EFFECT OF ENHANCED SOLAR UV-B (280-320) RADIATION ON
PHYSIOLOGY AND VOLATILE OIL SYNTHESIS IN OCIMUM
BASILICUM L.

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ABSTRACT
Ocimum basilicum is one of the most widely used traditional Indian herbal medicine. It is used for bronchitis, bronchial asthma, skin disease, arthritis, inflammation, fever etc. Basil grown for essential oil from leaves and stem. The plants grow well in full sunlight condition when the plant is exposed to enhanced solar UVB radiation under field condition for four hours various changes were observed. The result showed UVB has significant effect on basil morphological and biochemical characteristics. Compared to control, the UVB radiation had stimulating effect in the height, fresh and dry weight, leaf area, pigment biosynthesis and volatile oil content were significantly increased. Electron microscopic study showed that UV-B also stimulates the synthesis of volatile oil composition. The size and number of oil glands was also increased in UV-B doses. The present finding suggested that UV-B radiation promote the growth and volatile oil content of the plant which are economically important.

KEY WORDS: Ocimum basilicum, essential oil composition, UV-B.

INTRODUCTION
The stratospheric depletion cause by components such as CFCs and BFCs lead to an increase in amount of UV-B (280-320 nm) radiation at the earth surface. This increased UV-B radiation will influence the growth and metabolism of tropical plants due to their requirement of sunlight for photosynthesis, and even a slight increase in UV-B intensity will have a
disproportionately large photobiological effect because it is absorbed by important macromolecules (Jansen et al., 1998). Excess UV-B radiation acts as an environmental stress on plants, altering their physiological functions and ultimately slowing plant growth, damaging photosynthetic pigment and lowering CO2 assimilation; these alterations reduce biomass productivity (Teramura, 1990). It affects plants by modifying both their biological and chemical environment. Damage may occur in many places, including the direct destruction of the DNA, deactivation of protein and enzymes, disruption of membranes and other cell structures and the generation of highly reactive chemical agents known as 'free radicals' (Caldwell et al.1989, Chang,2004). Plant species vary greatly in their response to UV-B. Some plants tolerate enhanced UV-B irradiation by acquiring protective modifications such as increased leaf thickness, higher production of UV-B filters (flavonoids), and stimulated antioxidant activity acting to quench free radicals (Caldwell et al., 2003; Agrawal and Mishra, 2008).The effect of enhanced UV-B on plant growth and physiology has been the subject of a number of studies (Jansen, 2002).

*Ocimum* groups are reported to be widely distributed in the tropical, sub tropical and warm temperate regions of the world (Paton et al., 1999).They are grown for the essential oils in leaves and stems. Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties (Lemos et al., 2005),antinociceptive property (Rabelo et al., 2003),anticonvulsant,antioxidant (Javanmardi et al.,2003;),germicidal (Holetz et al., 2003),antimalarial activity (Ezekwesili et al., 2003) and has found wide use in toothpastes and mouth washes as well as some topical ointments and used as a general promoter for health in herbal medicine. Many medicinal plants, especially those that belong to the Lamiaceae family, contain large amounts of antioxidants other then Vitamin C, Vitamin E, flavonoids and carotenoids. Basil has been shown to produce high levels of phenyl- opanoids, e.g. eugenol and methyleugenol, and terpenoids, e.g. linalool and 1, 8-cineole. Both oil yield and herb yield vary greatly under different environmental conditions.

UV-B not only enhanced the total oil content twofold but also altered the oil composition in fresh basil leaves. This paper reports UVB induced changes in plantphysiology and volatile oil content of basil leaves grown under enhanced solar UV-B light in natural environment condition This results confirms that basil grown under enhance solar UV-B radiation increase in the volatile oil content in leaves.
MATERIALS AND METHODS

Plant materials: Certified seeds of Ocimum basilicum L. obtained from the Agriculture Department, Madurai were sown in experimental plots in The American College, Botanical Garden. One set of plants was grown under ambient solar radiation and other under 20% UV-B enhanced solar radiation.

Plant growth and UV-B treatment: The seeds were soaked overnight in running water. Separate soil beds were prepared for control (ambient) and UV-B treatment and seeds were sown in these experimental plots. The plants were watered regularly and care was taken to avoid microbial or pest infection during the experimental period. Plants with the first foliage leaf stage were used for UV-B treatment. UV-B treatment was given to these plants for 4 hours daily from 10 a.m to 2 p.m. Treatment was continued under ambient solar radiation and 20% UV-B enhanced solar radiation supplemented by a Philips TL40W/12 sunlamp (Gloelampenfabrieken, Holland). The first formed leaves were collected at different time periods and all the physiological and biochemical analyses were carried out.

Measurement of radiation: A Li-Cor Li-188B quantum/radiometer (Li-Cor., Inc., USA) with suitable photo detector was used to measure all the visible and photo synthetically active radiation. Radiation below 400 nm was determined by an IL 700 radiometer with a SEE 400 photodiode detector (International Light Inc., USA).

Determination of growth: Shoot lengths were determined soon after the seedlings were uprooted. All measurements were the mean of twenty randomly selected samples.

Estimation of pigments: Chlorophylls: Pigments were extracted in 80% acetone and the amount of total Chl, Chl a, Chl b and carotenoid was quantified using the formulae of Wellburn and Lichtenthaler (1984).

Carotenoids: The concentration of total carotenoids was estimated in the 80% acetone extract by measuring the absorbance at 480 nm. Mackinney’s (1941)

Flavonoids: Fresh leaf samples equivalent to 100 mg were cut into small pieces and incubated overnight in 5 ml of 80% acidified methanol (80:20:1 of methanol:water:HCl) at 4°C in dark. After centrifugation to remove debris, the absorbance at 315 nm was taken and the flavonoid content was expressed as µg/g leaf fresh weight (Mirecki and Teramura, 1984).
Analysis of leaves for volatile oil content and composition
The volatile oils from fresh samples (5 g) of the fourth pair of leaves of randomly selected plants were collected and analyzed by THERMO GC -TRACE ULTRA VER: 5.0, THERMO MS DSQ II In the COLUMN : TR 5 - MS CAPILLARY STANDARD NON - POLAR COLUMN DIMENSION : 30 Mts, ID : 0.25 mm, FILM : 0.25 μm CARRIER GAS : He, FLOW : 1.0 ML/Min TEMP PROG : OVEN TEMP 40 C RAISED TO 250 C AT 5 C /MIN

RESULT AND DISCUSSION
The enhanced UVB radiation affects the plant growth and other metabolic process generally. But it depends upon the plant it varies. The basil plant exposed UVB radiation under field conditions and the changes in physiological, biochemical and volatile oil content were analysed in different time intervals.

Plant growth characteristics

![Image](Occimum basilicum plant grown under ambient and UV-B treatment)

The enhancement of solar UV-B radiation was 20% over the ambient level and was provided artificially by using sunlamps. After exposure to enhanced solar UV-B radiation, samples were collected at regular time intervals for various analyses. All the parameters were analysed 10 days after the onset of UV-B treatment. Fig.1 Basil were grown successfully under tropical climate.

CHANGES IN PLANT HEIGHT
The changes in seedling growth of plants grown under enhanced UV-B radiation was compared with those grown under ambient light conditions. Fig.2.
In Basil, an increase in fresh weight and dry weight of the shoot was observed. The plant height and leaf area was increases slowly during the first 20 days of UV-B treatment and rapid increase was seen in the later stage. As much as three-fold increase on plant height was observed on the 40th day. Enhanced UV-B radiation increase elongation of the main stem of basil plants, resulting in more compact plants, possibly due to changes in the phytohormones, especially IAA that has been shown to play a role in stem elongation in sunflower. This indicates that peroxidases also play a role in UV protection and enhanced growth in higher plants. (Johnson et al., 1999). The apparent increase in the number of lateral shoots could be a consequence of the elimination of apical dominance due to the decrease in IAA concentration in the apex of the main stem, since some studies have indicated a breakdown of IAA on exposure to UVB radiation (ROS and Tevini, 1955. Huang et al. 1993) Currently Sakalauskaitė 2012 reported the supplementary UV-B irradiation had beneficial influence for increasing basils growth and some biochemical constituents.

**CHANGES IN PHOTOSYNTHETIC PIGMENTS**

Photosynthesis is very important metabolic process. Photosynthetic process is based on chlorophyll system. If the chlorophyll system is unchanged plant resistant to UVB radiation. The changes in photosynthetic pigments were analysed at different growth periods in plants exposed to ambient and enhanced UV-B irradiation (.Fig. 3) shows the effect of enhanced UV-B radiation on the total Chl content increased up to the full growth period.
Basil plants grown under UV-B enhanced radiation maintained 20-30% of more total Chl over the respective ambient light grown plants at all stages of growth.

![Graph showing changes in chlorophyll content under UV-B and ambient light](image)

**Fig.3.** Changes in the chlorophyll pigment content in *Ocimum basilicum* plants grown under ambient and enhanced UVB radiation. The values represent an average of 3 independent measurements. Mean ±SE, n=20

The total Chl content showed a significant increase in Basil grown under supplemental UV-B and such increase may be due to the stable organization of Chl and β-carotene in the light harvesting domains. Recently Sakalauskaitė 2013 reported supplement UVB radiation increase chlorophyll content. Increase in Chl content in some plant species was also shown to be due to the enhanced synthesis of Chl *a* rather than Chl *b*. Enhanced UV has also been shown to cause damage to the Chl *b* rather than Chl *a* which could be due to its direct absorption or due to inhibition of Chl biosynthesis (Teramura and Sullivan, 1994). UVR under constant PAR did not cause any changes in chlorophyll nor carotenoid contents in Halophila stipulacea. (Sharon et al., 2011)

**CHANGES IN CAROTENOIDs CONTENT**

Carotenoids act as a UVB screening pigment and also important class of phytonutrients. The changes in the carotenoid content followed a similar pattern as that of total chlorophyll. (Fig.4). As much as 50% increase in carotenoid level was found in Basil, particularly at the later stages of plant growth. The carotenoids under UV-B enhanced radiation play a photoprotective role in the photosynthetic apparatus (Midleton and Teramura, 1993) and any loss in their level would lead to the loss of photosynthetic activity. Both Chl and carotenoids were adversely affected by relatively large amounts of UV-B radiation with carotenoids
generally being less affected than the Chl (Chang et al., 2005). The UVB radiation increase synthesis of carotenoids levels would be beneficial to human health via enhancing plant nutritionals status (Kumari and Prasad 2013).

**Fig. 4.** Changes in the carotenoids content in *Ocimum basilicum* plants grown under ambient and enhance UVB radiation. The values represent an average of of 3 independent measurement. Mean ±SE, n=20

**CHANGES IN FLAVONOID CONTENT**

The flavonoids are UV-B protecting pigments and they are known to accumulate under enhanced UV-B radiation. The increase in flavonoid content was more in Basil throughout the plant growth. (Fig. 5) The chemical evolution and significance of flavonoids has been assumed to play an important role in overcoming the oxidative stress in cells. Evidences suggest that the presence of flavonoids in UV-B irradiated leaves could alter the perception or response of other defense mechanisms. (Nijvedt et al., 2001). Barnes et al. (1987) reported that the photosynthetic apparatus of some plants from high elevation tropical area was inherently more resistant to UV-B radiation which was apparently not due to increase in flavonoid concentration. However, flavonoid concentration may be higher (Ziska et al., 1992) in tropical species growing at high elevated regions. The greater increase in the leaf UV-B absorbing compounds occurred when the plants were grown in relatively low PAR/UV-B (Wilson and Greenberg, 1993, Alexieva et al., 2001). Increased level of flavanoids and pal activity are considered important defence response of UVB tolerant species. (Wilson et al., 2002).
Fig. 5 Changes in flavanoids content *Ocimum basilicum* plants grown under ambient and enhance UVB radiation. The values represent an average of 3 independent measurement. Mean ±SE, n=20

**CHANGES IN VOLATILE CONTENT**

Fig. 6 Changes in volatile oil content in *Ocimum basilicum* plants grown under ambient and Enhanced UVB radiation condition.

There was significant differences in total volatile oil content between UV-B treated and ambient grown plants at the later stages of the plant growth (Fig. 6). Overall, enhanced solar UV-B light effectively increased the total volatile oil content of basilicum leaves. There have been two reports on the effects of UV-B on volatile oils in basil plants. Johnson et al. (1999) reported that UV-B not only increased the total content but also changed the composition of
these oils. In contrast, Ioannidis et al. (2002) stated that neither the quality nor the quantity of the volatiles was affected by UV-B. The essential oil content was slightly increased by UVB radiation (Maffei, Scannerini 2000). Daphane 2002 reported the UVB is required for normal development of oil glands in Ocimum basil. The coleus species produce essential oils, reported as an effective invitro antimicrobial natural molecules against fungi and bacteria (Khare et al., 2011). The UVB radiation can increase the essential oil yield in Japanese mint plant (Hikosaka et al., 2010).

CONCLUSION
This research suggested that enhanced UV-B significantly increased plant height but increased the number of shoots and plant dry matter. The total volatile oil content in basil fresh leaves was increased by enhanced UV-B radiation; however there was change on the composition of the compounds.

REFERENCES


