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RHODOTORULA SPP. AS A POTENT ANTIMUTAGEN TO PREVENT CHROMOSOMAL ABERRATION IN *ALLIUM CEPA* AS A RESULT OF PROLONGED UV EXPOSURE

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ABSTRACT

The worldwide influx of solar UV radiation poses a threat to the growth of agricultural crops and also phyllospheric organisms except the epiphytic yeasts like *Rhodotorula* which can resist UV damage due to higher torularhodin concentrations which enhances UV-B survival in these yeasts. As onion root tips are used for radiation cytogenetic studies for assessment of chromosomal damage to plant cells, here in this study we have used *Allium cepa* root meristem cells. A karyotype denotes a full set of chromosomes from an individual species which is generally compared to a normal karyotype of the same species for confirmation of the detected chromosomal anomaly which occurs usually when there is an error in cell division following meiosis or mitosis. The karyotype analysis (2n=16) of root tip cells of onion showed normal mitotic metaphase, anaphase, telophase chromosomes in contrast to those onion root tips which after pretreatment were exposed to UV radiation of 254 nm for a duration of 5 and 10 minutes. Various types of chromosomal alteration were observed in terms of appearance of laggard, deletions, duplications, inversions, ring formations, and translocations. These numerical or structural aberrations reported was thus tested again using the same protocol of staining with aceto-orcein and karyotype analysis after squashing the root tip cells of onion which were exposed to UV and later on allowed to grow in *Rhodotorula* culture suspension. Karyogenesis and ideogram thus made, helped us conclude that the total number of cells studied was 90 out of which abnormal cells were 71% and 91% of the total cell population in case of onion root tips exposed to UV for a duration of 5 and 10 minutes respectively and this figure showed a 15%

reduction for 5 minute UV radiation exposure and 49.5% reduction for 10 minute UV radiation induction when treated with *Rhodotorula* yeast, the net result being reduction of chromosomal aberration in root tip cells treated with *Rhodotorula* suspension which reveals the fact that if onions are allowed to grow in soil rich with phyllospheric organisms like *Rhodotorula* spp. then the probable UV damage caused to onion can be reduced as there is high accumulation of carotenoid pigments which provides photoprotection against lethal photooxidation in these UV resistant yeasts.

KEYWORDS: *Rhodotorula* spp., photoprotection, karyotype, ideogram, *Allium cepa*, chromosomal aberration.

INTRODUCTION

UV radiations induce changes in molecular organization of chromosomes known as chromosomal aberrations which occur when there is an alteration in surrounding environment of a cell. Ultraviolet radiation comprises of 8-10% of total solar radiations and plants as require sunlight for photosynthesis are exposed to it continuously (Micron 33-2002). Like all living organisms plants do respond negatively to UV radiations by exhibition of damage to various plant processes especially DNA damage by inducing abnormalities in terms of appearance of polycentric, clumped chromosomes in metaphase cells and laggard, polycentric and non-dysjunction in those of anaphasic chromosomes. As the world climate is affected by global warming influence, the damaging effect of prolonged UV exposure could lead to reduction in agricultural production and pose a deleterious effect on biologically active molecules in the coming years.

To protect the chromosomal damage in onion root tip meristem some antimutagen is required to inhibit the cytotoxic effect and protect the plant against environmental mutagen like UV which not only induces chromosomal damage but also interferes with DNA repair mechanism finally leading to apoptosis of the affected cells. Phyllosphere harbours wide variety of microorganisms and yeasts like *Rhodotorula* spp. which have unique capacity to adapt to harsh environment due to their reorganization of gene expression (Pubmed-2010). Synthesis of antioxidants and carotenoids by these organisms impart photoprotective characteristics. These compounds absorb light in the UV wavelength with subsequent photodecomposition and thereby protect the onion from DNA changes thus preventing altered cell functioning.

Though the antimutagenic potential of Rhodotorula yeast has been proved previously but this study showed a significant reduction in percentage of abnormal cells in onion root tips constantly exposed to UV in terms of reduction of aberrations in *Allium cepa* root meristem cells. So, if such organisms colonize the environment where there is large scale production of *Allium cepa*, they offer protection to host plants through natural biological control (British microbiology research journal-2014). The paper focuses on effect of UV radiation on *Allium cepa* and the control of UV induced damage by *Rhodotorula spp*.

MATERIAL AND METHODS

PLANT MATERIAL

Onion bulbs *(Alium cepa*) were purchased from the local market. They were put in a sand pot and sand dried for 5 days. The dried roots from the base of the onion bulbs were shaved off with a sharp blade and taken for further processing.

PRE-TREATMENT

Pre-treatment is done for disorganising the spindle fibres to spread out and flatten the cells in metaphase. This causes the clearing of the cytoplasm and solidifies the cytoplasmic background. This is done at low temperature. The root tips were dipped in p-dicholobenzene for 5 hours at 12-13 degree C.

FIXATION

Fixation is done for the selective preservation of morphological organisation and chemical composition of tissues. It is done to prevent bacterial decomposition and make the tissue suitable for staining. It increases the visibility of cell contents. 1:3 acetic acid: alcohol (contains glacial acetic acid one part and absolute ethyl alcohol three part) was used as fixative for the root tips. Acetic acid swells and softens the tissues, destroys the Golgi apparatus and mitochondria. Ethyl alcohol precipitates the proteins and shrinks the tissues. The roots are fixed overnight in acetic acid: ethanol (1:3) mixture.

STAINING

The root tips were kept in 45% acetic acid for 5 – 10 minutes and heated for few seconds in 2% orcein: N/50 HCL (9:1) mixture and kept for 45 minutes.

SQUASH TECHNIQUE AND CAMERA LUCIDA DRAWING

The tips were then squashed in 45% acetic acid on a clean grease free slide by covering with a cover slip and sealing with paraffin wax. The mitotic plate was observed with 16 chromosomes. The selected plate was focused under oil immersion objective of the lens and finally was drawn with the help of a drawing prism.

KARYOTYPING

The length of the chromosomes is measured in milimeters from the drawn metaphase plate. Then the lengths are converted in micron. The lengths of the short arm and long arm are calculated separately. The ideograms were prepared consequently.

This was the control of our experiment. For the next setup.

UV EXPOSURE

The roots of the onion bulbs were taken in a petri dish and separated in two sets. The first set was exposed to UV B for 5 minutes and the next set was exposed to UV B for 10 minutes, both at a distance of 20 cm from the UV lamp. UV causes DNA breakage and causes abnormalities in the cellular component.

Pre-treatment, fixation, staining and camera lucida drawing was done in the same way like our control set up. For the next set up.

CELL COUNT

A small amount of the *Rhodotorula* spp. pure culture suspension was taken in micropipette and loaded in the wells of the hemocytometer. Then we put the cover slip on it and saw them under the microscope.

The root tips of Alium cepa were dipped in *Rhodotorula* sp. suspension for some time and being dipped in the suspension the tips were exposed to UV in two sets for 5 minutes and 10 minutes respectively. Pre-treatment, fixation, staining, camera lucida drawing and karyotyping was done as before.

RESULTS

The experiment was done to show that the *Rhodotorula* spp. can prevent the chromosomal DNA damage caused by UV B.

1. The control was taken as the normal squashing of the *Alium cepa* roots in the absence of any mutagen. It was found that the maximum number cells were found in the metaphase stage, followed by anaphase and telophase stage. There was no remarkable abnormality in them. Following closer investigation of a single cell in mitotic phase, a karyotype was made (Fig-2). The normal cell showed the presence of 8 pairs of chromosomes out of which 3 were found to be metacentric, 3 submetacentric and 2 acrocentric. The average length of each pair of chromosome was used as a reference to calculate the extent of the aberrations in the mutagen treated cells (Fig-3).

2. In the UV B 5 minute set, the percentage of abnormal cells in the metaphase was 70% while the percentage of abnormal cells in anaphase cells was 71.4% (Table-3). Further, there was 10% shortening of the length of the long arm in 2 submetacentric and 1 metacentric chromosomes in the short arm of one submetacentric chromosome (Fig-6).

3. In the UV B 10 minutes set, the percentage of abnormal cells in the metaphase was 100% while the percentage of abnormal cell in the anaphase set was 81.5% (Table-5). This proves that with increase in the exposure to UV, the cellular abnormality increases. There was 10% shortening of the length of the long arm in 1 submetacentric and 1 acrocentric chromosomes in the short arm of 1 submetacentric and 1 acrocentric chromosome and 40% shortening of long arm of 1 submetacentric chromosome which now appeared like a metacentric one. Moreover 1 of the metacentric chromosome appeared to be deleted as observed in the karyotype (Fig: 9).

4. In some root tip cells exposed to UV radiation for 10 minutes, 8 were found to have satellite chromosomes and 6 other had slow divisional rate apart from other abnormalities.

5. Hemocytometer count was done and it was found that there was 55*10^ 4 *Rhodotorula* spp. yeast cells in the pure culture suspension.

6. When the root tips dipped in the yeast cell suspension was exposed to UV for 5 minutes, it was found that the abnormal cells in metaphase stage is 60% while the percentage of abnormal cells in anaphase stage is only 52% (Table-7) which is much less than the abnormality percentage found when the root tips was not dipped in yeast cells suspension. Moreover there was only 10% shortening of the length of the long arm in 1

submetacentric chromosome. Hence the damages under normal UV treatment appeared to be almost reversed.

7. When the root tips dipped in the yeast cell suspension was exposed to UV for 10 minutes, it was found that the percentage of abnormal cells in metaphase stage is 33% while the percentage of abnormal cells in anaphase stage is only 50% (Table-9). There was 10% shortening of the length of the long arm in 2 metacentric, 2 submetacentric and 1 acrocentric chromosome and in the short arm of 1 acrocentric and 1 submetacentric chromosome which consequently appeared like an acrocentric one. Hence the damages under normal UV treatment for the same duration appeared to be lessened to a considerable extent.

OBSERVATION TABLES AND FIGURES

Table-1: Cell count for karyotype analysis of normal onion root tip cells.

Number of cells studied	Number of cells in metaphase	Number of cells in anaphase	Number of cells in telophase
38	27	7	4

Table-2: For root tip cells exposed to UV radiation for 5 minutes.

No. of cells studied	Number of cells in metaphase	Number of cells in anaphase	Number of cells in telophase	Number of normal cells in	
				Metaphase	Anaphase
90	50	35	5	15	10

Table-3: Abnormalities observed.

No. of abnormal cells	Number of metaphase cells		Number of anaphase cells		
	Polycentric	Non formation of proper metaphase plate	Laggard	Non-dysjunction	Polycentric
60	15	20	15	5	5

Table-4: For root tip cells exposed to UV radiation for 10 minutes.

No. of cells studied	Number of cells in metaphase	Number of cells in anaphase	Number of cells in telophase	Cells in normal metaphase	Cells in normal anaphase
90	25	65	-	-	12

Table-5: Abnormalities observed.

No. of abnormal cells	Number of metaphase cells	Number of cells in anaphase			
	Polycentric	Laggard	Non dysjunction	Polycentric	Deletion
78	25	24	18	10	1

Table-6: For root tip cells treated with *Rhodotorula* spp. suspension exposed to UV radiation for 5 minutes.

No. of cells studied	Number of cells in metaphase	Number of cells in anaphase	Number of cells in telophase	Number of normal cells in	
				Metaphase	Anaphase
90	40	50	-	16	24

Table-7: Abnormalities observed.

No. of abnormal cells	Number of metaphase cells		Number of anaphase cells		
	Polycentric	Non-dysjunction	Laggard	Non-dysjunction	Polycentric
50	16	8	8	16	2

Table-8: For root tip cells treated with *Rhodotorula* spp. suspension exposed to UV radiation for 5 minutes.

No. of cells studied	Total no of cells in metaphase	Total no of cells in anaphase	Number of cells in normal metaphase	Number of cells in normal anaphase
90	30	60	20	30

Table-9: Abnormalities observed.

No. of abnormal cells	Number of metaphase cells		Number of anaphase cells		
	Polycentric	Clumping	Laggard	Non-dysjunction	Polycentric
40	10	-	20	10	-

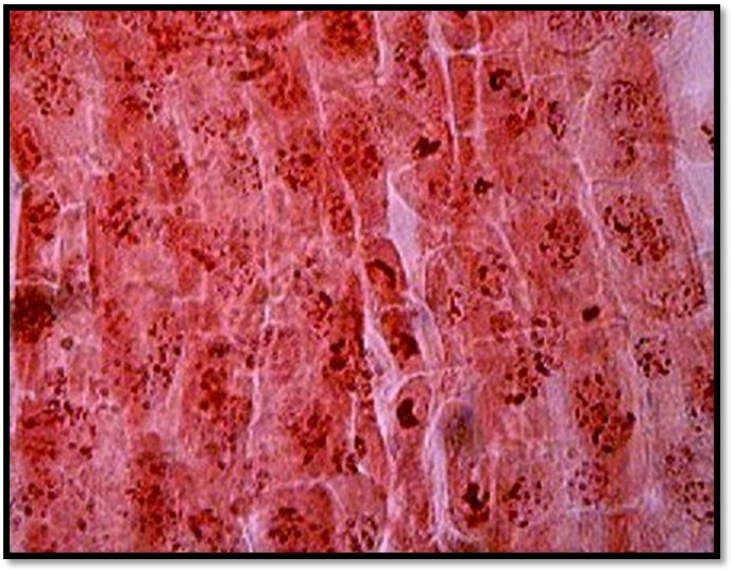

Fig-1: Microscopic view of *Allium cepa* squash.

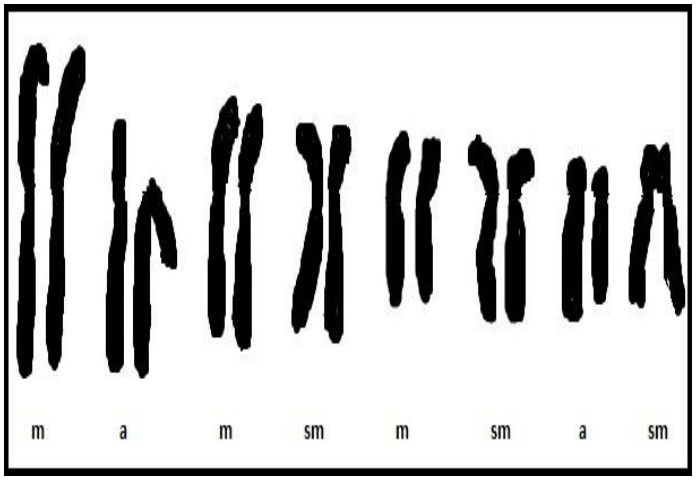

Fig-2: Karyotype of *Allium cepa*.

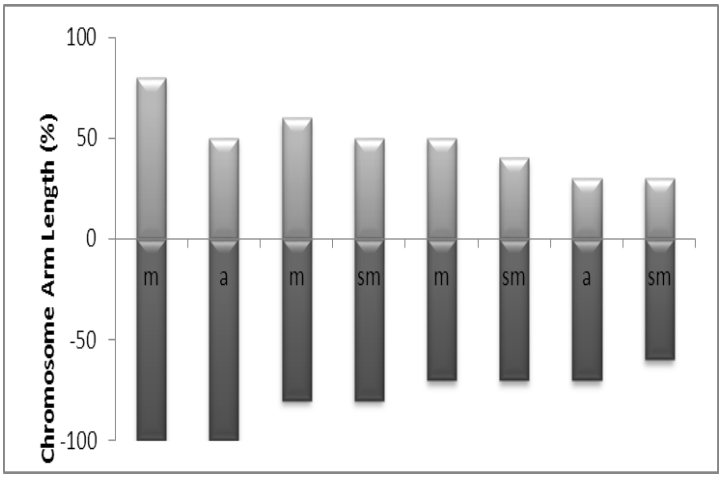

Fig-3: Ideogram of *Allium cepa*.

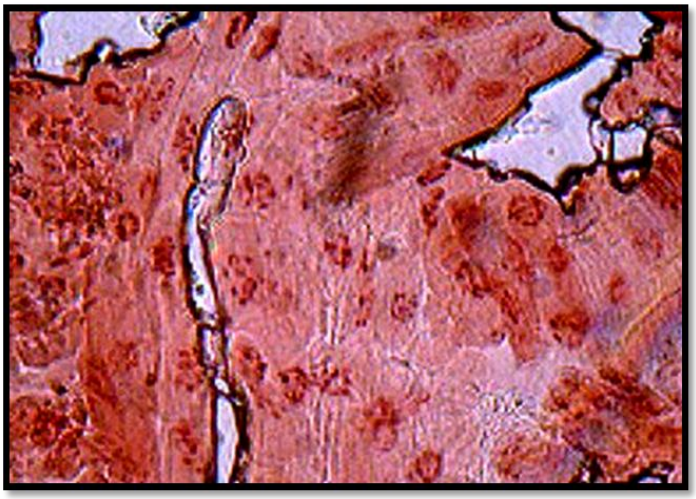

Fig-4: Microscopic View of UV Treated (5min) *Allium cepa* Squash.

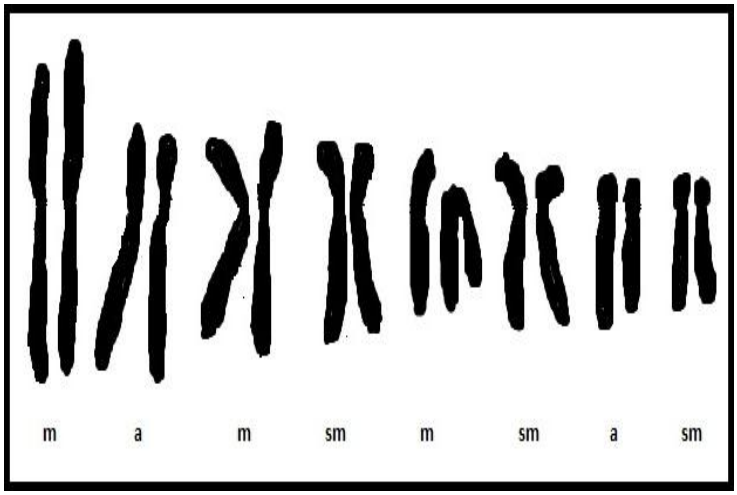

Fig-5: Karyotype of UV Treated (5min) *Allium cepa.*

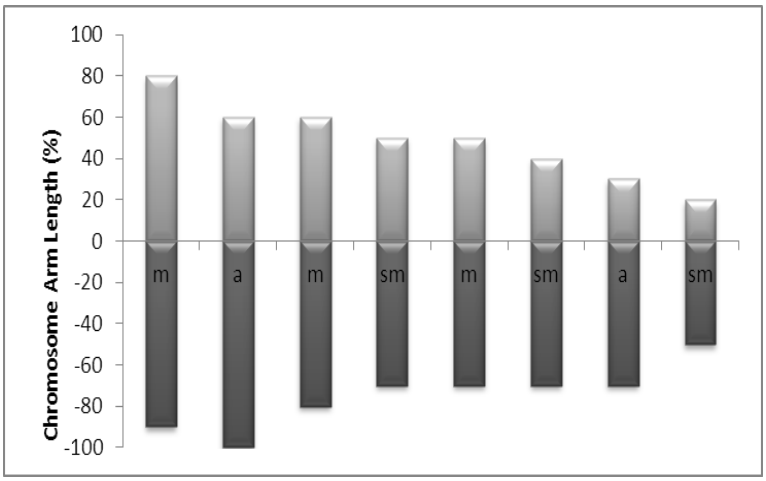

Fig-6: Ideogram of UV Treated (5min) *Allium cepa.*

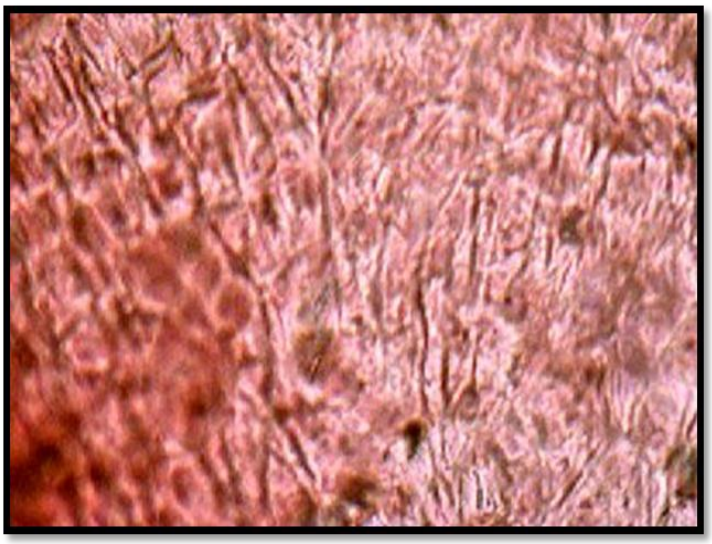

Fig-7: Microscopic View of UV Treated (10min) *Allium cepa* squash.

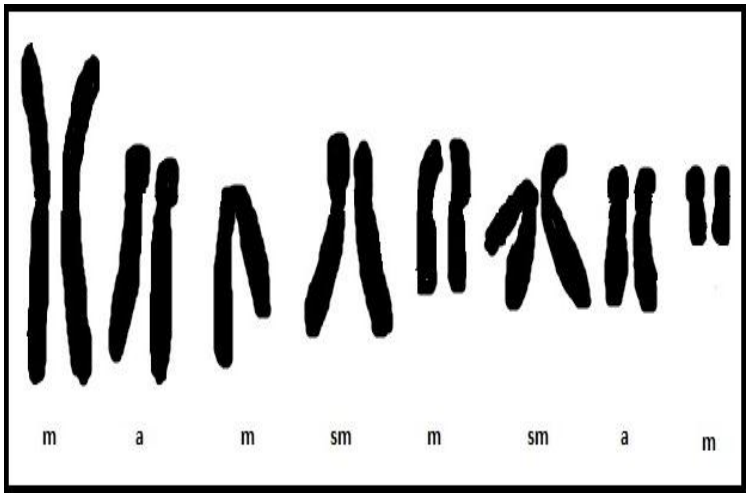

Fig-8: Karyotype of UV Treated (10min) *Allium cepa.*

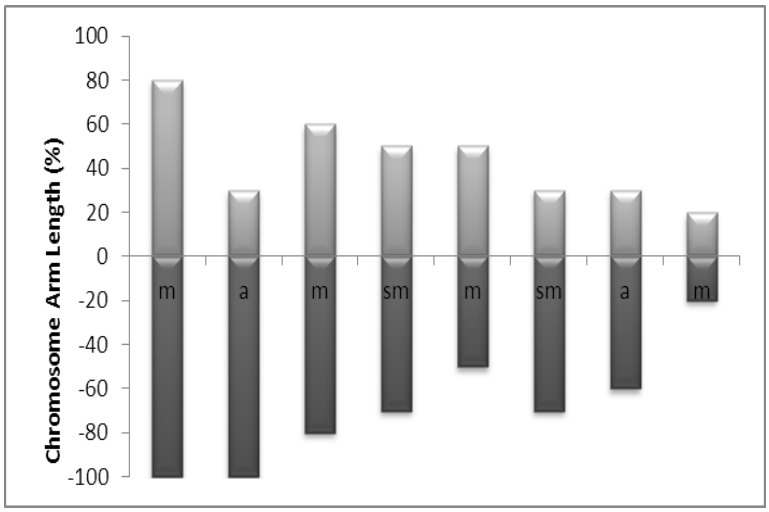

Fig-9: Ideogram of UV Treated (10min) *Allium cepa.*

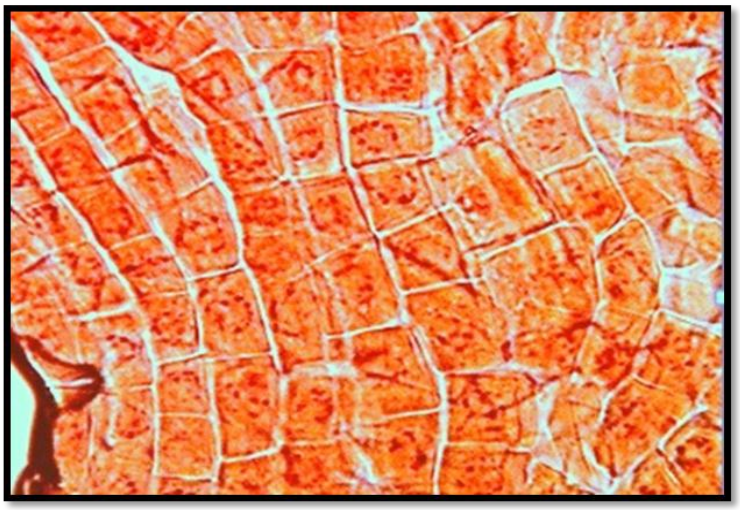

Fig-10: Microscopic view of *Rhodotorula* spp. protected+UV treated (5min) *Allium cepa* squash.

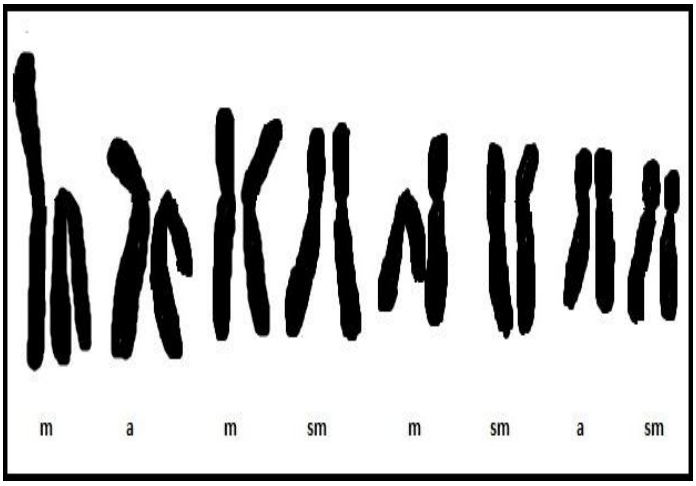

FIG-11: KARYOTYPE OF *Rhodotorula* spp. PROTECTED+UV TREATED (5min) *Allium cepa.*

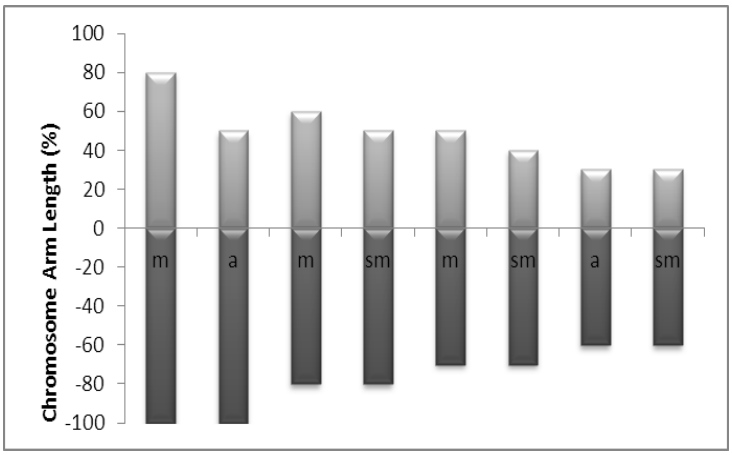

FIG-12: IDEOGRAM OF *Rhodotorula* spp. PROTECTED+UV TREATED (5min) *Allium cepa.*

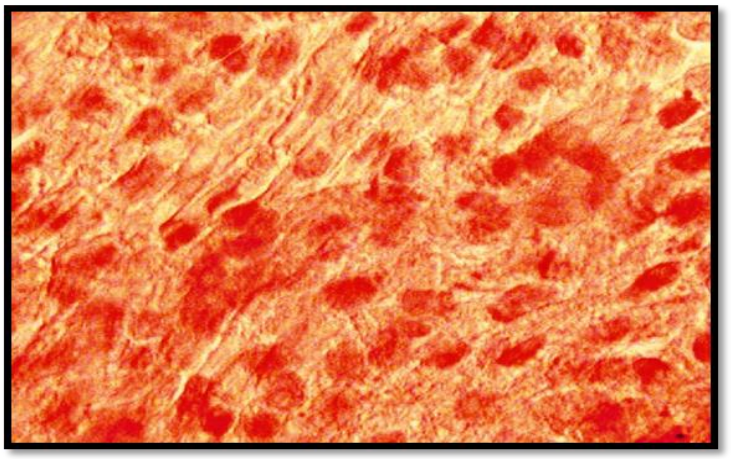

FIG-13: MICROSCOPIC VIEW OF *Rhodotorula* spp. PROTECTED+UV TREATED (10min) *Allium cepa* SQUASH.

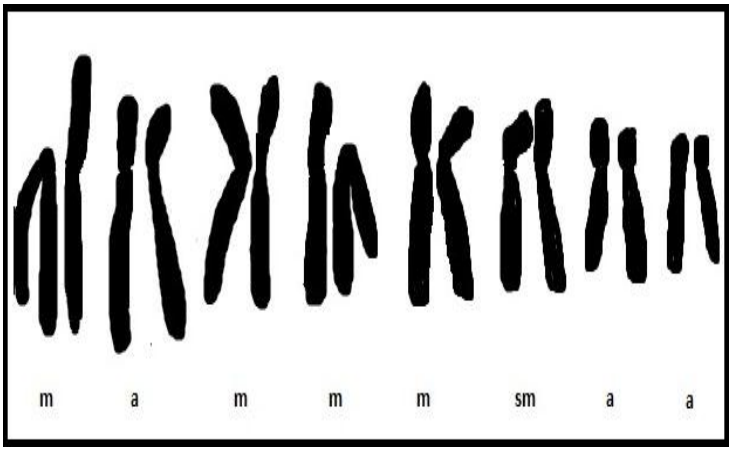

FIG-14: KARYOTYPE OF *Rhodotorula* spp. PROTECTED+UV TREATED (10min) *Allium cepa.*

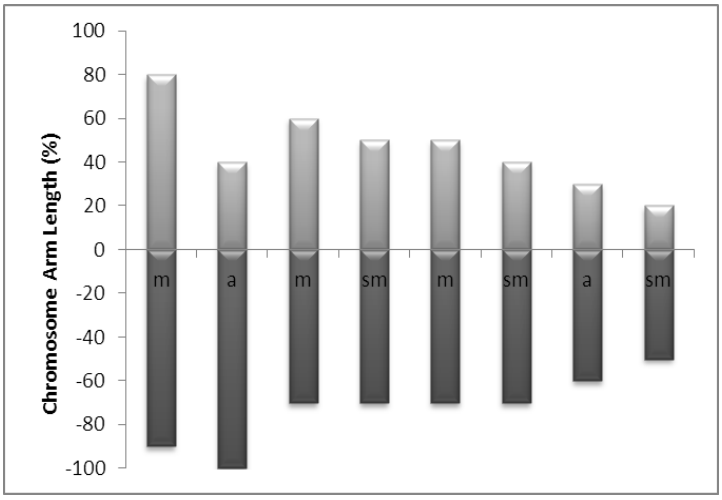

FIG-15: IDEOGRAM OF *Rhodotorula* spp. PROTECTED+UV TREATED (10min) *Allium cepa.*

DISCUSSION

In this investigation, it was proved that *Rhodotorula* spp. could act as a potent chromosomal abnormality reducer resulting from prolonged UV exposure. Plant system have a wide variety of genetic endpoint in chromosomal aberration and sister chromatid exchange so is the possible best available resource for conducting this experiment (Grant, 1994). In spite of vast research in UV induced chromosomal damage, the exact mechanism of damage is yet to be elucidated and the experiment needs to be carried out in field condition to check temperature dependence of chromosomal damage due to these radiations. UV rays cause damage not by direct damage but indirect damage by induction of ROS (Photochem. photobio. L 2002). As the increased UV radiation levels from ozone layer reduction reaches the plants, it has potency to cause chromosomal breakage, induce abnormalities like many number of

polycentric chromosomes and laggard chromosomes during anaphase in root tip cells exposed to UV radiation for a duration of 5 and 10 minutes. Apart from these abnormalities, presence of satellite chromosomes and slow divisional rate was also observed in root tip cells exposed to UV radiation for 10 minutes. Stickiness of chromosome causing chromosome clumping have been attributed to disruption of bonds between protein and nucleic acid constituents (Cohn 1969).

Rhodotorula spp. is known to produce torulene and torularhodin and pigmentation capability was exploited (Simpton 1964) to resist the UV damage to onion root tip cells. The photoprotective role of carotenoids in yeast has been shown in *Cystofilobasidim capitatum* and *Sporobolomyces ruberrimus* (Photochem. photobio. 2009). Similar such experiments were carried out using caffeine which reduced mutagenic effect (Hereditas 1976). Similarly, the protective effect of curcumin due to its antioxidant nature, trapping of free radicals and formation of complex with mutagens (Premkumar 2004) and the curcumin thus has been proposed to have antimutagenic potential against sodium azide inducing chromosomal aberration in root tip cells of onion (PMC 2007). As the yeast community has been found to be resistant to not only UV-B in onion cells but previously has been reported to exhibit photoprotection in phyllosphere of strawberry (British microbio research journal, 2014). So the cell suspension of *Rhodotorula* spp. could reduce the chromosomal aberration resulting from Physical mutagen like UV radiation. From the result obtained it could also be concluded that the yeast could significantly reduce the number of abnormal cells in root tip cells exposed to UV radiation for a greater time interval that is 10 minutes rather than exposure at 5 minutes. Since mutations are induced at chromosomal level and are probable cause of cancer related disease, the inhibition or slight reduction in chromosomal damage by *Rhodotorula* spp. suggests its antimutagenic anticarcinogenic activity. This study thereby could open up a new scope of using yeasts as a remedy against UV induced damage in higher plants.

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