Evaluation of changes in oil cells and composition of essential oil in lemongrass (*Cymbopogon citratus* (D.C.) Stapf.) due to supplemental ultraviolet-B irradiation

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Field grown lemongrass (*Cymbopogon citratus* (D.C.) Stapf.) was exposed for a short duration (3 h/day) to supplemental ultraviolet-B (sUV-B) to evaluate its effect on oil cells and chemical composition of essential oils. Histochemical studies showed an increase in number of oil cells in tissues under sUV-B treatment. Estimation of essential oil content also demonstrated an increase of 25.7% in sUV-B treated plants over its control. Gas chromatography-mass spectrometric (GC/MS) analysis of essential oils demonstrated significant variations in qualitative and quantitative expression of oil constituents of sUV-B exposed plants. sUV-B irradiation induced the formation of major compound z-citral within the investigated oil samples by 117.56% as compared to oil obtained from non-irradiated plants. Geraniol formate, pulegol, linalyl formate and other compounds also showed significant variations after sUV-B treatment. Results suggest that sUV-B exposure stimulated the production of oil cells vis-à-vis positive change in quality and percentage of essential oil contents of lemongrass. The results suggest some future prospects for better economical utilization of lemongrass after irradiation with low doses of sUV-B for its commercial exploitation.

Keywords: Citral, *Cymbopogon citratus*, essential oil, oil cells, sUV-B.

LEMONGRASS (*Cymbopogon citratus* (D.C.) Stapf.) is a commercially important aromatic tropical C_4 grass of the family Poaceae, commonly known as sweet grass family. Leaves of this plant contain high citral content in the essential oils with a typical strong lemon-like aroma[1]. As a medicinal herb, lemongrass has been considered as carminative, insect repellent and widely used as herbal tea. Lemongrass oil is one of the most important essential oils produced in the world. India is a major producer of this oil. Chemical compounds present in varying concentrations in lemongrass have a great demand due to their use in perfumery, flavour and pharmaceutical industry. The oil extracted from leaves of lemongrass is used for its spasmolytic, analgesic, anti-inflammatory, antipyretic, diuretic and tranquilizing properties in treating various digestive disorders, inflammation, diabetes, nervous disorders and fever as well as other health problems[1,2]. It is a tonic for the body which boosts up the parasympathetic nervous system and helps to combat nervous exhaustion and stress-related conditions.

Composition of volatile constituents of lemongrass has been reported[3,4]. The essential oil of lemongrass is characterized by a high content of citral[5,6] (>45%) and its quality is generally determined by the amount of citral present in the oil. Citral is a mixture of two stereoisomeric monoterpene aldehydes; geranial (trans-citral, called citral a) and neral (cis-citral, called citral b). Essential oil of *C. citratus* is mainly composed of citral (30–93.74%) with general predominance of geranial[2].

Earlier reports indicate special histochemical localization of citral accumulating essential oil sites in lemongrass using Schiff's reagent[7]. The specific staining of these oil cells is due to the presence of citral containing aldehyde group which reacts with Schiff's reagent to form Schiff's base that produces intense and typical purple-red colouration[8].

Interest in the effects of UV-B on tropical plants has increased considerably in recent years where a small increase in UV-B intensity may affect the plants significantly. Plants which use sunlight for photosynthesis and are unable to avoid exposure to enhanced level of UV-B radiation are at greater risk[9]. Extensive studies have been done on the biological and ecological effects of the expected increase in UV-B radiation on the agricultural crops of economic importance[10]. However, scientific research on the UV-B induced changes on medicinal plants is comparatively limited. Evidences are available indicating positive effects of UV-B on volatile oil production in *Mentha spicata*[11], *Mentha piperita*[12] and *Ocimum basilicum*[13,14]. Possible involvement of UV-B in volatile oil production and in inducing changes in specialized oil sacs have been studied in *O. basilicum*[15].

To the best of our knowledge, UV-B induced changes in specific oil cells of lemongrass and its effects on chemical composition and yield of its essential oils have

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Table 1. Meteorological data of the study site during the experimental period

Month/year	Total rainfall (mm)	Temperature (°C)		Relative humidity (%)		Total sunshine (h)
		Maximum	Minimum	Maximum	Minimum	
March 2007	17.7	29.35	15.7	74.6	47.16	278.6
April 2007	4.7	39.23	23.3	50.96	37.9	271.8
May 2007	18.6	39.32	25.6	56.7	38.4	275.7

Figure 1. Experimental plot showing treatment of lemongrass (*Cymbopogon citratus* (D.C.) Stapf.) with supplemental UV-B radiation.

not yet been reported. Therefore, the present study was undertaken with the objective to find out the effects of sUV-B on specialized oil cells of lemongrass by using histochemical techniques and on the quantity and chemical composition of the essential oil. The results of this study suggest some future prospects for better commercial utilization of this important tropical grass.

Experimental

Experimental site and growth condition

The experiment was conducted at the Botanical Research Garden, Department of Botany, Banaras Hindu University (BHU), Varanasi. The bulbous stems of lemongrass (*Cymbopogon citratus* (D.C.) Stapf.) were collected from the Department of Horticultural Science, Institute of Agricultural Sciences, BHU, and transplanted on 8 March 2007 by adopting conventional methods with proper irrigation. The experiment was carried out in March to May 2007.

This period of the year is characterized by mean monthly maximum temperature ranging between 29.35 and 39.32°C and mean monthly minimum temperature between 15.7 and 25.6°C. Photosynthetic active radiation (PAR) ranged between 1100 and 1200 $\mu mol\ m^{-2}\ s^{-1}$. Total mean rainfall was 41 mm. Maximum relative humidity varied from 56.7 to 74.6% and minimum relative humidity ranged from 37.9 to 47.16%. There were no significant differences in temperature, relative humidity and PAR for sUV-B exposed plants and the control. Monthly variations in climatological data during the experimental period are given in Table 1.

Plant material

C. citratus (locally grown cultivar) is a clump forming grass, native to India and Sri Lanka. It is found growing naturally in tropical grasslands. In India, it is cultivated as a medicinal herb and also for perfume. Lemongrass is mildly diuretic and a stimulant when used as herbal tea, and promotes digestion of fats. An essential oil distilled from the leaves is used as flavouring agent and in medicines. The essential oil of the lemongrass and the plant itself is characterized by its lemony aroma. The important constituent of the oil is 'citral', a monoterpene aldehyde, which is mainly used for the manufacture of vitamin A.

Experimental design

UV-B irradiation: Supplemental level of UV-B over the ambient level was provided artificially by 'Q Panel UV-B 313, 40 W fluorescent lamps' (Q Panel Inc., Cleveland, OH, USA). Plants were irradiated with sUV-B after leaf emergence for 3 h/day (10.00 a.m.–1.00 p.m.) up to 80 days after transplantation. Cellulose diacetate and polyester films of 0.13 mm thickness were used to transmit UV-B (cut off ca. 292 nm) and exclude UV-B (cut off ca. 318 nm) respectively for sUV-B treatment and control set up. Filters were changed frequently to avoid ageing effects on the spectral transmission of UV-B.

Field setup: The experimental plots were randomly designed with three replicates for each treatment. Each of the plots was of 1 × 1 sq. m area. Banks of three UV lamps (120 cm long) fitted 45 cm apart on a steel frame were suspended above and perpendicular to the planted rows of each plot (Figure 1). The 45 cm distance between the top of the plant canopy and UV lamps was kept constant by adjusting the steel frame. Plots were properly irrigated at fixed time intervals and identical water regime was maintained for plants of both control and sUV-B treatments. No fertilizer was added during the experiment. Soil of the

experimental site was sandy loam in texture (sand 45%, silt 28% and clay 27%). Based on analysis, water holding capacity of the top horizon of soil of study site was 48.7%. Soil of the experimental plots was characterized by neutral pH (7.1–7.4), organic C 0.684 g kg^{-1}, total N 0.214 g kg^{-1} and available P 52.53 g kg^{-1} soil.

Measurement of sUV-B exposure: UV-B irradiance at the top of the plant canopy was measured with Ultraviolet Intensity Meter (Model UVP Inc. San Gabriel, (A), USA). The readings was converted to biologically effective ($UV\text{-}B_{BE}$) values by comparing with the Spectro Power Meter (Model Scientech, Boulder, USA). Plants under polyester film only received ambient UV-B (8.6 kJ m^{-2} $UV\text{-}B_{BE}$) on the summer solstice weighted against generalized plant response action spectrum of Caldwell[16] and designated as control plants. The plants beneath cellulose diacetate filter lamps (i.e. treated plants) received sUV-B (ambient + 1.8 kJ m^{-2} $UV\text{-}B_{BE}$) that mimicked 5% reduction in stratospheric ozone at Varanasi (25°14′N, 82°03′E) during clear sky conditions on the summer solstice[17], normalized at 300 nm. The O_3 column thickness was assumed at 3.0 mm, the albedo 0 and the scatter 1.0.

Tissue preparation and examination of oil cells: Healthy leaves of similar age were collected from both control and sUV-B treated plants. Hand-cut sections were prepared from fresh leaves and incubated in Schiff's reagent (Sigma, USA) at room temperature. The sections were then washed three times (10 min each) with a freshly prepared solution of 5% (w/v) sodium metabisulphite in 1% HCl[8], and examined under Olympus BX51 microscope using transmitted light. The number of oil cells per unit surface area was quantified and expressed as oil cell frequency.

Isolation of essential oil: Fresh leaf sections of lemongrass (300 g each) from control and sUV-B treated samples were processed to hydrodistillation for three hours in a Clevenger type apparatus[18]. Both control and sUV-B treated samples were extracted in triplicate. The essential oil obtained was then separated, dehydrated over anhydrous sodium sulphate and then stored in a sealed vial, at 4°C until the time of analysis. The essential oil obtained from both samples were measured and percentage oil contents determined in relation to the weight of fresh leaf samples.

Head space gas chromatography and mass spectrophotometric analysis

Chemical characterization of essential oils: The essential oil samples were analysed by gas chromatography (Perkin Elmer Auto XL GC) equipped with a flame ionization detector. The GC conditions were as follows: column, EQUITY-5 (60 m × 0.32 mm × 0.25 μm); H_2 was the carrier gas; column head pressure 10 psi; oven temperature program isotherm 2 min at 70°C, 3°C/min gradient to 250°C, isotherm 10 min; injection temperature 250°C; detector temperature 280°C.

GC/MS analysis was also performed using Perkin Elmer Turbomass GC/MS. The GC column was EQUITY-5 (60 m × 0.32 mm × 0.25 μm) fused silica capillary column. The GC conditions were as follows: injection temperature, 250°C; column temperature isothermal at 70°C for 2 min, then programmed to 250°C at 37°C min^{-1} and held at this temperature for 10 min; ion source temperature 250°C. Helium was used as the carrier gas. The effluent of the GC column was introduced directly into the source of MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 500 amu for 2 sec.

Identification of compounds: Identification of components of the essential oil was based on comparison of their relative retention indices of peaks on BP-1 column and its computer matching with the Wiley 275.1 and NIST libraries as well as by comparison of fragment patterns of mass spectra with those of authentic reference compounds reported in the literature[19]. The quantity of oil components was compared using peak area measurements.

Statistical analysis

Means and standard errors (SE) of all the data were calculated and values are reported as mean of three replicates. Mean differences were determined by using Student's *t*-test at 5% level of significance. All statistical analyses were performed using SAS version 8.2 (SAS Institute, 1999–2001).

Results and discussion

Oil cell frequency

Results of microscopic examination of specialized oil cells in leaf sections of control and sUV-B treated leaf samples are shown in Figures 2 and 3. Leaf sections of sUV-B treated plants clearly show the presence of specialized purple red stained fully dense oil cells in mesophyll tissues in between the vascular bundles (Figure 3). sUV-B exposed leaves contained more number of oil cells. In control leaf samples (sUV-B unexposed), oil cell frequency was 2.84 mm^{-2}, which increased to 4.01 mm^{-2} under sUV-B treated condition, thus showing an increase of 41.19% after treatment (Figure 4).

The oil cells stained purple-red with Schiff's reagent indicate the presence of a major aldehyde group of compounds in its essential oil (Figure 3 *a*). It is also

noteworthy that the observed staining of sUV-B treated leaf cells was more rapid and deep whereas staining was comparatively slow and light in sections of control plants. In leaves of control plants, partial fulfillment of oil cells was observed whereas in sUV-B treated leaves, oil cells were fully filled and having dense content (Figures 2 and 3). The difference in stainability duration indicated the presence of high concentration of aldehyde group containing compounds in the oil present in oil cells of sUV-B treated leaves.

Quantification of oil

The content of the essential oils on fresh weight basis (v/w) from leaves was 0.35% in control and 0.44% in sUV-B treated plant leaves (Figure 5). This clearly indicates that plants with moderate dose of sUV-B (+1.8 kJ m^{-2} d^{-1} above ambient) resulted in higher content of essential oils as compared to untreated plants. Short daily treatments with sUV-B also led to a substantial increase in essential oils in *M. piperita*[12] and *O. basilicum*[13,15]. These reports not only indicate the changes in total content of essential oil, but also show changes in its volatile composition.

Qualitative and quantitative analysis of oil composition

Eleven compounds, accounting for more than 87.03% of the total volatiles in leaves of control plants and 15 compounds representing about 96.63% of the total volatiles in sUV-B treated plant samples were detected and identified (Table 2). Table 2 shows that z-citral was identified as the major compound that accounted for 35.7% in the essential oil of the control plants. The local variety of *C. citratus* showed less content of citral in comparison to other Indian varieties released through clonal selection and interspecific hybridization. An Ethopian variety of *C. citratus* also showed only 13% citral (citral a + citral b)[20]. The percentage of z-citral increased to 77.69 in the essential oil of sUV-B treated samples. Citral content higher than 75% in the essential oils of *C. citratus* is considered as a high quality product[21,22]. Higher percentage of z-citral in sUV-B treated samples suggests the improved quality of oil after treatment. The current pharmacological status of citral lists it as a generally recognized as safe (GRAS) chemical on the FDA list. The literature points that the volatile properties of essential oil in *C. citratus* are mainly due to its major component, citral[4,23], an

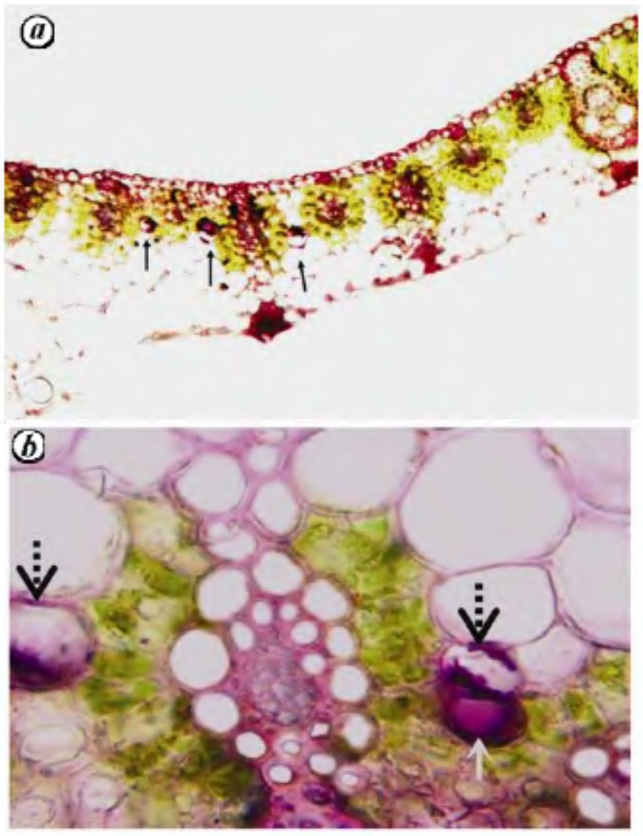

Figure 2. Cross-section of a fresh leaf blade of *C. citratus*, stained with Schiff's reagent. Figures show the microscopic study of red stained citral accumulating oil cells (partially filled) of control samples in low (***a***) and magnified view (***b***) (shown by arrows).

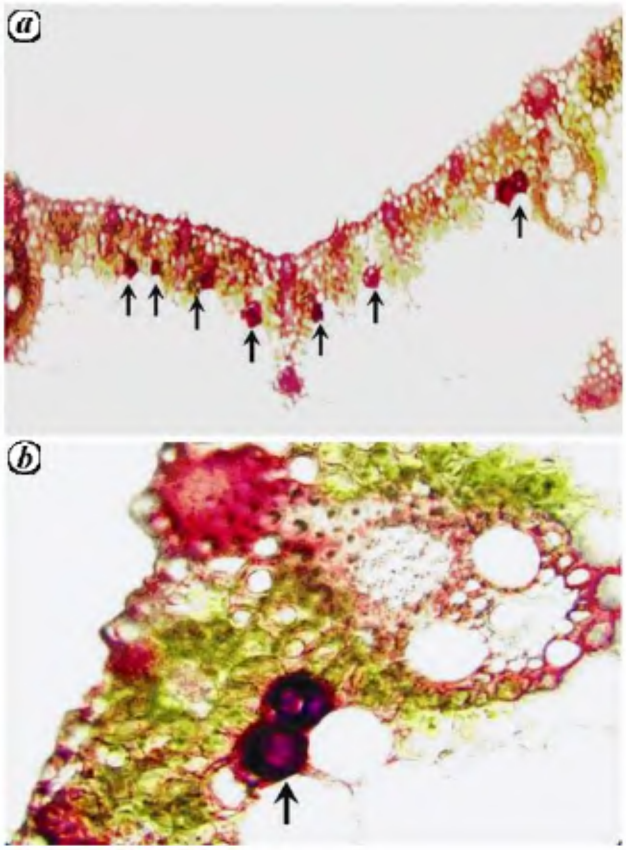

Figure 3. Cross-section of a fresh leaf blade of *C. citratus*, stained with Schiff's reagent. Figures show the microscopic study of red stained citral accumulating fully filled oil cells (designated by arrows) of sUV-B treated samples in low (***a***) and magnified view (***b***) (shown by arrows).

Table 2. Comparative changes in chemical composition of volatile oils obtained from leaf samples of control (C) and sUV-B treated *Cymbopogon citratus* (D.C.) Stapf.

Compound	Relative peak area (%)[+]		Change in area % after sUV-B treatment
	C	sUV-B	
6-Methyl-5-hepten-2-one	$0.95^{NS} \pm 0.04$	$1.09^{NS} \pm 0.06$	14.74% ↑
beta-Myrcene	$10.34^{NS} \pm 0.39$	$10.84^{NS} \pm 0.55$	4.8% ↑
cis-Ocimene	$0.42^{NS} \pm 0.04$	$0.39^{NS} \pm 0.03$	7.1% ↓
1,3,6-Octatriene,3,7-dimethyl	0.25** ± 0.02	–	n.d.
trans Ocimene	$0.21^{NS} \pm 0.01$	$0.23^{NS} \pm 0.01$	9.5% ↑
Linalyl formate	–	0.24** ± 0.1	d.
beta-Bisabolene	–	0.31** ± 0.02	d.
Pulegol	–	0.45** ± 0.02	d.
bicyclo [3.1.1]hept-2-ene-2-methanol,6,6-dimethyl	–	1.21*** ± 0.02	d.
trans-d-Dehydrocarveol	1.04*** ± 0.01	–	n.d.
10,12-Octadecadiynoic acid	1.57*** ± 0.01	–	n.d.
1H-3A,7 Methanoloazulene, octahydro-1,4,9,9-tetramet	–	1.85*** ± 0.02	d.
Nerol	$0.50^{NS} \pm 0.03$	$0.54^{NS} \pm 0.02$	8.0% ↑
2,6-Dimethyl-1,3,5,7-octatetraene	33.52** ± 1.16	–	n.d.
Geraniol formate	2.52** ± 0.01	1.79** ± 0.01	28.96% ↓
z-Citral	35.71*** ± 1.15	77.69*** ± 2.30	117.56% ↑

[+]Average of three repetitions (mean ± 1 SE). The variation coefficient was less than 10%.
Level of significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS, not significant; ↑, represent increase; ↓, decrease.
n.d., not detected after sUV-B treatment; d, detected after sUV-B treatment.

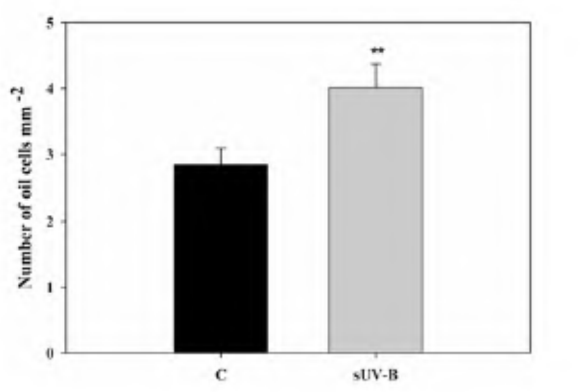

Figure 4. Comparative change in number of oil cells mm^{-2} in fresh leaf sections of control (C) and sUV-B treated plants of *Cymbopogon citratus* (D.C.) Stapf. (mean ± 1 SE). Levels of significance; *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$.

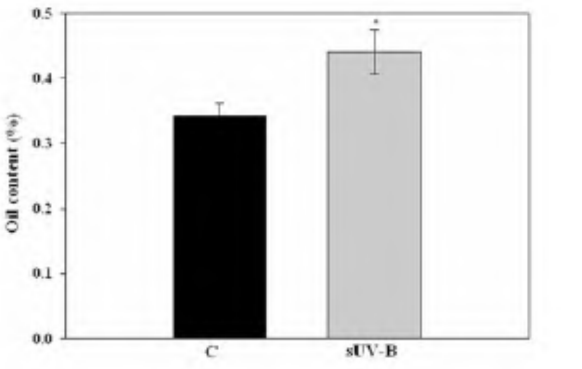

Figure 5. Comparative change in essential oil content (%) in fresh leaf samples of control (C) and sUV-B treated plants of *Cymbopogon citratus* (D.C.) Stapf. (mean ± 1 SE). Levels of significance; *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$.

active ingredient of lemongrass oil with characteristic lemony aroma. Very little is known about the effects of UV-B on essential oils. However, there is evidence for control by light acting via phyto-chrome on monoterpene levels in *Satureja douglasii*[24]. In another report, Ramani and Chelliah[25] have demonstrated that calcium dependent protein kinase (CDPK) and mitogen activated protein kinase (MAPK) play significant roles in UV-B signalling leading to enhanced production of catharanthine in *Catharanthus roseus* cell suspension culture by stimulating *Tdc* and *Str* genes.

Citral is used as a basic raw material for synthesis of β-ionone used for synthesis of a number of useful aromatic compounds and vitamin A and E, carotenoids, pharmaceuticals and fragrances[26,27]. Citral also possesses antioxidant activities which may be associated with some of the reputed beneficial effects on human health[28]. Compound 2,6-dimethyl-1,3,5,7-octatetraene was found to be the second main compound in the essential oil that accounted for 33.5% of the total oil, but it was detected only in the oil obtained from the leaves of control plants and was totally absent from sUV-B treated plant samples. A delayed activation of the biosynthetic pathway for these compounds could be the reason for the mentioned changes in oil constituents after sUV-B treatment. Beta-myrcene was identified as the third main constituent and its relative percentage accounted for 10.34 in the control and 10.84 in sUV-B treated samples. These compounds are followed by geraniol formate (2.5%) that reduced to 1.8% after sUV-B treatment. Other major compounds detected in oil from control plant leaves were 10,12,-octadecadiynoic acid (1.57%) and trans-d-dehydrocarveol

(1.04%) but these were not present in sUV-B treated samples. Other compounds detected, viz. 6-methyl-5-hepten-2-one, *cis* ocimene and *trans* ocimene having 0.95 and 1.09, 0.42 and 0.39%, and 0.21 and 0.23%, respectively in control and sUV-B treated samples showed little variation. Linalyl formate, beta-bisabolene and pulegol not detectable in control samples, were detected after sUV-B treatment. The changes manifested in various constituents of essential oil may be due to the effects of sUV-B on activation/suppression of various enzymes involved in eight reactions of plastidic non-mevalonate (MVA) or 2C-methyl-D-erythritol-4-phosphate (MEP) pathway responsible for the synthesis of various terpenoids.

To our knowledge, no data has been reported so far about the effects of UV-B radiation on modification of the chemical composition of essential oil of *C. citratus*.

Conclusion

Exposure of the lemongrass (*C. citratus*) plants to a mild supplemental UV-B dose resulted in increase in number of oil cells as well as increased production of essential oil. sUV-B exposure also modified the qualitative and quantitative composition of oil constituents. The important component, z-citral increased by 117.6% after sUV-B exposure. Increased synthesis of citral due to exposure of sUV-B may prove to be of high medicinal value. Recommendations can be made only after further investigations by evaluating the quality of essential oil obtained by the control and the sUV-B exposed plants; using some important parameters such as antimicrobial and antifungal activities.

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