2022) especially in plants because their survival depends on sunlight (Strid et al., 1994; Mpoloka, 2008). Additionally, UVC rays are released from germicidal lamps used for sterilization of foods, hospital rooms and equipment, cell culture media and for purification of drinking water (Yen et al., 2014; Verdes-Teodor et al., 2019; Çavuşoğlu et al., 2022). UV lamps are preferred over chemicals for sterilization as the latter can produce hazardous substances and also leave residue (Mori et al., 2007).

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UV radiation can cause damage to living beings as it gets easily absorbed by cell molecules such as amino acids, nucleic acids and proteins (Hollośy, 2002). UV radiation has been implicated in several types of damage to cells and tissues. Djordavic & Tolmach (1967) reported that exposure of HeLa cell cultures to UV radiation increased the duration of DNA synthesis resulting in a delay in mitosis. A similar delay in cell division in Petunia hybrida protoplasts was noted by Staxén et al. (1993). UV radiation reduces photosynthetic capacity by damaging photosystem II and Rubisco (Teramura & Sullivan, 1994). Reboredo & Lidon (2012) observed that UV irradiation causes decline in chlorophyll content in plants. Exposure to UV radiation for long durations can lead to excessive skin pigmentation and cancer in humans due to mutations caused in DNA (Vechtomova, 2021). UV radiation is described as a “complete carcinogen” because of its mutagenicity and properties of tumour initiation and tumour promotion (D’Orazio, 2013).

UVC radiation emitting lamps are greatly germicidal because 253.7 nm is the wavelength absorbed specifically by DNA molecule (Çavuşoğlu et al., 2022). In this study, the damage caused to meristematic cells of roots by UVC radiation of 254 nm was assessed through the Allium cepa assay. The **A. cepa** test was developed by Levan (1938). The assay is regarded as a sensitive assay for screening toxic effects of chemicals on mitotic division (Grant, 1982; Fiskesjö & Levan, 1993; Rank, 2003; Trushin et al., 2013; Timothy et al., 2014; Palmieri et al., 2016; Macar et al., 2019) and its results correlate well with those obtained from mammalian and other eukaryotic systems (Leme & Martin-Morales, 2009; Macar 2020; Öztürk et al., 2020; Srivastava & Singh, 2020).

The assay was performed using A. cepa roots subjected to exposure to 254 nm UV radiation for periods of 0.5, 1, 2 and 4 hours, respectively. The parameters used to evaluate genotoxicity of UVC radiation were mitotic index and frequency of chromosomal abnormalities during mitosis. Although there is a similar study on effect of UV radiation on roots of Allium cepa (Çavuşoğlu et al., 2022), that study reports genotoxicity of UV radiation after exposure of roots to UV for a long duration of 72 hours. In the present study, the authors report the cytotoxic and genotoxic effect of UV exposure as brief as half an hour.

Materials and Methods:-

Experimental design and treatment

The older roots and basal tissue of Allium cepa L. bulbs were removed with the help of a blade to expose root primordia. Rooting was induced in the Allium cepa bulbs by placing them on beakers filled with distilled water with the base of the onion in contact with the surface of water. The beakers with the onion bulbs were placed in a plant growth chamber maintained at 26^{o}C, 60% RH and a daily cycle of 16h light and 8h dark periods. Water was topped up in the beakers every day. On the sixth day, the beakers with rooted bulbs were exposed to UVC radiation of 254 nm for periods of half, one, two and four hours, respectively. Roots from the unexposed bulbs served as the control. Three replicates of the experiment were set up. Roots were randomly selected from the bulbs immediately after each treatment, and their tips excised and put into vials containing fixative for 24 h. Acetic alcohol (45% acetic acid: ethanol, 1:3) was the fixative used. After fixation the root tips were transferred to vials containing 70% ethanol and stored in the refrigerator.

Mitotic studies and analysis of cytogenotoxicity

For cytogenetic analysis, softening of root tips was carried out by treatment with a mixture of 1N HCl: 45% acetic acid (3:1). Root tips were then squashed on microslides and stained either with 2% acetocarmine or 2% acetoorcein, and observed under the microscope at 400X magnification. Sixteen random optical fields were scanned for each treatment to record observations. Mitotic index (MI) and chromosomal aberration frequency (CA) were the parameters used to assess cytogenotoxicity of UV radiation. MI and CA were calculated by the equations used by Verdes-Teodor et al. (2019) as given below:

$$\text{MI} = \frac{\text{number of dividing cells}}{\text{total number of cells}}$$

$$\text{CA} = \frac{\text{number of cells with chromosomal aberrations}}{\text{number of dividing cells}}$$

Statistical Analysis

The means for MI values were analysed by performing one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests at significance level $p < 0.05$. The IBM SPSS Statistics-21 was used to perform statistical analysis and plot the graphs.

Results:-

Mitotic Index (MI)

The values for MI of root tip cells of A. cepa are given in Table 1 and shown as the mean ± Standard Error (SE). The average MI value for control was 0.11, while those for 0.5h, 1h, 2h and 4h exposure to UV radiation were 0.08, 0.05, 0.06 and 0.05, respectively. A decrease was observed in the MI of UV-treated roots in comparison to the control at $p < 0.05$ (Fig 1). The difference between mean MI of 0.5h and 4h UV exposure was significant. However, the difference between means MI of 0.5h and 1h as well as 2h UV exposure was not significant. Thus, the 1h and 2h duration of exposure to UV did not affect MI values more than that for 0.5h duration. However, 4h exposure to UV radiation lowered the MI much more than the other three durations in comparison to the control.

Table 1:- Mitotic index and chromosomal aberration frequency of root tip cells of A. cepa following exposure to UVC radiation for different durations.

Duration of UV Exposure	Mean MI ± SE	Mean CA ± SE
Control	0.1066 ± 0.00409	0
0.5h	0.0776 ± 0.00407^{a}	0.3450 ± 0.0327^{b}
1h	0.0539 ± 0.00350^{a}	0.4538 ± 0.03145^{b}
2h	0.0561 ± 0.00333^{a}	0.4790 ± 0.03573^{b}
4h	0.0474 ± 0.00554^{a}	0.4767 ± 0.03078^{b}

$^{a, b}$ Values are significantly different from control at $p < 0.05$

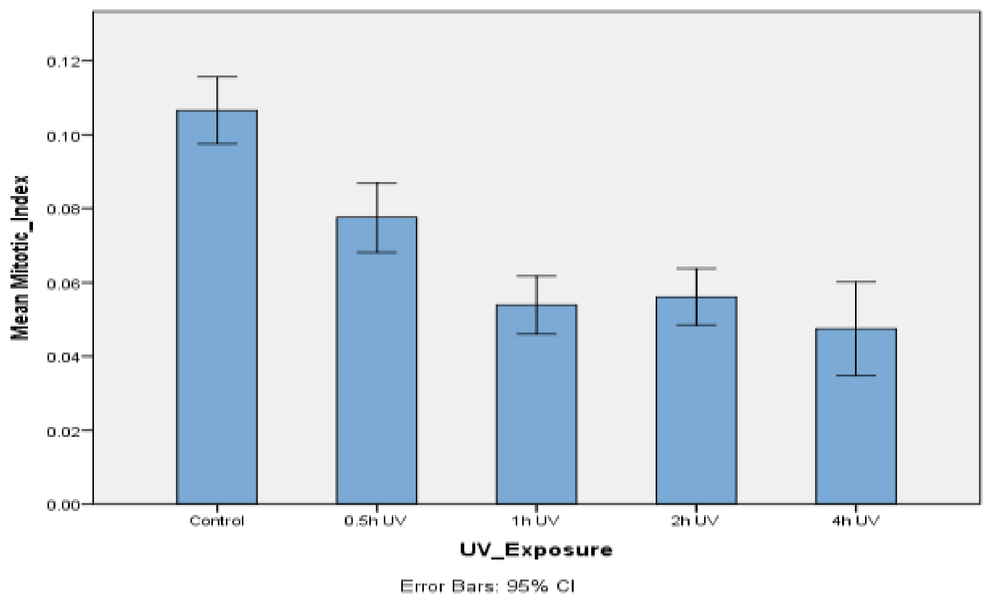

Figure 1:- Effect of exposure to UVC radiation for different durations on MI of A. cepa root cells. Error bars denote standard error of the mean.

Chromosomal Aberration frequency (CA)

No chromosomal aberrations were observed in control root tips (Fig 2). All roots exposed to UV radiation for different durations showed the presence of chromosomal aberrations. The type of chromosomal aberrations observed after different durations of UV exposure are summarised in Table 2. In the irradiated roots, c-metaphase and vagrant chromosomes were the most frequent aberrations observed after UV exposure for all durations. The other aberrations observed were sticky chromosomes, irregular anaphase, chromosomal bridge, laggards and multipolar anaphase (Fig 3).

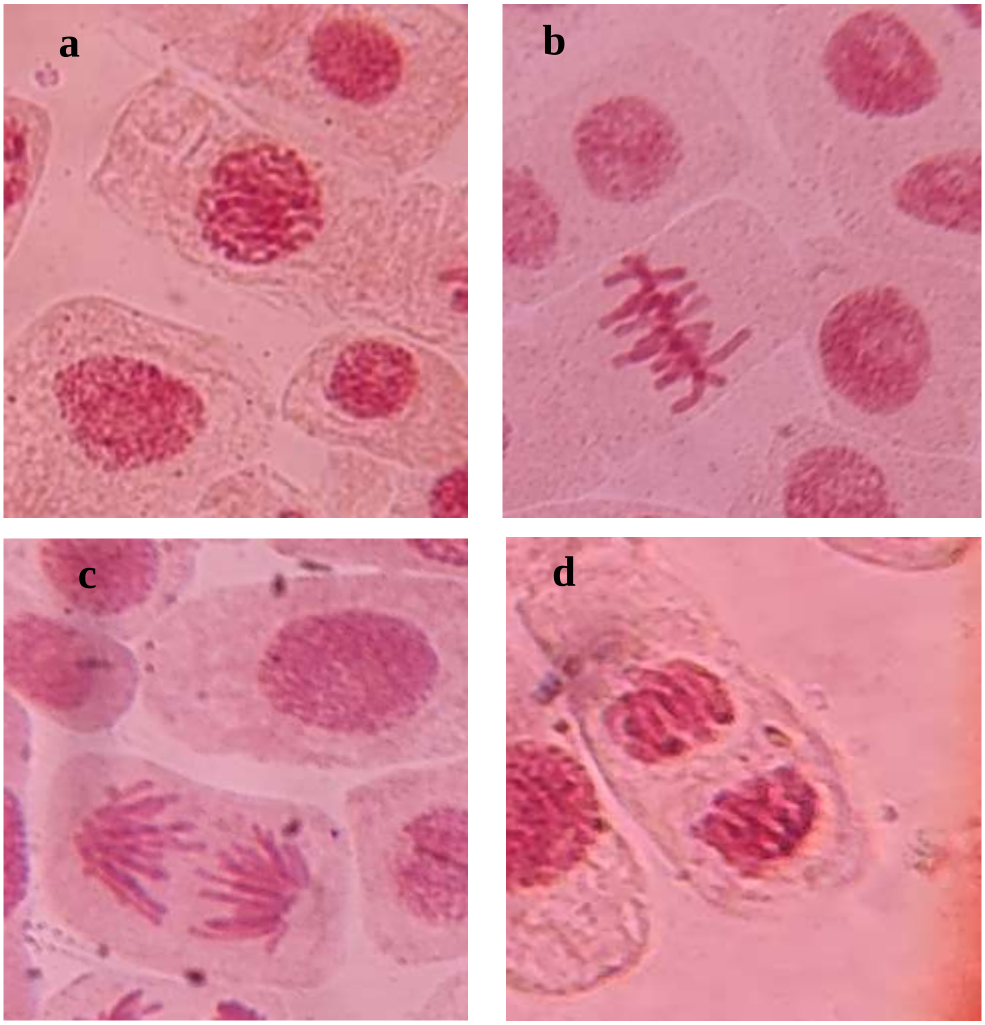

Figure 2:- Stages of normal mitosis in control roots of A. cepa. **a** prophase, **b** metaphase, **c** anaphase and **d** telophase (All images at 400X magnification).

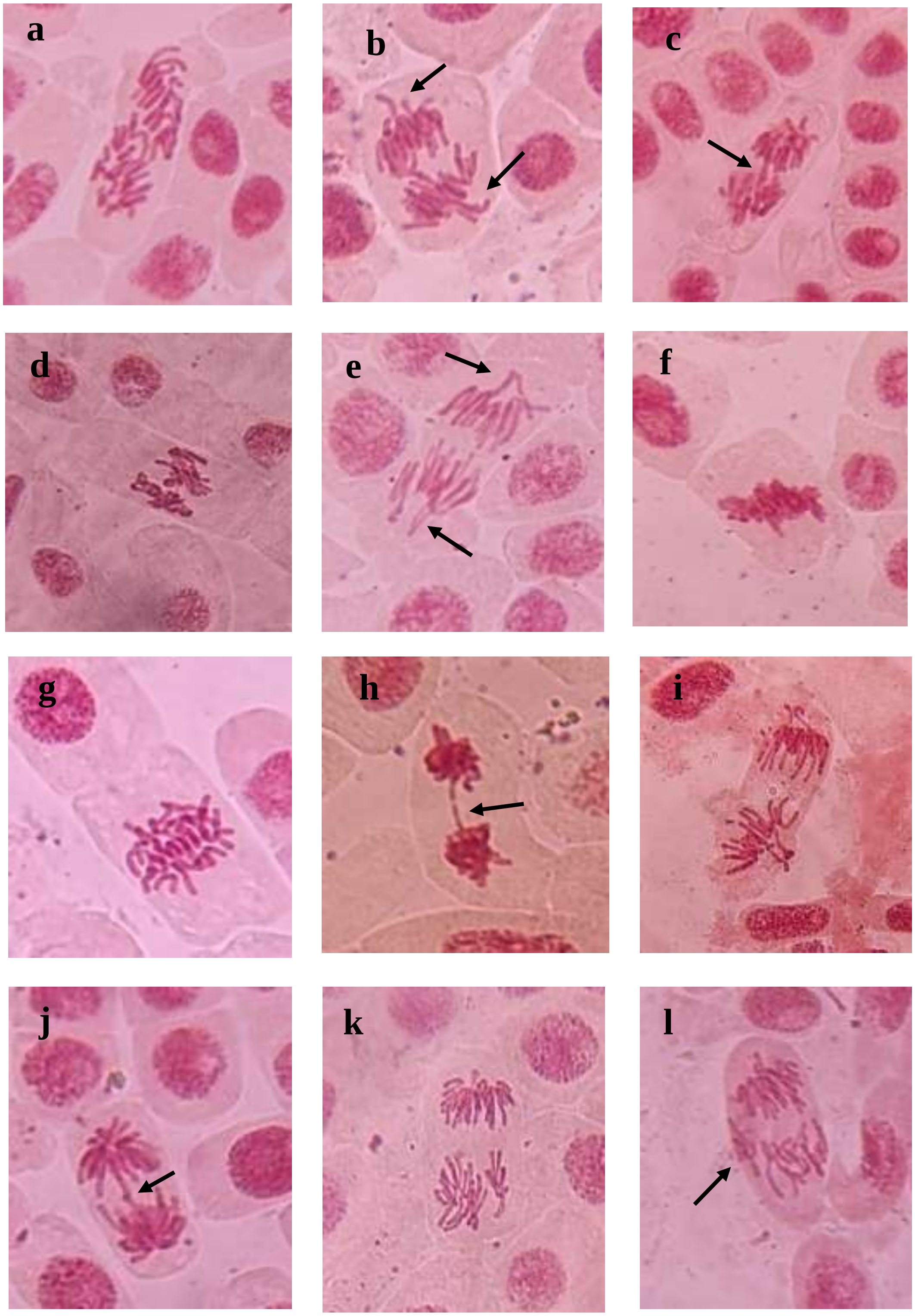

Figure 3:- Chromosomal aberrations observed in UV irradiated cells of A. cepa root meristem. **a**, **b**, **c** after 0.5h exposure to UV radiation: **a** c-metaphase, **b** vagrant chromosomes at anaphase, **c** chromosomal bridge at anaphase; **d**, **e** after 1h exposure to UV radiation: **d** irregular metaphase and sticky chromosomes, **e** vagrant chromosomes at anaphase; **f**, **g**, **h** after 2h exposure to UV radiation: **f** sticky chromosomes at metaphase, **g** irregular metaphase, **h**, chromosomal bridge at telophase; and **i**, **j**, **k**, **l** after 4h exposure to UV radiation: **i** irregular anaphase with vagrant

chromosomes, **j** chromosomal bridge at anaphase, **k** multipolar anaphase, **l** laggard chromosomes at anaphase (All images at 400X magnification).

Table 2:- Different chromosomal aberrations observed in A. cepa root tip cells after exposure to UV radiation.

S. No.	Duration of UV exposure	Chromosomal aberrations
1.	Control	None
2.	0.5h	c- mitosis, irregular anaphase, vagrant chromosomes, chromosomal bridge, multipolar anaphase
3.	1h	Delayed metaphase, sticky chromosomes, c-mitosis, irregular anaphase, vagrant chromosomes, chromosomal bridge
4.	2h	Delayed metaphase, sticky chromosomes, c-mitosis, irregular anaphase, vagrant chromosomes, chromosomal bridge
5.	4h	c- c- mitosis, irregular anaphase, vagrant chromosomes, chromosomal bridge, laggard chromosome, multipolar anaphase

The mean CA values were 0.35, 0.45, 0.48, and 0.48, for 0.5h, 1h, 2h and 4h exposure to UV radiation, respectively, while the value for control was zero (Table 1). The CA of UV-treated roots showed an increase with duration of exposure at $p < 0.05$ (Fig 4). The difference between mean CA of 0.5h and 2h as well as 0.5h and 4h UV exposure was significant. However, the difference between mean CA of 0.5h and 1h as well as between 1h and 2h UV exposure was not significant. Thus, the 1h and 2h duration of exposure to UV affected CA values more than that for 0.5h duration. Both 2h and 4h exposure to UV radiation resulted in similar CA frequency values.

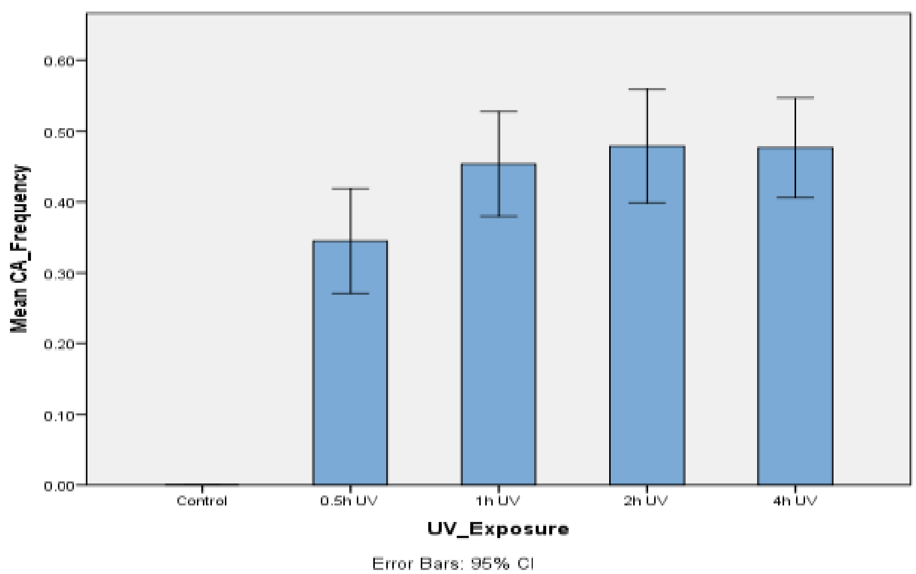

Figure 4:- Effect of exposure to UVC radiation for different durations on mean CA in A. cepa root cells. Error bars denote standard error of the mean.

Discussion:-

MI and CA were determined to assess the genotoxicity of UVC radiation on irradiated A. cepa root meristem cells. The MI has been used to monitor the cytotoxicity and genotoxicity of test chemicals, environmental pollutants and radiation (Rančelienė & Vyšniauskienė, 2012; Șuțan et al., 2014; Haq et al., 2017; Dutta et al., 2018; Macar et al., 2019; Macar, 2020; Nashnoush & Rodgers, 2020; Srivastava & Singh, 2020; Çavuşoğlu et al., 2022).

In this study all durations of irradiation of A. cepa roots with UVC were found to reduce MI. Çavuşoğlu et al. (2022) found that A. cepa roots exposed to 254 nm UVC radiation for 72h had a much lower mitotic index than unirradiated controls. Similarly, Nashnoush & Rodgers (2020) also noted that MI declined in meristematic cells of A. cepa roots subjected to UVB radiation. As early as 1967, Wolff et al. reported that even near UV radiation suppressed mitotic division in onion root tips.

A decrease in mitotic activity in root meristem cells of Crepis capillaris exposed to UVB radiation was observed by Rančelienė & Vyšniauskienė (2012). In Cucurbita pepo L. roots, reduction in dividing cell percentage was directly proportional to UVC exposure time (Verdes-Teodor et al., 2019). Likewise in roots of Phaseolus vulgaris L. a decrease in cell division frequency occurred after exposure to UVA, UVB and UVC radiation (Bara & Tiganas, 2005). Irradiation of Tobacco BY-2 (Nicotiana tabacum L. cv. Bright Yellow 2) suspension-cultured cells with UV-B resulted in inhibition of cell proliferation and arrest of cell cycle (Takahashi et al., 2015). Staxén et al. (1993) reported that irradiation of isolated Petunia hybrida mesophyll protoplasts with 280-360nm UV led to the fragmentation of microtubules and also delayed cell division. The disruption of microtubules by UV radiation could be a likely cause of low cell division (Çavuşoğlu et al., 2022). Damage caused to DNA and tubulin dimers by UV and the consequent chromosomal aberrations could lead to delay in cell cycle and thus mitosis (Staxén et al., 1993).

It has been suggested by de Oliveira et al. (2015) that in roots the development of meristematic tissue is less due to c-metaphases because c-metaphases can result in arrest of cell cycle at metaphase and thus nuclear division will not take place. This also probably explains the lowering of mitotic index.

UV radiation has been known since long to induce dimerization of adjacent pyrimidines in DNA molecule. Two kinds of photoproducts are formed: pyrimidine 6-4 photoproducts and cyclobutane pyrimidine dimers. The DNA molecule with such lesions is not replicated properly and mutations may result (Strid et al., 1994; Hollośy, 2002; Mpoloka, 2008).

Besides reduction in MI, several types of chromosomal aberrations were also observed in A. cepa root cells after UVC exposure in the present study while the unirradiated roots did not show any aberrations. These include sticky chromosomes, c-metaphase, irregular anaphase, chromosomal bridge, laggards, multipolar anaphase, and vagrant chromosomes. The lower MI and occurrence of chromosomal aberrations clearly indicate the cytotoxicity (reduction in mitotic division), genotoxicity (induction of chromosomal aberrations) and clastogenicity (fragmentation of chromosomes resulting in laggards) of UVC radiation. UVC radiation has been shown to induce chromosomal aberrations in cells of root meristem of A. cepa (Çavuşoğlu et al., 2022), C. pepo (Verdes-Teodor et al., 2019) and maize caryopses (Uta et al., 2016).

Clastogenic agents cause breaks in DNA strand and if these remain unrepaired acentric fragments of chromatid or chromosome may be formed. Acentric chromosome fragments usually lag behind during anaphase and fail to be included in the daughter nuclei in telophase. Another outcome is the formation of multicentric chromosomes which form chromosomal bridges as their centromeres are pulled toward opposite poles of the cells during anaphase (Fenech et al., 2020). As a consequence of breakage and fusion of either chromatids or chromosomes, loss of genetic material may occur which may produce changes such as recombination or deletion of genes (Ignacimuthu & Babu, 1989). Laggards as well as c-metaphases can alter chromosome number in daughter cells and hence lead to aneuploidy (Verdes-Teodor et al., 2019).

Vagrant chromosomes, which migrate towards the pole of the cell ahead of other chromosomes during anaphase, most likely result from non-disjunction of chromatids or unequal chromosome distribution (Dutta et al., 2018). Multipolar anaphase is believed to be attributed to formation of multipolar spindle and may lead to aneuploidy and cell death in the ensuing cell cycles (Vitre et al., 2020).

Conclusion:-

This study validates the genotoxicity and clastogenicity of UVC radiation, which is commonly used in various purification and germicidal procedures, in root meristematic cells of A. cepa. A UV exposure as brief as half an hour also caused significant decrease in mitotic index as well as occurrence of chromosomal abnormalities. The study shows that UVC radiation, though widely used for sterilization, can prove to be highly dangerous to non-target organisms that may be irradiated inadvertently.

Acknowledgement:-

The authors are thankful to the Principal, Miranda House, University of Delhi, for providing the facilities for this research work under the aegis of DS Kothari Centre for Research and Innovation in Science Education, Miranda House.

Conflict of interest

The authors declare that they have no conflict of interest.

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