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Effect of UV-B Radiation on Chromosomal Organisation and Biochemical Constituents of *Coriandrum sativum* L.

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Abstract

Stratospheric ozone depletion due to pollution has long been recognised as a threat to human health as well as to the earth's ecosystem. UV-B radiation being a part of solar electromagnetic radiations reaches earth's surface at an elevated level due to ozone depletion thereby imparting its ill impacts on flora and fauna. Hence, keeping UV-B as an important key of environmental factors inducing stress and disturbance on biodiversity the present experimental work has been designed to study the effect of UV-B on chromosomal organisation and biochemical contents of *Coriandrum sativum* L. Four sets have been maintained *viz.* set A for control, set B for 20 minutes treatment, set C for 40 minutes and set D for 60 minutes. All sets, excluding A, were irradiated with supplemental UV-B radiation along with visible lights for 1st, 2nd and 3rd day treatment after seed germination. For cytological study, irradiated germinated seeds of each set (B, C and D) were fixed in carnoy's fixative along with control (set A). For biochemical study few germinated irradiated seeds of each set (A, B, C and D) were transplanted in field for further growth and development. It was found that lower doses are stimulatory in its action while as the treatment time along with duration was increased, the rate of Mitotic Index (MI %) were decreased and Total Abnormality Percentage (TAB %) was increased in all the treated sets as compared to control set. Regarding biochemical constituents, the proline content was increased while photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoid) and total carbohydrate contents were declined at higher treatment doses. Hence, it was concluded that low levels of UV-B exposures are not inhibitory in its action and promote metabolic processes on the way in contrary high levels of elevated UV-B radiation are genotoxic and causes cell disruptions by inducing chromosomal aberrations increasing TAB (%), declined MI (%), decreased photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoid) and carbohydrates while in response to stress, *Coriandrum* adapts protective mechanism thereby elevated proline accumulation.

Key words- UV-B radiation, Mitotic Index (MI %), Total Abnormality Percentage (TAB %), Proline, Photosynthetic pigments, Carbohydrate, *Coriandrum sativum* L.

1. Introduction

Biodiversity in plants with its variety and variability contains enormous potential in meeting humans growing economic needs. Several hundreds of species have served as bio-resources of great potential, during the course of time as human civilization grows. Human intervention through centuries for food, fibre, shelter and medicine has altered the dynamic relationship among the various ecosystems leading to disturbed natures functioning. Ozone depletion is an outcome of modern scientific and technological advances that results into more penetration of Ultra-Violet (UV) radiation on earth's surface thereby imparting ill-effects on biological components. UV radiation is part of the sun's electromagnetic radiation, classified into UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm). UV-B is of particular interest because this wavelength represents near about 1.5% of the total spectrum but can induce a variety of damaging effects. As plants are the primary producers in an ecosystem and form the basis of bioactive systems hence they are more threatened to adverse effects of radiation. The sessile lifestyle of plants particularly necessitates the evolution of a number of strategies for adaptation to an ever-changing environment. Of utmost importance is light, which is not only a source of energy but also provides informational signals concerning the surrounding natural setting, influencing plant growth and development. UV-B can cause severe deleterious effects in biological organisms, despite representing only a small amount of total solar radiations. UV-B can adversely react with many biological molecules including amino acids, nucleic acids, proteins, lipids and elicits stress responses at molecular, cellular and whole organism levels. Garinis *et al.* (2005) stated that UV-B can damage DNA by creating cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidine dimer, which can lead to point and break mutations if not correctly repaired. Certain responses were elicited by plants including inhibition of hypocotyls

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elongation and root growth, cotyledon opening stomatal closure and anatomical changes associated with UV-B protection. Different species have different responses to the level of UV-B irradiation (Matthew *et al.*, 1996; Skorska 1996 a, b). The changes in plants morphology induced by UV-B may affect competition for light (Barnes *et al*., 1988). The negative effect of UV-B radiation results in deformed morphological parameters. Exposure to UV-B decreased plant height, leaf area and plant dry weight increased auxiliary branching and leaf curling (Dai *et al*., 1995; Greenberg *et al*., 1997; Furness *et al.*, 1999). Dai *et al.*, (1995) reported that after a few weeks of UV-B exposure, leaf area and plant dry weight of rice were significantly reduced. High levels of UV-B clearly decreased the relative growth rate and nitrogen productivity, as leaf area ratio, leaf area productivity and leaf nitrogen productivity were all decreased (Zuk-Golaszewska *et al*., 2003). Research studies had traditionally focussed on staple crops while little attention has been given to minor crops. The limited information available on many important and frequently basic aspects of underutilized crops hinders their development and their sustainable conservation.

Coriandrum sativum L. commonly called as Coriander is an important spice crop of Apiaceae possessing 2n=22 chromosomes having diverse economical uses. It has been widely used as a culinary ingredient as well as traditional remedies for the treatment of different disorders, like hyperglyceamia, antispasmodic, carminative, stimulant, cytotoxic, lipolytic, fungicidal and stomachic compound. Coriander also possesses hypolipidemic, antibacterial, antimutagenic activity, insecticidal and aflatoxin controlling effects primarily due to its essential bioactive compounds.

Considering the aforesaid features, in the present study coriander is selected as an experimental model as it is easily available throughout the year. Hence, the present research work has been designed to screen out the effect of UV-B radiation on biochemical constituents and chromosomal organisation of coriander. Chromosomal study was done in root meristems of coriander as these are the first to emerge and interact with environment. After an extensive review of literature, it was found that the present study is the first one on coriander of its kind, which will elicit further light on this subject.

2. Material and Methods

2.1. Seed Procurement

Seeds of Coriander were collected from research institute *viz.*, CRSS, Jagudan, Gujarat, *variety* CO-2. Seeds were consistently selected and proper washing was done for 10 minutes with distilled water and 0.1% $HgCl_2$ was utilized for sterilization.

2.2. Experimental Design

Fresh Coriander seeds were pre-soaked in distilled water for 12 hours and kept in seed germinator at 25 ± 20^0C with humidity 60-80% in sterilized petriplates with wet whatmann filter paper. Whatmann filter papers were regularly allowed to change and distilled water was sprinkled periodically. Four sets were prepared, i.e., Set A for control, Set B for 20 minute, Set C for 40 minute and Set D for 60 minute. The experiment was conducted by keeping nine replicates.

2.3. UV-B Treatment

Sets B, C and D having early roots of length between 5mm to 25mm were irradiated with fluorescent UV-B (280-320 nm) lamps along with visible light. Firstly, 9 replicates of each set were irradiated for1 day of time duration 20, 40, 60 minute along with supplementation of visible light. Out of which three replicates from each set were removed off from UV-B chamber for fixation. On second day next 6 replicates of each set were again irradiated for respective time duration and three replicates were removed off for fixations. For remaining three replicate out of 9 same procedures was followed for each set. Set A was remained untreated as standard. Radiation was started in the morning on each day.

2.4. Fixation

After one hour of recovery, all the irradiated germinated seeds of set B, C, D along with control set A were fixed in Carnoy's fixative in their labelled bottles for cytological study. After 24 hours of fixation, Carnoy's fixative was decanted off and sets were transferred into bottles containing only 90% alcohol.

2.5. Mitotic Preparation

For cytological study, squash technique was applied. Staining was done with 2% acetocarmine for half hours. Slides were prepared and cells were observed and snapped under Nikon Research Electron Microscope using Olympus PCTV Vision Software. Nearly 10 microscopic field views were recorded from each slide. Data were scored from 3 roots of each replicates.

2.6. Formula Used for Scoring of Data

To calculate Mitotic Index (MI %) and Total Abnormality Percentage (TAB %), the following formulas were followed **:-**

Mitotic index (MI) % = (Total number of dividing cells/Total number of observed cells)*100

Total abnormality percentage (TAB) % = (Total number of abnormal cells/Total number of observed cells)*100

2.7. Biochemical Analysis

2.7.1. Estimation of Proline

Total proline was estimated by using Bates *et al.* (1973) method.

2.7.2. Carbohydrate Estimation

Determination of total carbohydrate was done by using Hedge and Hofreiter (1962) method and absorption was taken at 630 nm.

2.7.3. Determination of Photosynthetic Pigments

Chlorophyll a, b, and carotenoids were extracted from fresh leaves of coriander with 80% acetone and determined according to Lichtenthaler method (1987).

2.8. Statistical Analysis

The data obtained were analysed using statistical software, SPSS 16 and means were compared using Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$). All the results were expressed in form of Mean ± Standard Error. The graph was plotted by using Sigmaplot 10.00 software.

3. Results

3.1. Cytological Observations

Mitosis was found to be normal in the control sets and showed regular arrangements of chromosomes at metaphase (2n=22) and having equal separation (22:22) at anaphase. However, various chromosomal abnormalities were recorded in root meristems of sets B, C, D raised for supplemental UV-B treatments.

3.1.1. Effect on Mitotic Index (MI)

The collected data infer that lower doses of UV-B are less significant at 1st day treatment while imposes impairment in regular cell division at 3rd day treatment. But higher doses of UV-B treatment caused a strong mitodepressive effect on meristematic cells of root tips of Coriander documented in table 1. In control set, MI was 12.64 ± 0.14^{a} however, the rate of MI (%) declined as the treatment duration increased. On 1st day at 20 min treatment MI was 11.42 ± 0.26^{b} which was reduced to 10.41 ± 0.13^{b} on 3rd day treatment that envisaged less effect of lower doses of UV-B treatment. Meanwhile, at 60 min treatment MI was recorded to be 8.19 ± 0.33^{d} on 1st day compared to 20 min it shows steep declined and on 3rd day MI was steadily declined to 6.75 ± 0.11^{d}. Hence, from above results, it was clearly inferred that higher doses of UV-B treatment are mitodepressive. The pattern of declined MI along with UV-B treatment was shown in Figure 2 and Table 2.

Table 1. Total account of Mitotic Index and Total abnormality percentage after UV-B radiations on root meristems of *Coriandrum sativum* L.

TREATMENT (UV-B)*	DOSES (Minutes)	MI (%)** (Mean ± S.E.)	TAB (%) *** (Mean ± S.E.)
1st DAY	Control	12.64 ± 0.19^{a}	-
	20	11.42 ± 0.26^{b}	2.53 ± 0.14^{c}
	40	10.12 ± 0.24^{c}	3.31 ± 0.26^{ab}
	60	8.19 ± 0.34^{d}	4.16 ± 0.32^{a}
2nd DAY	Control	12.64 ± 0.14^{a}	-
	20	11.21 ± 0.38^{b}	3.54 ± 0.12^{c}
	40	9.13 ± 0.38^{c}	4.84 ± 0.31^{b}
	60	7.74 ± 0.02^{d}	6.34 ± 0.12^{a}
3rd DAY	Control	12.64±0.14a	-
	20	10.41 ± 0.13^{b}	4.64 ± 0.33^{c}
	40	8.43 ± 0.11^{c}	6.70 ± 0.26^{b}
	60	6.75 ± 0.11^{d}	8.58 ± 0.37^{a}

Abbreviations: UV-B- Ultraviolet B radiation, MI (%) **- Mitotic Index, TAB (%) ***- Total abnormality percentage. Means are followed by lowercase letter is statistically significant at $p<0.05$.

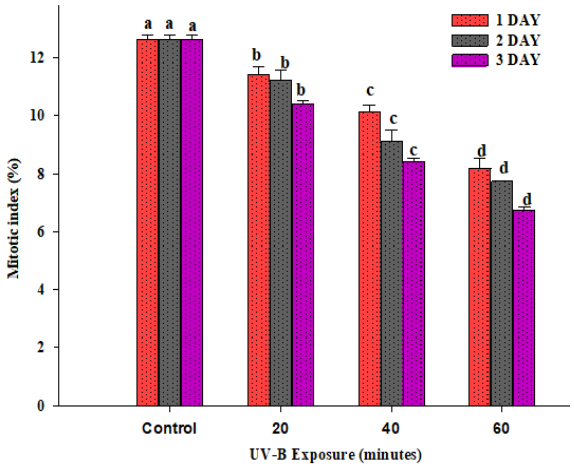

Figure 2. Comparative trend of MI on different doses of UV-B treatment on root meristems of *Coriandrum sativum* L.

Table 2. Metaphasic and Anaphasic Abnormalities induced by UV-B radiations in root meristems of *Coriandrum sativum* L.

TREATMENT (UV-B)	DOSES (Minutes)	METAPHASIC ABNORMALITY (%) (Mean ± S.E.)					ANAPHASIC ABNORMALITY (%) (Mean ± S.E.)				
		ST	CM	PR	SC	UN	BRDG	ST	UN	LG	OTH
1st DAY	20	0.63 ± 0.12^{a}	0.13 ± 0.12^{a}	0.12 ± 0.12^{a}	0.25 ± 0.12^{a}	0.25 ± 0.12^{a}	0.50 ± 0.12^{a}	0.12 ± 0.12^{a}	0.38 ± 0.21^{a}	0.00±0.00	0.12 ± 0.12^{a}
	40	0.51 ± 0.09^{a}	0.39 ± 0.01^{a}	0.14 ± 0.13^{a}	0.39 ± 0.19^{a}	0.13 ± 0.10^{a}	0.27 ± 0.13^{a}	0.27 ± 0.13^{a}	0.51 ± 0.99^{a}	0.37 ± 0.20^{a}	0.27 ± 0.13^{a}
	60	1.05 ± 0.25^{a}	0.21 ± 0.10^{a}	0.23 ± 0.11^{a}	0.45 ± 0.96^{a}	0.34 ± 0.19^{a}	0.69 ± 0.19^{a}	0.00±0.00	0.32 ± 0.18^{a}	O.36±0.22^{a}	0.45 ± 0.10^{a}
2nd DAY	20	1.13 ± 0.21^{ab}	0.63 ± 0.12^{a}	0.12 ± 0.11^{b}	0.50 ± 0.12^{ab}	0.50 ± 0.13^{a}	0.38 ± 0.02^{b}	0.00±0.00	0.25 ± 0.12^{ab}	0.00±0.00	0.00±0.00
	40	0.65 ± 0.14^{b}	0.36 ± 0.20^{a}	1.27 ± 0.09^{a}	0.13 ± 0.12^{b}	0.50 ± 0.11^{a}	0.13 ± 0.12^{b}	0.13 ± 0.12^{a}	0.64 ± 0.13^{a}	1.02 ± 0.12^{a}	0.00±0.00
	60	1.67 ± 0.31^{a}	0.76 ± 0.03^{a}	0.23 ± 0.21^{b}	0.63 ± 0.11^{a}	0.26 ± 0.12^{a}	0.18 ± 0.19^{a}	0.24 ± 0.12^{a}	0.12 ± 0.11^{a}	0.36 ± 0.20^{b}	0.26 ± 0.13^{a}
3rd DAY	20	0.63 ± 0.12^{b}	0.50 ± 0.25^{a}	0.37 ± 0.21^{a}	0.37 ± 0.21^{a}	0.25 ± 0.12^{a}	0.62 ± 0.12^{b}	0.62 ± 0.12^{a}	0.37 ± 0.21^{a}	0.62 ± 0.12^{a}	0.25 ± 0.12^{b}
	40	1.88 ± 0.35^{a}	0.41 ± 0.24^{a}	0.00 ± 0.00^{a}	0.54 ± 0.15^{a}	0.66 ± 0.25^{a}	1.62 ± 0.27^{a}	0.39 ± 0.22^{a}	0.40 ± 0.02^{a}	0.53 ± 0.13^{a}	0.26 ± 0.21^{b}
	60	1.94 ± 0.11^{a}	0.91 ± 0.11^{a}	0.34 ± 0.19^{a}	0.34 ± 0.01^{a}	0.46 ± 0.30^{a}	1.83 ± 0.11^{a}	0.45 ± 0.29^{a}	0.57±0.23a	0.57 ± 0.11^{a}	1.14 ± 0.10^{a}

*****Abbreviations: **ST**-Stickiness, **CM**-C-metaphase, **PR**-Precocious movement, **SC**- Scattering, **UN**-Unorientation, **BRDG**-Bridge, **LG**-Laggards, **OTH**-Other abnormalities. Means followed by lowercase letter is statistically significant at $p<0.05$.

3.1.2. Effect on Chromosomal Organization

From present study it was recorded that as the treatment duration increases the rate of chromosomal aberrations was also increased. Mitotic disturbances after UV-B treatments were dominantly confined at metaphase and anaphase as shown in Figure 1. The rate of chromosomal aberrations was documented in the form of Total Abnormality Percentage (TAB %) in Table 1. It was increased from 2.53 ± 0.13^{c} to 4.16 ± 0.32^{a} on 1st day, 3.54 ± 0.12^{c} to 6.34 ± 0.12^{a} on 2nd day and 4.64 ± 0.33^{c} to 8.58 ± 0.37^{a} on 3rd day, respectively. From the results, it was inferred that higher doses are more genotoxic and impose more aberrations in treated sets as compared to lower doses and control. The wide range of chromosomal aberrations observed were precocious movement of chromosomes (Figure 1.D), c-metaphase (Figure 1.E), clumping (Figure 1.F), scattering (Figure 1.G) at metaphase while forward movement (Figure 1.H), unorientation (Figure 1.I), laggard (Figure 1.J) and multiple bridge (Figure 1.K) at anaphase, respectively. The percentage of chromosomal aberrations increased as the time and duration of supplemental UV-B treatment was increased. Stickiness was found to be most dominant anomaly (1.94 ± 0.11^{a}) induced by UV-B at metaphase recorded in 3rd day treatment at 60 minutes while bridges were recorded as 1.83 ± 0.11^{a} on 3rd day treatment, precocious movement (1.27 ± 0.09^{a}) and laggards were next to induced at a higher frequency in 2nd day treatment at 40 minutes duration. UV-B induces lesser telophasic and other cellular abnormalities. The trend of increasing Total Abnormality Percentage (TAB %) along with UV-B treatment was shown in Figure 3.

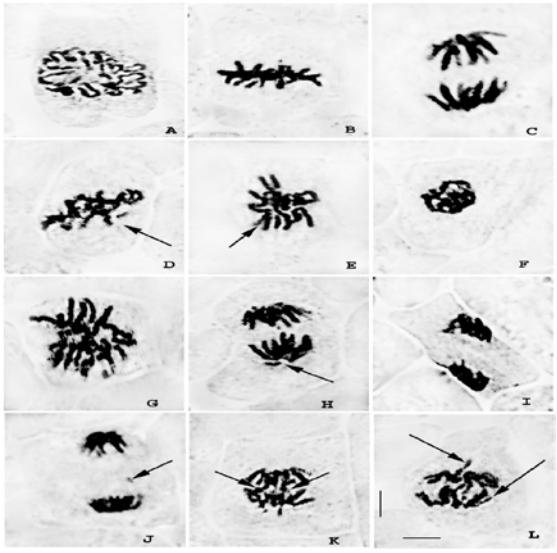

Figure 1. Different types of chromosomal aberrations induced by UV-B irradiation in root meristems of *Coriandrum sativum* L.- **Legends of figure- A:** Normal prophase**, B:** Normal metaphase **(2n=22), C:** Normal anaphase **(22:22), D:** Precocious movement of chromosomes with unorientation at metaphase**, E:** C-metaphase, **F**: Clumping at metaphase, **G:** Scattered chromosomes at metaphase, **H**: Forward movement at anaphase, **I**: Unorientation at metaphase, **J**: Laggard at anaphase, **K**: Multiple bridge at anaphase, **L**: Broken bridge with forward movement at anaphase.**[Scale bar: Length (1 cm) = 3.7 µm, Width (1 cm) =2.2 µm]**

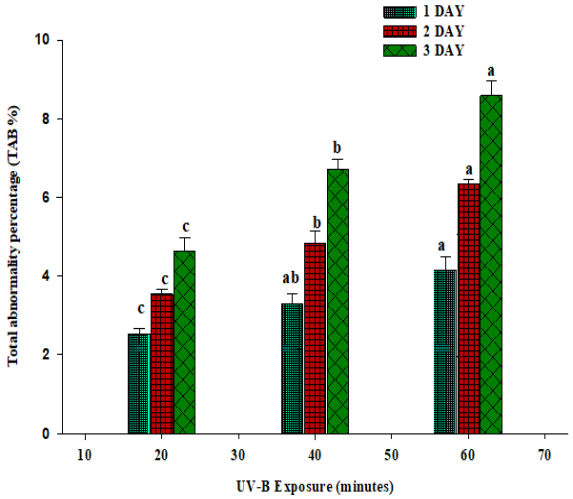

Figure 3.Comparative trend abnormalities induced at different doses of UV-B treatment on root meristems of *Coriandrum sativum* L.

3.2. Biochemical Observations

3.2.1. Effect on Proline Content

A sharp increase in levels of proline was observed in leaves of Coriander upon exposure to UV-B radiations. Along with the increase of time and duration of UV-B treatment the accumulation of proline content was also increased to 4.97 ± 0.70^{a} as compared to control 2.56 ± 0.36^{a}. Figure 4 shows the trend of proline content on 1st, 2nd and 3rd day UV-B treatment.

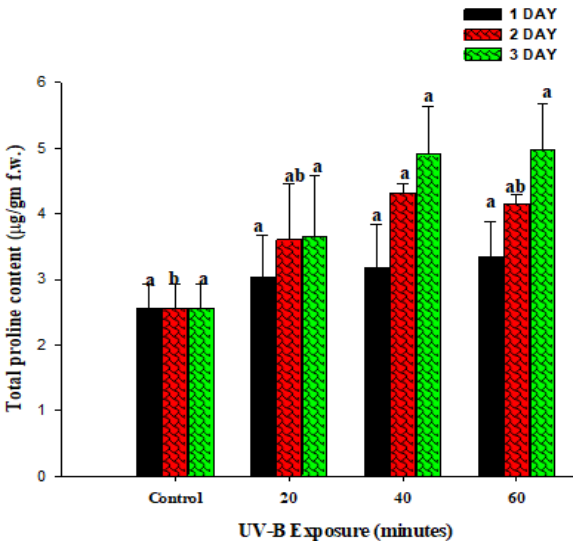

Figure 4. Showing an account of total proline content (mg/gm fw.) after UV-B treatment on 1st 2nd and 3rd day on *Coriandrum sativum* L.

3.2.2. Effect on Carbohydrate Content

There is a maximum level of increased carbohydrate content 6.51 ± 0.22^{a} at 60 minute in coriander over control 5.90 ± 0.34^{ab} on 1st day UV-B and it declined on 3rd day exposure. The trend of carbohydrate content in Coriander has been shown in Figure 5.

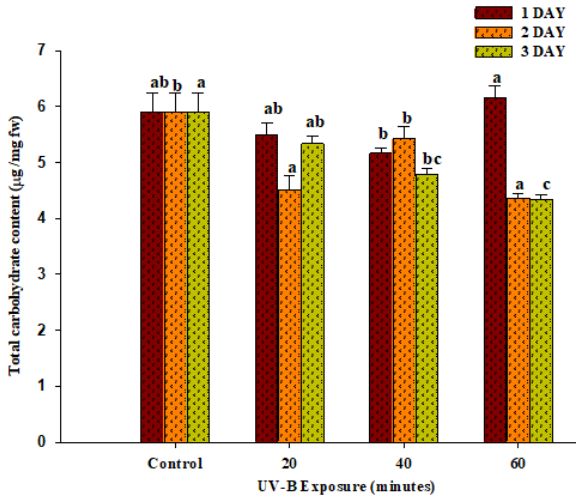

Figure 5. Showing the comparative trend of total carbohydrate content (mg/g f.w.) after UV-B exposure on 1st, 2nd and 3rd day treatment on *Coriandrum sativum* L.

3.2.3. Effect on Photosynthetic Pigments

The data scored envisaged that the supplemental UV-B radiation causes reduction in photosynthetic pigments of coriander leaves.

3.2.3.1. Chlorophyll a

Inhibition in chlorophyll a content was observed as the exposure time and duration of UV-B treatment was increased (Figure 6). Maximum inhibition (1.56 ± 0.27^{b}) was recorded at 60 minutes exposure on 2nd day treatment. A steady decline of 23% in chlorophyll a contents was observed on 1st day treatment over control.

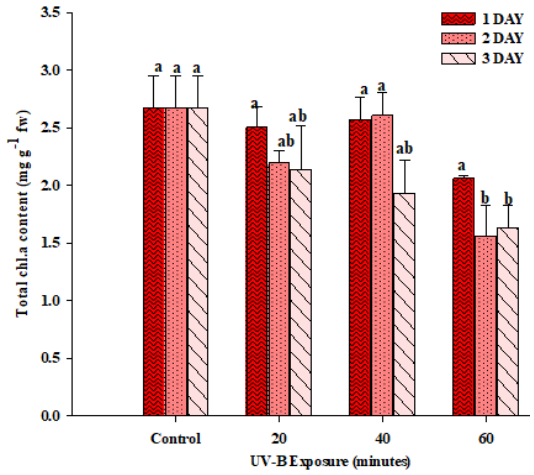

Figure 6. Showing the trend of chlorophyll a content (mg/g f.w.) after effect of UV-B treatment for 1st, 2nd and 3rd day exposure on *Coriandrum sativum* L.

3.2.3.2. Chlorophyll b

It was found to be increased by 5% over control set and reached to maximum level of 1.82 ± 0.10^{a} as compared to control 1.73 ± 0.24^{a} (Figure 7). An abrupt decline (1.07 ± 0.02^{b}) was recorded on 2nd day treatment at 40 minute exposure over control.

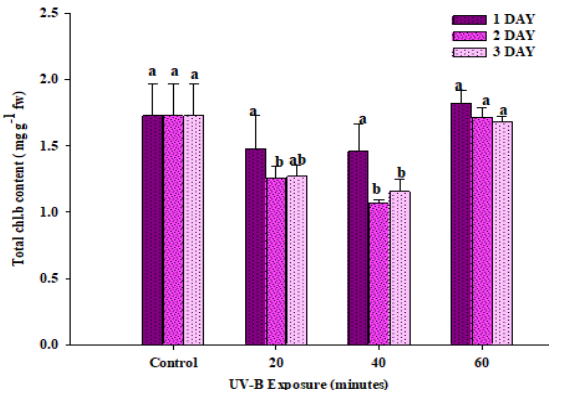

Figure 7. Showing the trend of chlorophyll b content (mg/g f.w.) of *Coriandrum sativum* L. after UV-B treatment for 1st, 2nd and 3rd day exposure duration of 20, 40 and 60 minutes.

3.2.3.3. Carotenoid

A significant decrease in carotenoid contents was recorded in UV-B treated sets over control Figure 8. A maximum increase was recorded in 20 minutes treated sets on 1st and 2nd day treated sets. However, there is constitutive decline by 27% on 1st day, 35% on 2nd day and 72% on 3rd day.

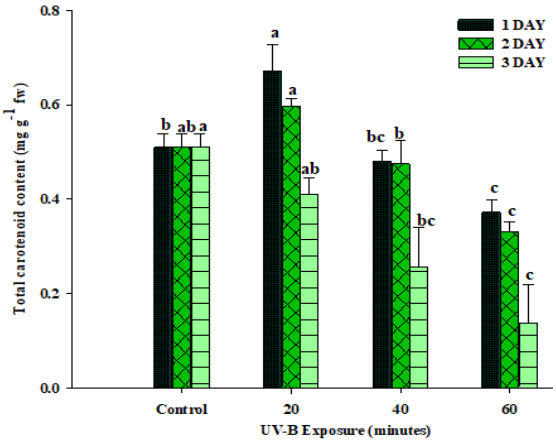

Figure 8. Showing an account of total carotenoid content (mg/g f.w.) after 1st, 2nd and 3rd day impact of UV-B exposure on *Coriandrum sativum* L.

4. Discussion

UV radiation plays an important regulatory role in plants growth and development. However, due to its putative functioning higher exposure of UV-B can cause stressful conditions leading to damaging impacts on physiological process and genome instability which in turn concerned with the human health and also plant production and quality measures.

In the present study, we have investigated the effect of UV-B on the root meristems of *Coriandrum sativum* L. with a wide range of doses and compared its action on 1st, 2nd and 3rd day exposure duration.

4.1. Cytological

The data gathered from present study show a significant decrease in mitotic index along with dose dependent increase in chromosomal aberrations in sets raised with

supplemental UV-B radiations. Similar results were recorded by Csilla (2009) and Hopkins *et al.* (2002).

Slowing of mitosis results in decreased MI % which is a protective mechanism acquired by plants to cope up with higher doses of UV-B radiation stress as DNA is most sensitive to UV-B during replication. According to Liu *et al*. (2015) reduced MI may be the outcome of breakdown of plant self-protection system and further inhibition of cell DNA replication, transcription and protein synthesis. In *Picea abeis*, Bavcon and Gogala (1996) also reported a decreased mitotic activity and lesser vitality due to influence of UV-B radiation. Arrested interphase due to damaging action of UV-B might leads to decline in cell division. Due to decreased ATP levels and pressure excursion by energy producing centre, probably inhibits the DNA synthesis and reduced ATP causing low MI (%). Reduced mitotic index is due to chromosome condensation in early prophase of the mitotic cycle, but prior to breakdown of nuclear membrane. It could be revealed that declined MI is due to mitodepressive actions of higher UV-B exposure duration and inhibition of DNA synthesis at telophase (Sudhakar *et al*., 2001). UV-B radiations are known to be a physical mutagens producing wide variety of chromosomal aberrations producing abnormal cells. In present investigations a vast spectrum of mitotic chromosomal aberrations were recorded in sets raised to UV-B treatment in Coriander.

CAs is of 2 types, chromosomal and chromatid type, but increased pool of chromosome type aberrations elucidates the genotoxic activity of UV-B radiation. Similar results were recorded by Ranceliene and Vysniauskiene, 2012. According to Cieminis *et al*. (1987), UV-B induced photoproducts could induce the formation of chromosomal aberrations; some of them could be cyclobutane-pyrimidine dimmers that prove to be a genetical danger for plants. Stickiness was found to be most dominant anomaly at metaphase chromosomal stickiness leads to inactivation of DNA replication, increased chromosomal contraction and condensation or nucleoproteins probably leading to cell death (Han *et al*., 2007). It could be due to depolymerisation of nucleic acid caused by mutagenic treatments or due to partial dissociation of the nucleoproteins and alterations in their pattern of organisation (Evans, 1962). Precocious movement of chromosomes at metaphase might be formed due to malformed homology of chromosome pairing or spindle mechanism whereby one or few chromosomes floats in the cytoplasm rather than arranged at equatorial plate. Probably, the disrupted spindle functioning causes precocious chromosomes. Spindle disruption also causes scattering, unorientation and c-metaphase. C-metaphase was first reported by Levan, 1938 in root tips of *Allium cepa* caused by inactivation of the spindle fibre followed by a random scattering of chromosomes over the cell. Unorientation and scattering of chromosomes at metaphase was observed in the present investigation which may be either due to inhibition of spindle fibre formation or destruction (Kumar and Rai, 2007). Chromatin bridges were another anomaly encountered dominantly at anaphase. It may occurred due to enhanced activity of UV-B radiations, making chromosome breaks, then the two chromosome sides are, respectively, healed, producing double centromere chromosomes i.e. "chromosome bridges." Bridges was also reported by Dhulgande (2015). Formation of bridges could be attributed to chromosomal stickiness (El-Khodary *et al*., 1990) and to chromosome breakage and reunion (Haliem, 1990) that may lead due to loss of genetic material. The loop forming laggards at anaphase (Figure 1.H) might have originated due to failure of kinetochores to attach with spindles and leading to the joining of ends forming loops. Such disorders may lead to mutations. A merotelic kinetochores orientation is a major cause of lagging chromosomes during mitosis. It was suspected that those chromosomes which do not active in bridge formation may sometimes get detached from the group and are remained as lagging in cell vicinity. DNA damages induced by UV-B radiation might have influenced the expression of number of genes leading to alterations in proteins that control many metabolic processes like plant Development, cell cycle, fertilisation and seed formation (Haliem *et al.*, 2013).

4.2. Biochemical Observations

4.2.1. Proline Content

Data of present study elucidate that the different time and duration of UV-B radiation induces increased proline contents in all treated sets as compared to control sets in *Coriandrum sativum* L. The findings of Demir (2000) and Amal *et al*. (2006) are in agreement with the present findings. In the seedlings of rice and mungbean accumulation of proline due to UV-B radiation has been reported. Masood *et al*. (2006) had also reported increased proline content in *Azolla pinnata* and *A. filiculoides* under UV-B treatment. Liang *et al*. (2013) and Saradhi *et al*. (1995) stated that accumulated proline is an adaptive measure of plants against adverse conditions and it involves stabilization of proteins and antioxidant enzymes, direct scavenging of ROS, balance of intracellular redox homeostasis (ratio of $NADP^+$/NADPH and GSH/GSSG) and cellular signalling promoted by proline metabolism and suppression of mitochondrial electron transport might be cause of proline accumulation. Stimulation of proline from abscissic acid, inhibition of proline oxidation to other soluble compounds and inhibition of protein synthesis are the causes of free proline accumulation.

4.2.2. Carbohydrate Content

These are the key source of energy for plants basic life functions. They harvest it by capturing incident solar radiation *via* photosynthesis. But elevated UV-B radiation disrupts the machinery significantly results in decreased soluble carbohydrates. The findings of Moghadam *et al*.(2012) are in agreement with the present findings. It may be suspected that UV-B distorts the grana causing inhibited photosynthesis leading to decreased rate of carbohydrate formation as exposure level increases. As UV-B treated plants have a tendency to lower sink capacity (Correia *et al.*, 2000), the observed decreased in the total carbohydrate content by UV-B indicates the main response is mediated by lower rate net photosynthetic rate (Correia *et al*., 2005). Similar results were observed by Musil (1996) and Mackerness *et al.* (1997). At 60 minutes, carbohydrate content gets increased on 1st day (Table: 3) but latter declined. Kovacs *et al*., 2002 stated that supplementary UV-B radiation damaged the structure of chloroplasts, as manifestated by dilations of thylakoids, a

progressive disruption of thylakoid structure and disintegration of the double membrane envelope surrounding the chloroplast, accompanied by the accumulation of large starch grains at higher level due to immobilization and then later declined.

4.2.3. Photosynthetic Pigments

Pigments of photosynthetic apparatus can be destroyed by UV-B radiation with comparative loss of photosynthetic capacity (Jordan *et al*., 1994). Chlorophylls and carotenoids were affected by differential UV-B radiation doses, while carotenoids are generally less affected than chlorophylls (Pfundel *et al.*, 1992). It has been reported that in tested plant (Table 3) the Chlorophyll a decreased as exposure duration increases but on 40 minutes treatment sets UV effect was regressed by plant but at 60 minutes causes reduction in chlorophyll a contents as compared to Chlorophyll b (Table 3). Similar findings were reported by Marwood and Greenberg (1996) that might point as more selective destruction of Chlorophyll a biosynthesis or degradation of precursors probably decrease in PSII due to higher UV-B radiations caused decreased Chlorophyll a. Decreased carotenoids may play a role in the decrease of chlorophyll concentrations since carotenoids protect chlorophyll from photo-oxidative damages (Singh, 1996). As compared to control at lower dose carotenoids increased to protects plants against UV-B radiation but at higher doses level decreases. The reduction in carotenoid content may result either from inhibition of synthesis or from breakdown of the pigments. Since carotenoids are involved in the light harvesting and protection of chlorophylls from photo-oxidative damages, any reduction in carotenoids could have serious consequences of chlorophyll pigments (Ravindran *et al*., 2010).

Table 3. Effect of UV-B radiations on proline, carbohydrate and photosynthetic pigments (chl a, chl b, & carotenoid) of *Coriandrum sativum* L.

Treatment (UV-B)	Doses (minutes)	Proline (µg/gm f.w.)	Carbohydrate (µg/gm f.w.)	Chl a* (mg/gm f.w.)	Chl b** (mg/gm f.w.)	Carotenoid (mg/gm f.w.)
1 DAY	Control	2.56±0.36^{a}	5.90±0.34^{ab}	2.67±0.28^{a}	1.73±0.24^{a}	0.51±0.03^{b}
	20	3.04±0.63^{a}	5.50±0.20^{ab}	2.51±0.17^{a}	1.48±0.25^{a}	0.67±0.05^{a}
	40	3.17±0.66^{a}	5.17±0.08^{b}	2.57±0.19^{a}	1.46±0.21^{a}	0.48±0.02^{bc}
	60	3.33±0.55^{a}	6.15±0.22^{a}	2.05±0.02^{a}	1.82±0.10^{a}	0.37±0.03^{c}
2 DAY	Control	2.56±0.36^{b}	5.90±0.34^{a}	2.67±0.28^{a}	1.73±0.24^{a}	0.51±0.03^{ab}
	20	3.60±0.86^{ab}	4.51±0.26^{b}	2.19±0.11^{ab}	1.26±0.09^{b}	0.59±0.02^{a}
	40	4.32±0.14^{a}	5.42±0.21^{a}	2.61±0.20^{a}	1.07±0.02^{b}	0.47±0.05^{b}
	60	4.16±0.13^{ab}	4.36±0.08^{b}	1.56±0.27^{b}	1.72±0.07^{a}	0.33±0.02^{c}
3 DAY	Control	2.56±0.36^{a}	5.90±0.34^{a}	2.67±0.28^{a}	1.73±0.24^{a}	0.51±0.03^{a}
	20	3.65±0.93^{a}	5.34±0.15^{ab}	2.14±0.37^{ab}	1.27±0.08^{ab}	0.41±0.03^{ab}
	40	4.91±0.73^{a}	4.78±0.10^{bc}	1.93±0.28^{ab}	1.16±0.09^{b}	0.26±0.08^{bc}
	60	4.97±0.70^{a}	4.34±0.09^{c}	1.62±0.19^{b}	1.68±0.04^{a}	0.14±0.08^{c}

1-Abbreviations*- Chlorophyll a, **- Chlorophyll b

2- Data are represented in Mean ± S.E. significantly different at $p<0.05$.

5. Conclusion

The results obtained from the present experimental work elucidate that higher doses of UV-B induces various cytological anomalies resulting into decreased MI (%) and exaggerated chromosomal aberrations as exposure duration increases. However, as concerned to biochemical responses of *Coriandrum sativum* L. against UV-B radiation the level of proline was increased to protect plant machinery but chlorophyll pigments (chl a, chl b and carotenoids) and total carbohydrate contents were declined which is correlated with decreased photosynthetic rates due to aberrations in cellular division rates in grana causing disruption and altered signalling during mechanism of photosynthetic process.

Hence, it can be concluded from the present result that the lower doses and exposure duration of UV-B are less significant in its action and promote some important metabolic processes in plants while higher doses are significant and induces toxicity. The present research work would be further helpful in selecting the doses which imparts characters of interest in *Coriandrum sativum* L. and for further study on UV-B responses of *Coriandrum sativum* L. after raising generations up to complete maturity stage and will provide an insight to understand the impact of UV-B on commercial crops and to devise necessary protective measures and strategy for conservation of spice crops of interest like coriander and provide awareness among farmers for ill effects of UV-B on crops.

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