Modulated UV-B radiation invoking cytomorphological and biochemical aspects of a medicinal plant, Bhringraj (*Eclipta alba* (L.) Hassk.)

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Abstract

Aim : The current experimental work has been designed to study the effect of UV-B exposure on the seedling growth and meiotic consequences of *Eclipta alba* (L.) Hassk.

Methodology : The seedlings were exposed to UV-B radiation for different time durations, i.e., 40, 80, 120 min along with control sets. UV-B irradiated seedlings along with respective controls were sown in the field and young floral buds were fixed in Carnoy's fixative for 24 hrs and preserved in 90% ethanol for meiotic study.

Results : Exposure of UV-B exposure resulted in various chromosomal aberrations like stickiness, laggards, bridges, unorientation, precocious, multivalents etc. Chromosomal stickiness was profound abnormality encountered at shorter duration (40 min) to UV-B exposure. The results revealed that UV-B exposure for shorter duration is quite beneficial to plant as it induces significant cytomorphological and biochemical variations.

Interpretation : Shorter exposure to UV-B radiation induced certain beneficial traits in *Eclipta alba*. Since, *Eclipta alba* is a medicinally significant plant, hence, it is essential to improve their quantitative and qualitative traits through induced mutagenesis using UV-B radiation to impel the novel characteristics of plant.

Key words: *Eclipta alba*, Meiotic aberrations, Seedling growth, UV-B radiation

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Introduction

The stratospheric ozone layer is continually being depleted primarily due to the contamination of anthropogenic chlorofluorocarbons. Long term depletion of stratospheric ozone layer contributes to an increase in terrestrial solar ultraviolet-B radiation. UV-B radiation damages nuclear, chloroplast and mitochondrial DNA. DNA damage under UV radiation has shown to effectively modulate the mutation rate, chromosomal aberration and viability of seeds and seedlings (Gill and Tuteja, 2015). UV- B radiations are well established agents for changing the morphological, cytological and biochemical aspects of plants. It was reported that UV-B radiation could promote growth, morphological, physiological and biochemical responses in plant (Zhang *et al.*, He *et al.*, 2003). But long term exposure to UV-B radiation induces disturbance in the plant metabolism and causing lethal effect on the plants (Mopoloka., 2008). There are mainly two types of damages inflicted by UV-B radiations, injury to DNA which leads to mutation and damage to plant's physiological and biochemical functions which include membrane disruption, effect on plant hormones and pigments that ultimately affect the plant growth, development, yield and numerous cellular processes such as photosynthesis and respiration (Zlatev *et al.*, 2012). Previous studies have reported that along with damaging effects at enhanced dosage of UV-B radiation; the ambient dosage have some beneficiary effects in plants like increase in plant height of tartary buckwheat (Yao *et al.*, 2007), increase in internodal length, leaf area, number of tillers in wheat plant (Kataria and Guruprasad 2012b), and increase in grain quality in maize (Yin and Wang 2012). *Eclipta alba* (L.) Hassk. (Bhringraj) is a medicinally important plant since antiquity belonging to Asteraceae family. Bhringraj has a broad spectrum of medicinal properties including antimicrobial, anti-inflammatory, antidiabetic, antihepatotoxic, antihemorrhagic, and hair growth rejuvenator (Yadav *et al.*, 2017; Mithun *et al.*, 2011; Dalal *et al.*, 2010). In view of the above, this study aimed to bring in advancements in plant genetic variability and evaluate different cytomorphological and biochemical aspects of *Eclipta* incurred as a result of differential exposure to UV-B radiation.

Materials and Methods

Experimental design: Seeds of *Eclipta alba* collected from the Central Council for Research in Ayurvedic Sciences (CCRAS), Jhansi, Uttar Pradesh were soaked in distilled water for 12 hrs and then kept in sterilized Petriplates for germination in a seed germinator at $25 \pm 2^\circ\text{C}$ and 80- 90% humidity. Moisture was maintained by regular sprinkling of water. Seedling stage was obtained after 3 days and Petriplates were taken out from seed germinator. Seedling were sown in four different sets for different exposure time viz. Set A for 40 min, Set B for 80 min, Set C for 120 min and set D as control. Three replicates were maintained for each treatment.

UV-B treatment: Sets A, B and C were kept in Ultra violet florescence cabinet and the seedlings were irradiated with UV-B

lamp along with white light (CX-20 equipped with internal 8 Watt tubes) for 40, 80, 120 min respectively. After completion of treatment, they were kept for recovery for half an hour, and after UV-B exposure seedlings were transplanted to their respective pots.

Cytological investigations: For cytological analyses, young floral capitula of Bhringraj of appropriate size were fixed in Carnoy's fixative for 24 hr and then preserved in 90% alcohol for meiotic study. Anthers were excised from young capitula and kept on a slide. Anthers were teased and stained in 2% acetocarmine, followed by squash preparation. Slides were observed under the microscope and pollen fertility was evaluated by acetocarmine stainability test. The snapshot of chromosomes were captured with Pinnacle PCTV software.

Morphological investigations

Survivability percentage data : The data was calculated on 14th day from the day of seedling emergence by the formula given below:

Survivability percentage = Number of seedling survival/Total no. of germinated seed*100

Plant height : Plant height was measured from the apex to the starting part of the stem with a measuring scale, and internodal length was measured when the plant height attained stability.

Leaf area: Leaf area of randomly selected leaves along with control was measured by using "SYSTRONICS LEAF AREA METER 211"(a set of 9 circular mask plates ; 10, 20, 50, 80, 100, 125, 150, 180 and 200 cm²) which is simple, quick and accurate.

Biochemical estimation: Biochemical study was based on the photosynthetic pigment quantification. Chlorophyll and carotenoid content was estimated by the method of Lichtenthaler and Welburn (1987). Leaf tissues (20 mg) were homogenized in 10 ml of 80% acetone. The homogenate was centrifuged and optical density was read at 663, 646 and 470 nm using a spectrophotometer. The chl 'a', 'b' and carotenoid content was calculated.

Statistical analyses: All the experiments were performed in three replicates for accuracy of the results. Statistical analyses was performed using SPSS 16.0 software. ANOVA and DMRT were used as statistical tools for mean separation and detailed study of morphological variable and graphical illustration of data was plotted usually Sigma Plot 10.0 Software.

Results and Discussion

In this study, the potency of UV-B exposure on Bhringraj plant was inspected at cytological, morphological and biochemical levels. UV-B exposure produced a large number of anomalies at prophase, metaphase and anaphase stages of meiotic cells. (Table 1). The detailed investigation showed that

Table 1: Cytological abnormalities and pollen fertility (%) in *Eclipta alba* induced by UV-B exposure

Dose (UV-B) Exposure Time (min.)	No. of PMC's observed	Metaphasic Abnormalities (%)				Anaphasic Abnormalities (%)				Pollen Fertility (%)	Oth	T.Ab. (%)
		St	Sc	Pm	Mv	Un	Lg	St	Fw			
Ct	361	-	-	-	-	-	-	-	-	95±1.08	-	-
40	371	1.17±0.09	0.54±0.14	0.44±0.82	-	0.45±0.09	0.36±0.99	0.45±0.09	0.35±0.08	92±2.44	0.53±0.14	4.31±0.48
80	383	2.60±0.42	1.89±0.28	0.77±0.14	-	0.77±0.14	0.70±0.24	0.95±0.10	0.79±0.28	86±2.90	1.38±0.61	9.9±0.84
120	359	3.23±0.38	1.29±0.16	1.57±0.25	1.47±0.22	1.02±0.35	1.02±0.94	1.11±0.58	0.83±0.11	74±3.80	1.38±0.30	14.82±0.20

Where, Sc- Scattering; Pm- Precocious movement of chromosomes; St- Stickiness; Fw- Forward movement of chromosome ; Mv- Multivalent; Lg- Laggard; Un- Unorientated; Asy- Asynchronous division; Dp- Disturbed polarity; Oth- Others; T.Ab. – Total abnormalities

Fig. 1: Chromosomal anomalies induced by UV-B exposure: (A) Normal metaphase ($2n=22$); (B) Normal anaphase; (C) Stickiness at Metaphase; (D) Precocious movement at metaphase II; (E) Stickiness at one pole; (F) Laggard at Anaphase I; (G) Disturbed polarity; (H) Stickiness at Anaphase II; (I) Fertile (Arrow) and sterile pollen. Scale bar- 10.89 μ m.

UV-B stress may invoke certain adaptive and ameliorative responses in regulation of cell cycle. Meiosis was found normal in the control plants ($2n=22$) Fig.1 (A and B). The present study reveals that with the increase in duration of UV-B radiation there was also increase in abnormality percentage (Table 1). Cytological explorations inferred that brief exposure to UV-B induced minimum chromosomal anomalies, however, abnormalities increased as duration of UV-B increased. Several anomalies such as stickiness, unorientation, laggard, precocious, disturbed polarity, etc. were also recorded (Fig. 1).

The most observed abnormality was stickiness at metaphase and anaphase stages (Fig.1 C,H). The major cause of

stickiness is alteration in the pattern organization of chromosomes (Sharma and Kumar, 2002; Kumar and Singh, 2003). Laggard chromosome may be due to delayed terminalisation, chromosome stickiness or failure of chromosome movement (Fig.1F) (Muniswamy Reddy and Munirajappa, 2012). Another abnormality observed was the precocious movement of chromosome, which may be due to the migration of chromosome to the poles which can result into early chiasma terminalization in diakinesis or metaphase I. (Srivastava and Kapoor, 2008) (Fig. 1D). Longer exposure to UV-B rays disturbs spindle formation which may cause disturbance in the polarity of chromosomes (Fig. 1G). Disturbed polarity has been reported in maize. (Kumar and Rai, 2007); Apart from these abnormalities, UV-B rays

Fig. 2: Effect of UV-B radiation on the morphological parameters of *Eclipta* (a) survival %; (b) plant height; (c) internodal length and (d) leaf area.

caused pollen sterility in plant, which was observed in plants exposed to radiation for longer durations. Chromosomal aberrations are probable reason for pollen sterility. Pollen sterility results due to interchange in the segments between non-homologous chromosomes. It is considered that the presence of laggards, univalents, micronuclei, and stickiness is closely associated with pollen sterility (Bhat *et al.*, 2007) (Fig. 1 I). UV-B radiation damages DNA by inducing stress condition, which affects pyrimidine bases to pyrimidine dimer, DNA replication and transcription process. It has been observed that UV-B exposure increases thymidine dimer level and, subsequently, increases damage, however, the repair mechanism is too slow that it can only repair low levels of thymidine dimer. Thymidine dimer have been found to be reduced under negative UV-B condition, hence,

it has been proved that DNA damage is stimulated at high exposure to UV-B radiation. (Rainer and Weissenbock, 2003).

Plant have advanced repair mechanisms to counter the damage induced by UV-B stress at lower duration of exposure (Frohnmeier and Staiger, 2003). It has also been studied that pyrimidine dimer causes cell death by interfering the cell cycle at longer duration of UV-B radiation. There are several reports on DNA damage due to UV-B stress, leading to cytogenetical anomalies and genetic variations in the plant system. Short duration exposure to UV-B radiation causes low abnormality and genetic variations in plants, which suggest that irradiation for short duration is beneficial for plants, and in case of *Bhringraj*, low exposure to UV-B was found promising in some aspects. In the

Fig. 3: Effect of UV-B radiation on morphological traits of *Eclipta alba*.

Fig. 4: Effect of UV-B radiation on *Eclipta alba* on (a) Chlorophyll a, (b) Chlorophyll b and (c) Carotenoid contents.

present study, plant height and internodal length, increased after UV-B exposure. Plants exposed to 40 min UV-B radiation showed increased plant height (28-34%) and internodal length (6.2-7.3%) as compared to control. However, vice-versa was observed in plants exposed to UV-B radiation for 80 and 120 min. Exposure to UV-B rays for 40 min resulted into increased plant height as compared to control and other treated sets. (Fig.3). Short term exposure to UV-B has been found to be quite beneficial (Thomas and Blake, 2003 and Yao *et al.*, 2007). An increase in plant height and internodal length may be due to regulation of photomorphogenic genes, i.e., HY5 and COP1, which play a crucial role in the growth and development of plants. They increase stress tolerance in plants at lower exposure to UV-B radiation and protects from photodamages, hence, exposure to short duration increases plant height and internodal length (Kacharava *et al.*, 2009).

These beneficial morphological traits may be attributed to the priming effects of UV-B radiation leading to activation of factors responsible for stress tolerance and increased production of auxin, cytokinin (increased plant height) and gibberellin (increased internodal length) however, with enhanced exposure to UV-B, reduction in both features were noted, which might be due to inactivation of photomorphogenic genes and damages caused by radiation at genetic and biochemical level (Thomas and Puthur, 2017). Some plant species are unaffected by UV-B irradiation and several are apparently stimulated in their growth, but most of species are sensitive, (Hidema *et al.*, 2007; Du *et al.*, 2011). The survival percentage and leaf area decreased with the increasing duration of UV-B radiation. The highest survivability (97%) was recorded in the control plants but with increase in exposure period, survivability continuously decreased. The lowest survivability percentage (60%) was recorded at 120 min of UV-B exposure. The survivability percentage decrease due to the destruction of growth hormone, i.e., IAA and formation of IAA photoproduct which decreases the survivability of plants (Kumar and Bhardwaj, 2019). Another reason for decreased survivability is DNA damage. Longer exposure to UV-B radiation disrupts DNA polymerase which leads to altered DNA replication and transcription, reducing plant survival (Britt and May, 2003).

The leaf area of *E. alba* was also influenced by UV-B exposure. The maximum leaf area was recorded in control plants and as the exposure UV-B radiation increased, the leaf area comparatively decreased and least leaf (17%) area was observed in plants exposed to 120 min of UV-B. The decrease in leaf area in response to UV radiation is a kind of adaptation to reduce the cellular damages in leaves. This morphological effect in UV irradiated plants may be attributed to the reduced rate of cell division and cell elongation in leaf tissues (Hopkins *et al.*, 2002). UV-B has a major impact on the rate of photosynthesis and its pigments. Several biochemical changes have been reported in plants due to UV-B exposure (Mpoloka, 2008; Fedina *et al.*, 2010). It was observed that with increase in the duration of UV-B exposure, there was decrease in chlorophyll *a* and chlorophyll *b*, and increase in carotenoids (Fig. 4 a,b,c). In control sets, amount

of chl *a* and chl *b* was found in its highest quantity i.e. 2.26% and 1.38% $\mu\text{g mg}^{-1}$ FW respectively where as carotenoid was found in its lowest amount i.e. 0.21% $\mu\text{g mg}^{-1}$ FW. However the amount of photosynthetic pigments(chl *a* and chl *b*) was found lowest in longest duration (120 min) exposure of UV-B radiation i.e. 0.75%, 0.32% $\mu\text{g mg}^{-1}$ FW respectively whereas the carotenoid content found increased 1.01% $\mu\text{g mg}^{-1}$ FW.

Elevated UV-B radiation reduces the chlorophyll content in many plant species which occur due to lipid peroxidation in chloroplast membrane (Rai and Agrawal, 2017). Photo-reduction of protochlorophyllide to chlorophyllide by protochlorophyllide oxidoreductase is one of the possible targets of UV-B (Erdei *et al.*, 2019). As this reaction is light driven, it is possible that UV-B can damage this enzyme resulting in lowering rate of chlorophyll accumulation. UV-B downregulates the expression of genes, which encodes for chlorophyll *a/b* binding proteins (Casti and Walbot, 2003., Lizana *et al.*, 2009).

Carotenoids play an important role against UV-B damage in higher plants. Carotenoids, scavenge singlet oxygen species formed during intense light, are involved in the light harvesting and protection of chlorophylls from photooxidative destruction. The reduction in carotenoid content may result due to inhibition of synthesis or due to breakdown of pigments or their precursors at lower exposure of UV-B radiation. (Singh *et al.*, 2011), however longer exposure of UV-B rays resulted into increased carotenoid content (fig. 4C). This increment might be a strategy for protection of chlorophyll against photooxidative damages caused due to exposure of UV-B rays by dissipating excess excitation energy (Yamamoto and Bussi, 1996). Thus, increased carotenoid content indicates an important stress tolerance strategy adopted by plants (Jaleel *et al.*, 2009). The present study indicated that shorter exposure to UV-B radiation through induced breeding has improved certain beneficial traits in *Eclipta alba*.

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