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Research Article

EFFECT OF ULTRAVIOLET C RADIATIONS ON POLYTENE CHROMOSOME AND DEVELOPMENT OF *DROSOPHILA MELANOGASTER*

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ABSTRACT
Various researchers have studied the effect of different stressors such as temperature, osmotic stress, hormones etc. on polytene chromosomes and development of *Drosophila melanogaster*. In the present studies, we have investigated how an exposure to UV radiations at 2nd and 3rd instar of *Drosophila melanogaster* affects puffing pattern in polytene chromosomes, an immediate survival, subsequent survival and emergence of the adult. There were alterations in the staining property of the polytene chromosomes after UV-C irradiation indicating the altered pattern of DNA packaging and transcriptional activity. Second instar larvae showed the lowest survival under UV stress at a distance of 30cm and 60cm and at 30,60,90 and 120 seconds of exposure, whereas, 3rd instar larvae were relatively tolerant.

Key words: *UV-C radiation, Polytene chromosome, Drosophila melanogaster*

INTRODUCTION
Drosophila melanogaster is an established model organism in various disciplines of biological science, such as Genetics, Developmental biology, Molecular Biology, Neuroscience, Behavioral Biology, aging, etc. It is the most popular because of its short life cycle, large number of offsprings and inexpensive cost of culture.

Polytene chromosomes are found in dipteran flies, the most studied examples are *Drosophila* and *Chironomus* (Zhimulev and Koryakov 2009). These chromosomes are commonly found in the salivary glands produced by the repeated rounds of DNA replication without cell division. The polytene chromosomes contain bands and interbands. The bands become enlarged to form swellings called puffs (Holden and Ashburner, 1972). The puffs are the sites of active transcription (Tissieres *et al.* 1974). The puffing pattern gets altered as per the environmental alterations (Vlasova and Zhimulev 1988). Studies by Clever and Karlson (1960) on *Chironomus* have demonstrated that the activity of many puffs arising at the end of larval development is induced by ecdysone which control metamorphosis in insects. The induction of puffs in the salivary gland chromosomes of *Drosophila* larvae by a temperature shock was first described by Ritossa (1962). Salivary gland chromosome of *Drosophila melanogaster* undergoes marked structural deformation when exposed to the action of solutions of trypsin containing electrolytes (Kaufmann 1995). In the present investigations effect of exposure to UV-C radiations was studied on the polytene chromosome, larval development and survival of *Drosophila melanogaster*.

MATERIALS AND METHODS
Animal Model:
For this study *Drosophila melanogaster* was used as an experimental model.Flies were obtained from Department of Zoology, Banaras Hindu University Varanasi. They were maintained at 21˚C in the BOD incubator on maize medium.

Preparation of maize medium:
1.5 gm of agar agar was dissolved in 50 ml of drinking water by boiling with constant stirring. Maize powder and crude sugar was mixed in another 50 ml of distilled water and kept for warming. As soon as

agar agar got completely dissolved, the mixture of Maize Powder and crude sugar was added into it and boiled with continuous stirring. Once the medium was prepared and cooled to 50˚C, the antifungal agents: sodium benzoate and propionic acid were added in the medium. The medium was poured into culture vials and bottles up to the level of 1 cm and 2.5 cm from the bottom respectively. After cooling and solidification of the medium, the inner surface of the vials/bottles was wiped off to prevent slipping of the flies. Finally, yeast granules were added to the surface of the culture medium. Propagation was done by transferring 10 females and 10males in the culture bottle. On 4th day the flies were removed from bottles.

Experimental Design:

The second instar and third instar larvae were used in the present invetigations. The larvae were randomly separated into two main groups:

- Group I : Without UV-C irradiation; this group was treated as a control group
- Group II : Exposed to UV-C radiations;

In this group, exposure of UV-C radiation was given to the Second Instar larvae and third instar larvae. The sets of 15 larvae in each were exposed to UV lamp in Laminar Air Flow for varying time interval viz. 30 seconds, 60 seconds, 90 seconds and 120 seconds. The exposure of UV light was with varying distance of 1 foot (30 cm) and 2 feet (60 cm) from the UV tube.

Exposure to UV-C radiations:

1. Control group: Larvae from this group were not exposed to UV-C radiations.
2. Experimental Group: Larvae from the culture medium were transferred to 200 µl Phosphate Buffered Saline of pH 7 in transparent plastic tissue culture plate and exposed to UV-C radiation in the laminar airflow.

Experimental group A: Exposure of Second Instar Larvae to UV-C

Set No.	**Number of larvae**	**Distance from UV Lamp**	**Sets according to Exposure time in Seconds**			
			i.	**ii.**	**iii.**	**iv.**
I.	15	30 cm	30	60	90	120
II.	15	60 cm	30	60	90	120

Three subsets of each of set I and set II were carried out. All 15 larvae of each set, after exposure to UV light were again transferred in small glass vials containing *Drosophila* culture medium to continue their life cycle.

Experimental group B: Exposure of Third Instar Larvae to UV-C

Set No.	**Number of larvae**	**Distance from UV Lamp**	**Sets according Exposure time in Seconds**			
			i.	**ii.**	**iii.**	**iv.**
I.	15	30 cm	30	60	90	120
II.	15	60 cm	30	60	90	120

Three subsets of each of a set I and set II were carried out. Five 3rd instar larvae from each set were sacrificed for the dissection of salivary glands for the observation of polytene chromosome. Remaining ten larvae were transferred into small glass vials containing *Drosophila* culture medium for further development.

Dissection and Staining:

30 minutes after the exposure of 3rd instar larvae to UV light, five larvae from each set were used for dissection of salivary glands. Larvae were taken in 0.7 % NaCl solution and salivary glands were dissected under stereomicroscope.

After dissection, salivary glands were fixed in 45% Acetic Acid for 1 minute. After 1 minute, 45% Acetic Acid was blotted out using blotting paper.
A drop of 1 % Aceto-orcein stain was added on to the fixed salivary glands and kept for 15 minutes. Then coverslip was placed on it and the salivary glands were gently teased by pressing the coverslip. The polytene chromosomes of salivary gland cells were observed under light microscope at 100x objective.

RESULTS

Behavior of larvae: The larvae exposed to UV radiations became hyperactive during the period of exposure to UV-C radiations, appearing to escape away from radiations. The larvae from the nonirradiated i.e. control group appeared to be calm.

Texture of salivary glands: The texture of salivary glands of larvae exposed to UV radiations for 30 seconds and 60 seconds was like that of the non-irradiated control. Whereas, salivary glands of the larvae exposed to UV-C radiations for 90 seconds and 120 seconds were constricted and shrinked. The glands became brittle and rod shaped. They were floating in the fixative.

Effect on polytene chromosomes: The salivary gland cells from the control group exhibited typical polytene chromosome with bands and interbands. At certain regions puffing could be distinctly seen. There was a change in the puffing pattern in the polytene chromosome of the larvae exposed to UV-C radiation. As the duration of the exposure was increased there was a decrease in the number of puffs (Plate I).

Effect on the life cycle: The larvae from control group, i.e. the larvae which were not exposed to UV-C radiations exhibited a typical life cycle. The second instar was of three days duration, third instar was of two days duration. Pupal period was of four days and adult survived up to 40 days. The results are displayed in table No.1

IInd instar 30cm group: The results in graphical form are represented in Graph 1 and 2. The results are displayed in table No.2

When the larvae from IInd instar-30cm group were exposed to UV-C radiations for 30 seconds, 78% died in the second instar stage itself (Chart 1), whereas 22% larvae after 4-5 days, entered into the third instar stage and finally 11% entered into pupation but no adult emerged out. The larvae remained in an extended second instar, suggesting the impaired growth and development due to exposure to UV radiations. The duration of the second instar stage was increased up to 50% (Graph 2).

When the larvae from IInd instar-30cm group were exposed to UV-C radiations for 60 seconds, 91% died in the second instar stage (Chart 2) and 9% after 4-5 days in the second instar stage entered into the third instar and then entered into the pupation. However, no any fly emerged out.

When the larvae from the IInd instar-30cm group were exposed to UV-C radiations for 90 seconds, 91% larvae died in the second instar stage (Chart 2) and 9% larvae entered into the third instar which later died in the third instar itself. No larva entered in the pupation.

When the larvae from the IInd instar-30cm group were exposed to UV-C radiations for 120 seconds, there was 100% mortality (Chart 3) and all larvae died in the second instar.

The mortality was directly proportional to the exposure period.

IInd instar 60cm group: The results in graphical form are represented in Graph 1 and 2. The results are displayed in table No.2

When the second instar larvae were exposed to UV radiations at a distance of 60 cm for 30 seconds, 53% died in the second instar stage (Chart 4) and 47% larvae entered into third instar. Amongst these surviving larvae, 10% died in the third instar stage and 90 % larvae entered into pupation, however no pupa emerged out into adult. This indicates failure of pupal-adult transition. The organs of the adult fly failed to develop.

The second instar larvae when exposed to UV-C radiations at a distance of 60 cm for 60 seconds, surprisingly only 33% larvae died in the second instar (Chart 5); while 67% larvae entered into the third

instar. However, among the third instar stage, 73% larvae entered into the pupation, while 27% died in the third instar itself. However, no adult emerged out.

The second instar larvae when exposed to UV-C radiations at a distance of 60 cm for 90 seconds, 78% (Chart 6) larvae died in the second instar and 22% entered into the third instar. Among the surviving population, only 4% could enter into pupation, while 96% died in the third instar. However, no adult emerged out.

The second instar larvae when exposed to UV-C radiations at a distance of 60 cm for 120 seconds, 89% larvae died in the second instar (Chart 7) and11% larvae completed second instar in 1-3 days and entered into third instar. However, all these larvae died in the third instar.

The mortality was directly proportional to duration of exposure and hormesis was observed at a distance of 60cm and 60 seconds exposure.

IIIrd instar 30cm group: The results in graphical form are represented in Graph 1 and 3. The results are displayed in table No.4

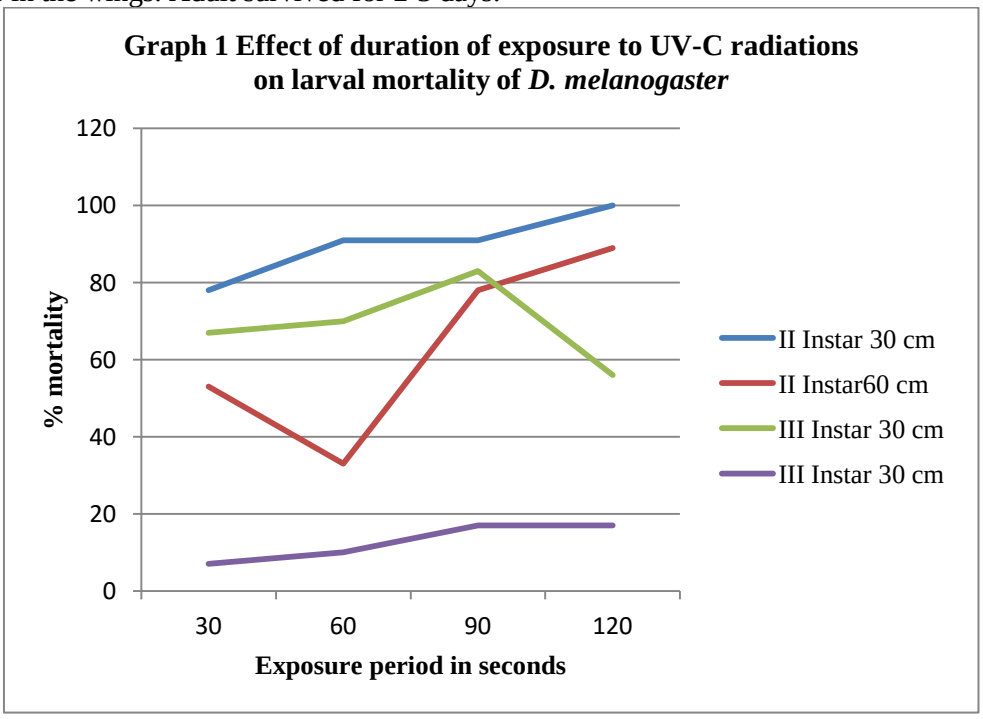
When the larvae from the IIIrd instar were exposed to UV-C radiations for 30 seconds, 67% larvae died in the third instar (Chart 8) and 33% larvae completed third instar for 1- 2 days of duration and entered into the pupation. Pupae lived for 2-7 days and then all pupae died. No any adult emerged out.

When the larvae from the IIIrd instar were exposed to UV radiations for 60 seconds,70% larvae died in the third instar (Chart 9) and 30% larvae survived in third instar for 1-2 days and entered into the pupation. Among the pupae, 78% pupae died and 22% adult emerged out. They had a life span of 2 days.

When the larvae from the IIIrd instar were exposed to UV radiations for 90 seconds, 83% larvae died in third instar (Chart 10)and 17% larvae survived in the third instar for 1-2 days. Among them 100% larvae entered into pupation, however, all the pupae died and no adult emerged out.

When the larvae from the IIIrd instar group were exposed to UV-C radiations for 120 seconds, surprisingly only 56% larvae died in the third instar (Chart 11) and44% larvae survived in the third instar for a day. There was a shortening of the length of the third instar. Among them 100% larvae entered into the pupation. 62% pupae died and 38% pupae survived. 38% adult emerged out. The adults were having deformities in the wings. Adult survived for 2-3 days.

Graph 1 Effect of duration of exposure to UV-C radiations on larval mortality of *D. melanogaster*

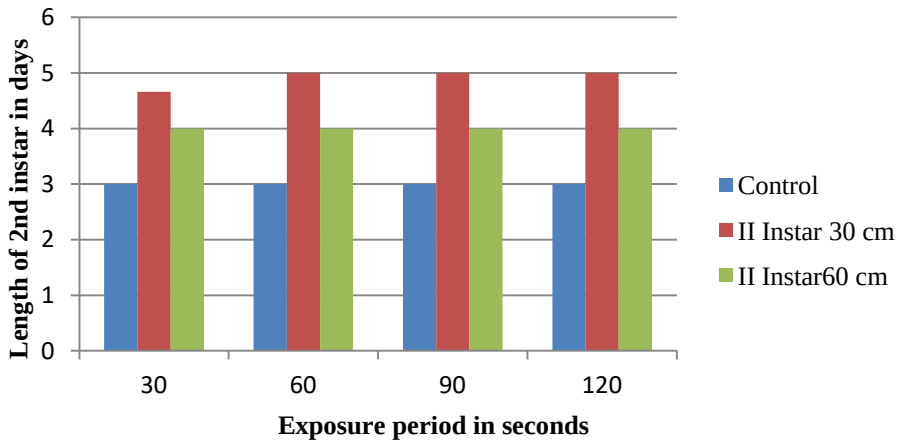


IIIrd instar 60cm group: The results in graphical form are represented in Graph 1 and 3. The results are displayed in table No.5

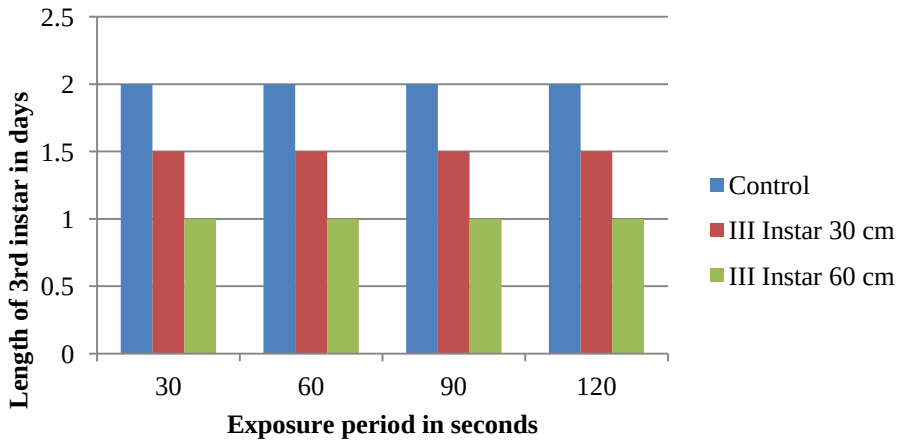
When the larvae from the IIIrd instar were exposed to UV-C radiations for 30 seconds, 7% larvae died in the 3rd instar stage after a day(Chart 12), and 93% larvae entered into the pupation. From these, 53% pupae died and 47% adult flies emerged out from the pupal case. These adults had a life span from 1 to 7 days. The adults were with deformities in the wings.

When the larvae from the IIIrd instar were exposed to UV-C radiations for 60 seconds, 90% larvae entered into the pupation (Chart 13)and 10 % larvae died in third instar stage after a day. From those pupae 93% pupae died and 7% adult emerged out having defective wings and had a life span of three days.

Graph 2 Effect of duration of exposure to UV-C radiations on the length of second instar of *D. melanogaster*

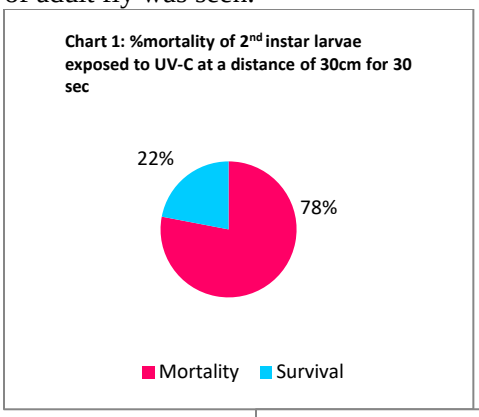


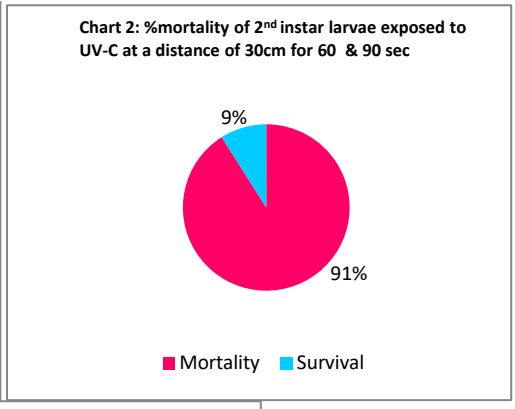
Graph 3 Effect of duration of exposure to UV-C radiations on the length of third instar of *D. melanogaster*

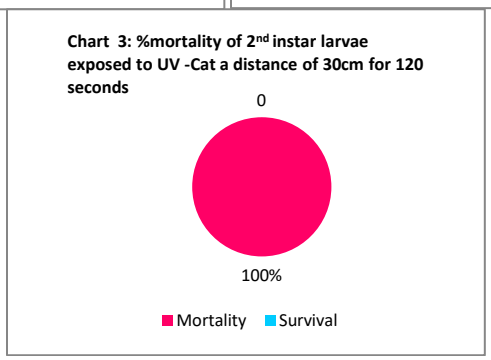


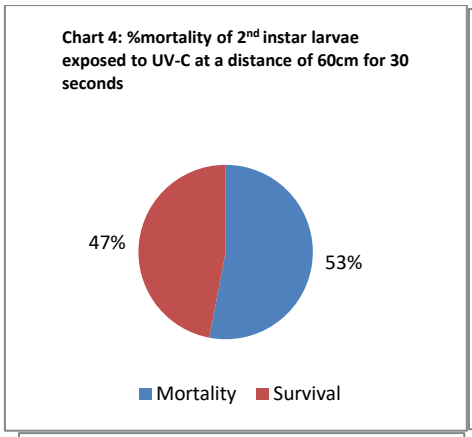
When the larvae from the IIIrd instar were exposed to UV-C radiations for 90 seconds, after a day 17% larvae died (Chart 14) and 83% larvae entered into the pupation and all pupae died. No adult fly emerged out in this set.

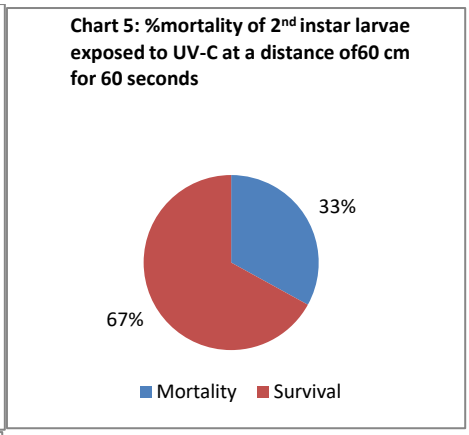
When the larvae from the III[rd] instar were exposed to UV-C radiations for 120 seconds, after a day 17% larvae died in the third instar (Chart 14) and 83% larvae became pupae. All the pupae died and no emergence of adult fly was seen.

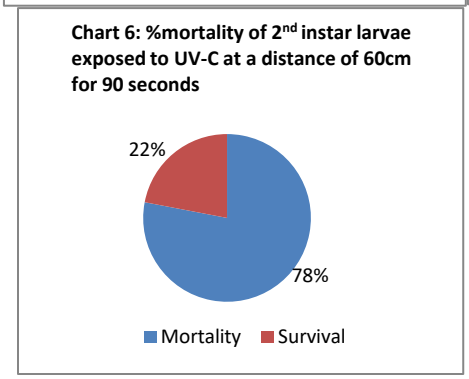


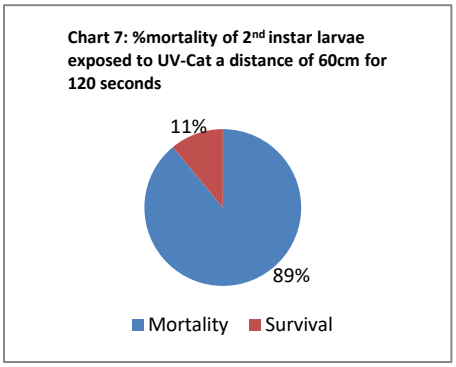












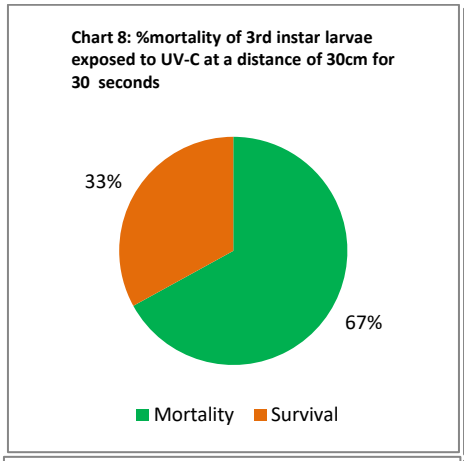


Chart 8: %mortality of 3rd instar larvae exposed to UV-C at a distance of 30cm for 30 seconds
33%
67%
Mortality
Survival

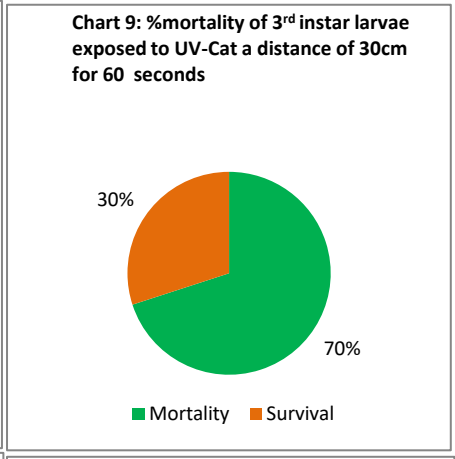


Chart 9: %mortality of 3rd instar larvae exposed to UV-Cat a distance of 30cm for 60 seconds
30%
70%
Mortality
Survival

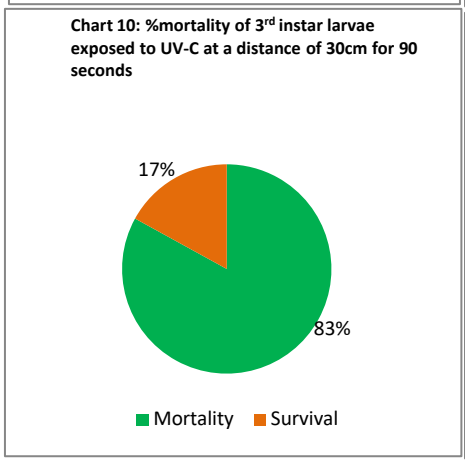


Chart 10: %mortality of 3rd instar larvae exposed to UV-C at a distance of 30cm for 90 seconds
17%
83%
Mortality
Survival

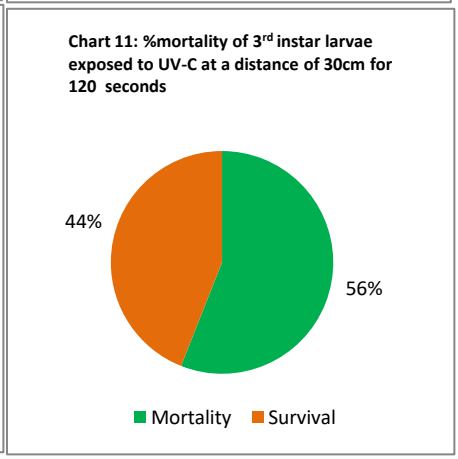


Chart 11: %mortality of 3rd instar larvae exposed to UV-C at a distance of 30cm for 120 seconds
44%
56%
Mortality
Survival

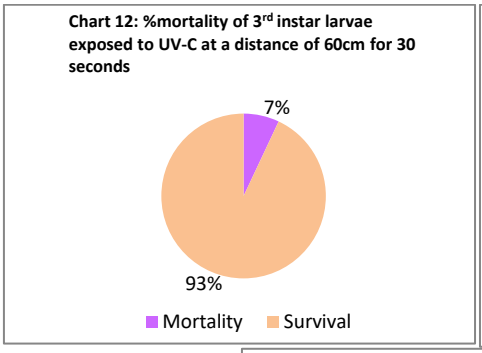


Chart 12: %mortality of 3rd instar larvae exposed to UV-C at a distance of 60cm for 30 seconds
7%
93%
Mortality
Survival

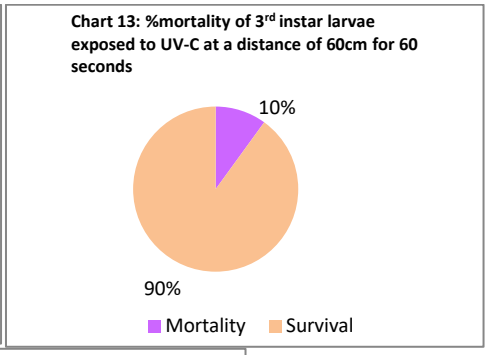


Chart 13: %mortality of 3rd instar larvae exposed to UV-C at a distance of 60cm for 60 seconds
10%
90%
Mortality
Survival

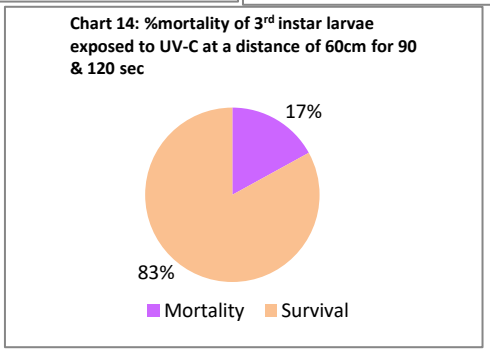
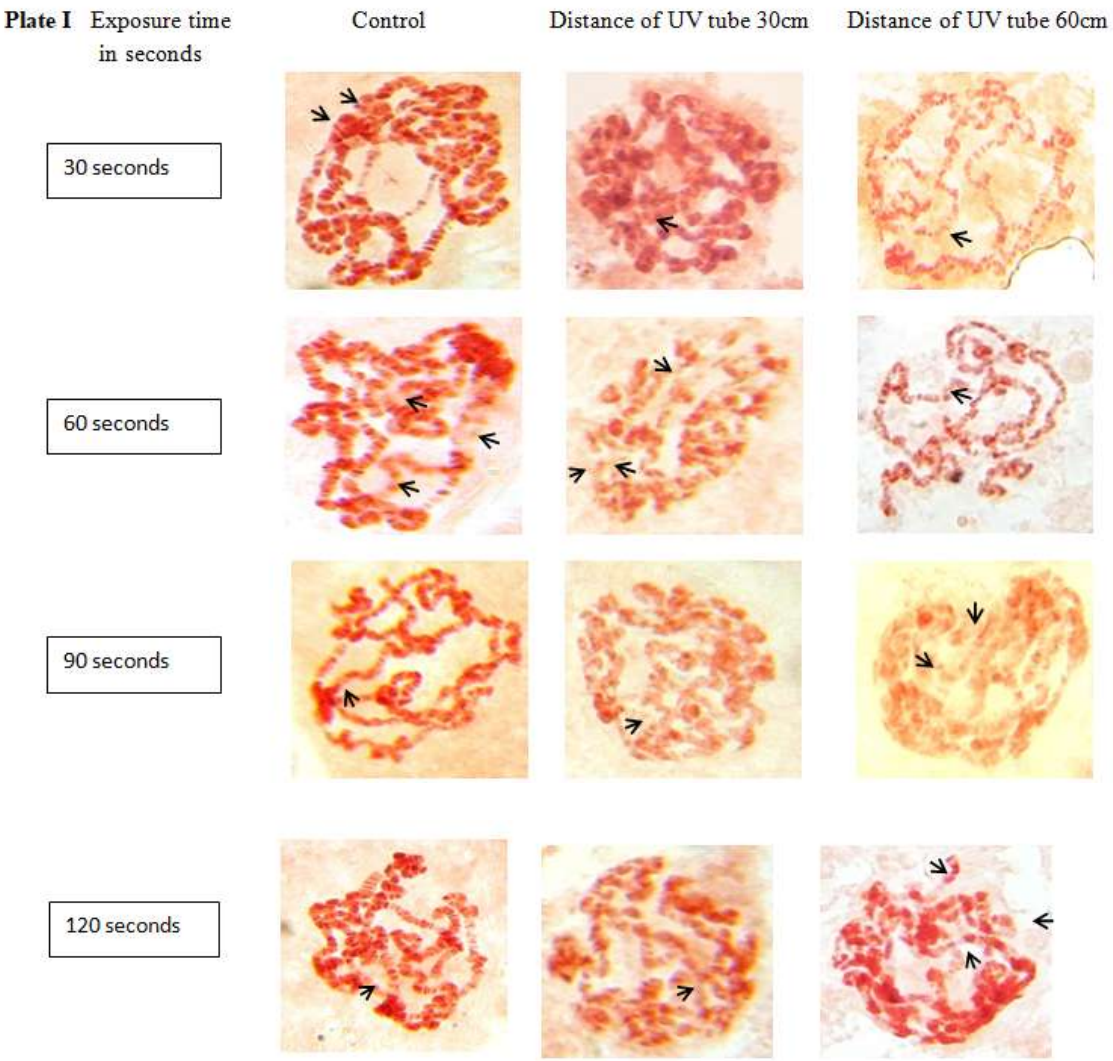


Chart 14: %mortality of 3rd instar larvae exposed to UV-C at a distance of 60cm for 90 & 120 sec
17%
83%
Mortality
Survival



↗ *Indicate puffs. Darkly stained regions are bands and lightly stained regions are interbands.*

Dorsal view			
Lateral view			
Ventral view			

DISCUSSION

The alterations in the staining property of the polytene chromosomes after UV irradiation indicate altered pattern of DNA packaging. The light bands i.e. interbands indicate loosely packed DNA involved in transcription. According to Gersh (1975) interbands are considered to be the regions in the chromosome having genes in a steady state of activity. Paul (1972) described that the interband regions are essentially polymerase-binding sites and transcription continues into band regions. The increased intensity of the staining at certain regions i.e. bands indicates tight compaction like heterochromatinization in which DNA is not transcriptionally active. Zhimulev (1999) and Zhimulev *et al.*(2014) There were certain puffs seen in the polytene chromosomes of UV irradiated larvae which were exposed to UV radiations at a distance of 60cm for 120 seconds. Bands are sites of inactivated genes Increase in the staining intensity indicates an increase in the heterochromatinization and thus transcriptional inactivation. The degree of heterochromatinization was directly proportional to the duration of the exposure to UV radiations. This alteration in the puffing pattern was phenotypically reflected in morphology of the wings. The deformities in the wings are the results of mutation in the genes responsible for development of the wings. UV induced mutagenic activity was studied by Negishi *et al.*, (2001) in *Drosophila* by spot test in which they have observed mutant wings, hair colonies (spots) on the wings of adult flies obtained from the treated larvae.

Increased percentage of survival of larvae from IInd instar 60cm- 60 seconds exposure group (67%) as compared to IInd instar 60cm-30 seconds exposure group (47%) could be a hormetic dose to trigger the stress response pathways. Hormesis is an adaptive response of cells and organisms to a moderate stress (Mattson 2008). Exposure to UV radiations for 60 seconds might have activated the adaptive mechanisms to combat and repair the harmful effects of UV radiations. Therefore, there might be increased percentage of surviving population even after 60 seconds of exposure as compared to 30 seconds exposure group. However, among the third instar larvae from IInd instar 30cm-60 seconds exposure group, 73% larvae could enter into pupation and 27% larvae died in the third instar itself. This suggests, even though hormesis could help survival of larvae but there might be certain damages which could not repair and thus inhibited the progression of 27% larvae which could successfully completed the second instar but failed to complete third instar. Organisms may die in unfavourable conditions and to survive in the unfavourable conditions. They have evolved various strategies. Some organisms, including nematodes, survive in unfavourable conditions by undergoing developmental arrest (Diaz and Viney 2015). *Caenorhabditis elegans* develops into the arrested dauer larva form in unfavourable conditions (Golden and Riddle 1984). The increased length of second instar in the larvae exposed to UV radiations is an adaptive response. Developmental arrest is a well-documented example, among invertebrates and is common among insects and nematodes, as well as other invertebrates (Denlinger 2002, Chen and Glazer 2004).

Interestingly, contrary to this, there was a decrease in the length of third instar in UV irradiated group. After completion of the third instar, there is pupation. So as to ensure the survival and escape from the stressful condition, there may be an intrinsic mechanism to accelerate the speed development. The ability of an organism to modulate the speed of development as per the environmental conditions is termed as developmental plasticity, which is exhibited by nematodes, insects and amphibians (Gomez-Mestre *et al.* 2013, Bekhet *et al.* 2014, Diaz and Viney 2015). This could be the reason of very negligible rate of eclosion of adults from the pupal case. Moreover, the adults that emerged out were phenotypically abnormal with wing mutations. There may be mutations in the metabolic pathways and abnormalities in the internal organs which could not be seen phenotypically. Therefore, the larvae died at an early age as compared to control. The rapid development came at the cost of a much shorter life span of 4 days as compared to 40 days in nonirradiated control. To determine the physiological effects UVC radiation on *Drosophila*, Masatoshi *et al.* (2014) exposed the drosophila during the larval and pupal stages when the organisms DNA is most vulnerable. UVC radiation induced a statistically significant mutation risk which was proportional to the duration of exposed radiation. Janssens and Stocks (2018) demonstrated that

accelerated larval development was associated with an increase in oxidative damage to lipids, proteins and DNA. This may be one of the reasons of shortening of adult life span in UV irradiated group.

REFERENCES

Bekhet GA, Abdou HA, Dekinesh SA, Hussein HA and Sebiae SS (2014). Biological factors controlling developmental duration, growth and metamorphosis of the larval green toad, *Bufo viridis viridis. J Basic & Appl Zoo*, **67** 67–82

Chen S and Glazer I (2004). Effect of rapid and gradual increase of osmotic stress on survival of entomopathogenic nematodes. *Phytoparasitica*, **32** 486–497.

Clever U and Karlson P (1960). Induction of puff changes in the salivary gland chromosomes of Chironomus tentans by ecdysone. *Exp Cell Res*, **20** 623–626.

Denlinger DL (2002). Regulation of diapause. *Annu. Rev. Entomol*,**47** 93–122.

Diaz SA and Viney M (2015). The evolution of plasticity of dauer larva developmental arrest in the nematode *Caenorhabditis elegans*. *Ecology and Evolution*, **5(6)** 1343-1353.

Gersh ES (1975). Sites of gene activity and of inactive genes in polytene chromosomes of diptera. *J Theor Biol*, **50(2)** 413–28.

Golden JW and Riddle DL (1984). The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food and temperature. *Dev. Biol*, **102** 368–378

Gomez-Mestre I, Kulkarni S, Buchholz DR (2013). Mechanisms and Consequences of Developmental Acceleration in Tadpoles Responding to Pond Drying. *PLoS ONE*, **8(12)** e84266. https://doi.org/10.1371/journal.pone.0084266

Janssens L and Stocks R(2018). Rapid larval development under time stress reduces adult life span through increasing oxidative damage. *Functional Ecol*, **32(4)** 1036-1045

Kaufmann BP (1952). Cytochemical studies of the action of trypsin 1 Digestion of salivary gland chromosomes. *Proc Natl Acad Sci USA*.**38(5)** 464-468.

Mattson MP (2008). Hormesis Defined. *Ageing Res Rev*, **7(1)** 1-7.

Negishi T, Nagaoka C, Hayatsu H, Suzuki K, Hara T, Kubota M, Watanabe M and Hieda, K (2001). Somatic-cell mutation induced by UVA and monochromatic UV radiation in repair-proficient and -deficient Drosophila melanogaster. *Photochem. Photobiol*, **73(5)** 493-498.

Paul J (1972). General theory of chromosomes structure and gene activation in eukaryotes. *Nature* **238** 444–446.

Rittosa F(1962). A new puffing pattern induced by temperature shock and DNP in *Drosophila.Experentia***18** 571-573.

Tissieres A, Mitchell HK, Tracy UM (1974). Protein synthesis in salivary glands of *Drosophila melanogaster* relation to chromosome puffs. *J. Mol. Biol*, **84(3)** 89-398.

Vlasova IE and Zhimulev IF (1988). Effect of transcription on RNA synthesis in the polytene chromosome of *D. melanogaster*. *Tsitologiia*, **30(5)** 568-72.

Zhimulev IF (1999). Genetic organization of polytene chromosomes. *Adv Genet*, **39** 1–589.

Zhimulev IF and Koryakov DE (2009). Polytene chromosome. *Encyclopedia of life science*, (ELC). DOI:10.1002/9780470015902.a0001183.pub2

Zhimulev IF, Zhimulev IF, Zykova TY, Goncharov FP, Khoroshko VA, Demakova OV, Semeshin VF, Pokholkova GV, Boldyreva LV, Demidova DS Babenko VN Demakov SA and Belyaeva ES (2014). Genetic organization of interphase chromosome bands and interbands in *Drosophila melanogaster*. *PLoS ONE*, **9(7)** e101631. doi:10.1371/journal.pone.0101631.