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RESEARCH ARTICLE

Effect of sUV-B on essential oil from aerial and sub-aerial parts of *Cymbopogonflexuosus*(Nees ex Steud) Wats.

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Manuscript Info ***Abstract***

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The effect of supplemental Ulatraviolet-B (sUV-B) radiation on essential oil yield and chemical composition were analysed in *Cymbopogonflexuosus* (Nees ex Steud) Wats. The exposure to sUV-B radiation for different intervals of time (15min, 30min and 1hr) showed increased percentage of essential oil in aerial parts of the plant. There was no significant variation was observed in essential oil percentage of sub-aerial parts.Theanalysis of essential oils was performed through Gas chromatography (GC) and Gas chromatography - Mass Spectroscopy (GC-MS),which indicated high citral (isomer of geranial and nearl) production in aerial part,an important compound in perfumery and pharmaceutical industry. The increase in citral percentage in UV treated plants ranged from 67.83% to 81.80% compared to control which showed 65%. The sub-aerial part of the control plantpossessed higher percentage of citral (30%)and sUV-B treated plants showed either one of the isomers in varying percentages.Neral (14.73%) and Junipene (52.70%) in sub-aerial part of sUV-B treated plants were found in higher percentages.

Introduction

Plants are exposed to different biotic and abiotic stresses (Hassan et al., 2012). These either directly or indirectly affect the rate of photosynthesis under environmental stress like drought (Sangwan NS et al., 1994; Rouhi et al., 2007; Hojati et al., 2011) and UV-B (Mishra and Agarwal , 2006; Singh R et al., 2011). Increased solar UV-B radiation (280-320nm) reaching the Earth's surface due to stratospheric ozone depletion (Caldwell et al., 1998) have raised concerns about UV-B impacts on plants. The plant species are specific to UV like other environmental stresses (Smith et al., 2000; Alexievaet al., 2001; Zu et al., 2004). Wide inter and intraspecific differences have been reported in response to UV-B for growth, plant morphogenetic response (Kramer et al., 1991; Rozema et al., 1997; Milchunas et al., 2004) and for physiological processes (Agarwal and Rathore, 2007; Tsormpatsidis et al., 2010). Some species exhibit varied degree of tolerance (Kolb et al., 2001) and others are sensitive to the existing level of UV-B radiation (Teramura and Sullivan, 1991;Zu et al, 2011). The tolerance is attributed to effective absorption of UV-B by a wide range of secondary metabolites produced by certain plants. The different sensitivities of plant are partially explained by their ability to respond to UV-B through the induction of defensive pathways (Hassan et al., 2012).

Increased UV-B radiation influences the growth and metabolism of terrestrial plants due to their requirement of sunlight for photosynthesis. UV-B is one of the important factors which stimulate the production of secondary metabolites**.** Many UV induced compounds such as UV-B absorbing anthocyanins, flavonoids, antioxidants such as ascorbate (Vit C), carotenoids (Vit A), glutathione and a broad range of metabolites (Sakalauskaite et al., 2012) provide protection against potential UV damage. Excess UV-B radiation acts as an environmental stress on plants altering their physiological functions (Kumari R et al., 2009).

Lemongrass is an important member of the tropical C_4 grass and belongs to the family Poaceae. It is grown mainly as a source of citral, as culinary herb or as an ingredient in herbal teas. Traditionally, southern India has been the main source of India's lemongrass oil obtained mainly from *C. flexuosus*that grows as a wild plant in the coastal forest region of Malabar (Stapf, 1906). Upon hydrodistillation, the members of the genus yield essential oils with a wide array of aroma chemicals. Besides citral the essential oil contains geranyl acetate, linalool, limonene, caryophyllene, pineneetc as important compounds. Citral a terpene aldehyde derived from lemongrass oil has a prominent position among the most widely used aroma chemicals in the world. It is the starting material for the preparation of important ionones. α-Ionone is used as a raw material in flavour, cosmetics and perfume industries; β-ionone is used for the synthesis of Vit-A (Pinder, 1960). The whole essential oil is used for its spasmolytic, analgesic, anti-inflammatory, antioxidant, antimicrobial, antipyretic, antinociceptive, anticancer, diuretic and transquilizing properties and used to treat various digestive disorders , inflammation, diabetes, nervous disorders and fever as well as other health problems (Kumari R et al., 2009; SharmaPR et al., 2009; Prasad C et al., 2011; Quintans L et al., 2012).

Vegetative aerial parts are commonly used for the production of essential oil in *C. flexuosus* (Ottavioli J et al., 2009; Parik and Desai, 2011; Adinarayan et al., 2012). The inflorescence of this plant is also used for the extraction of essential oil (Sarma A et al., 2011,).The essential oil from rhizome has been studied in *C. citratus* and *C. densiflorus* (Loumouamou AN et al., 2010; Andrade EHA et al., 2008).

Little is known about the impact of UV-B on medicinal and aromatic plants(Karousou et al., 1998; Ionnidis et al., 2002; Nitz and Schnitzler, 2004). Hence in the present investigation, the wild *C. flexuosus*were maintained in the departmental garden were studied for the effect of sUV-B irradiation on essential oil yield and composition in both aerial and sub-aerial parts.

Material and Methods

2.1 Collection and maintenance of plant:

C. flexuosus collected from HimavathGopala hills in Karnataka, India were maintained at departmental garden, Bangalore University, Bangalore. The plants were raised from slips for further studies. After three months of maintenance, the plants were selected for UV treatment.

2.2 UV treatment:

A pot scale experiment was conducted in growth chamber. The Plants were exposed to supplemental UV-B (sUV-B) irradiation for different intervals of time (15 min, 30 min and 1 hr) for three consecutive days. The growth chamber was fitted with UV-B fluorescent tubes (TL40W/12 RS UV-B Medical, Philips) with the output of 312 nm. Cellulose diacetate of 0.13mm thickness and polyester films were used to filter the transmission of wavelength below 290nm. Control plants were exposed to sunlight.

2.3 Extraction of essential oil:

The aerial part of both control and treated plants were cut into small pieces, air dried at room temperature for three days and then subjected to hydrodistillation using Clavenger's apparatus for 3 hours.

The sub aerial part of both control and treated plants were thoroughly washed, cut into small pieces, air dried at room temperature for 5 days and subjected to hydrodistillation for 9 hours in Clavenger's apparatus.

The fresh and dry weight of herbage and rhizome were noted down and given in the table I and II. The essential oils obtained were stored under anhydrous sodium sulphate and kept at 4^0C for further analysis.

2.4 Analysis of essential oil:

2.4.1 Quantitative analysis:

The essential oil yield was calculated on the basis of dry weight of the material (v/w) using the formula,

Essential oil content (V/W) = a x100 / b

Where a = volume of the oil in ml collected through hydrodistillation

b = weight in grams of the sample taken.

1.4.2 Qualitative analysis:

Chemical analysis was performed using GC and GC-MS.

a) GC method:

The GC analysis was performed on a Thermo GC-trace ultra ver: 5.0, equipped with a split/splitness injector (260^0C, split ratio 1:10) using DB-5 capillary standard non-polar column (30mx0.25mm, df: 0.25 µm). The temperature program was 70^0c (6 min) rising to 260^0C at a rate of 6^0C/min. Injector and detector temperature was 260^0C. Helium was used as carrier gas at a flow rate 1.0ml/min.

b) GC/MS method:

The GC/MS analysis was performed on a Thermo GC-trace ultra ver: 5.0, Thermo MS DSQ II using DB 5-MS Capillary Standard Non-Polar Column (30mts x 0.25mm x 0.25µm). The temperature program was 70^0c (6 min) rising to 260^0C at a rate of 6^0C/min. Injector and detector temperature was 260^0C. Helium was used as carrier gas at a flow rate 1.0ml/min.
Identification of the compounds was carried out by comparison of the mass spectral fragmentation patterns with those stored in MS database (National Institute of Standards and Technology).

Results

3.1 Essential oil yield:

3.1.1 Aerial part:

The essential oil yield from the aerial part of the control plants were found to be 1.27% and in UV treated plants itranged from 1.37% to 1.68% (Table I). Citral percentage was increased in UV treated plants which ranged from 67.83% to 81.80%, compared to control where it showed 65% (Table III).

3.1.2 Sub-aerial parts:

The essential oil content from sub aerial part of the control plant was 0.32% and in UV treated plants the yield ranged between 0.20 to 0.39% (Table II). The citral percentage in control was found to be 30% whereas in UV treated plants one of the isomers of citral either geranial or neral was found. In UV treatment for 15 min neral percentage was 14.73 (Table IV).The concentration of Junipene was increased by 50 fold i.e. 52.70% in UV treated plants whereas in control it was 1.36%.

3.2Chemical composition of essential oil:

The important compounds from aerial and sub-aerial parts of control and UV-treated plants were listed in table III and IV respectively. The compounds with higher percentages in aerial part were 1,7-Octadien-3-ol (13.97%), (1R,4S,6R,7S)- 1,7-Dimethyl 9-oxatricyclo [4.3.0.0 (4,7)] nonanone (12.44%), (+) (4R,5S) cis-4,5-dihydroxy-4,5-dihydrobenzofuran (11.96%), (E)-N,N-Dimethyl-4-phenyl-3-butenylamine (18.23%) and (1S,3S,4R, 6R) (+)-3,7,7-trimethyl bicycle [4.1.0] heptane, 3,4-transoxide (14.03%). In sub-aerial part the compounds with higher percentages were ciscarvyl propionate (4.43%), Junipene (52.70%), Bicyclo[3.3.1]non-6-en-3-ol (22.70%), 3-(Indol-3-yl)-5-(5-nitro-2-furyl)-1,2,4-oxadiazole (59.77%), Menthanol (1.48%), 2-Tetrahydrofurfuryl isothiocyanate (32.61%), 7,7-dimethylbicyclo[3.3.0]octan-2-one (2.39%), (-)-N-(tert-butoxycarbonyl)-l-alanyl-glycin-nitril, 2-Butenedioic acid (E) dimethyl ester (58.57%), 8-Ethoxy-6(2H)-isoquinolinone (3.50%) and 1-Butanol, 3-methoxy-, acetate (29.79%).

Table I. Weight of aerial parts with corresponding essential oil yield.

Sl No	Treatment	Fresh weight of herbage (gms)	Dry weight of herbage (gms)	Yield of essential oil (%)
1.	Control	281.18	178.86	1.27
2.	UV-15 mins	163.78	65.23	1.37
3.	UV-30 mins	161.59	84.19	1.55
4.	UV-1 hr	221.89	68.97	1.68

Table II. Weight of sub-aerial parts with corresponding essential oil yield.

Sl No	Treatment	Fresh weight of rhizome (gms)	Dry weight of rhizome (gms)	Yield of essential oil (%)

1.	Control	344.91	287.63	0.32
2.	UV-15 mins	102.32	68.97	0.20
3.	UV-30 mins	98.57	61.33	0.36
4.	UV-1 hr	100.48	65.02	0.23

Table III. Important essential oil compounds from aerial part of control and UV treated plants.

Sl no	Compound Name	Peak Area %			
		Control	UV(15 min)	UV(30 min)	UV(1 hr)
1.	Z-Citral (neral)	29.85	28.62	33.69	28.38
2	E-Citral (geranial)	35.13	40.12	48.11	39.45
3	Limonene oxide	0.02	1.18	1.29	_
4	å Caryophyllene	0.33	0.04	1.44	_
5	Caryophyllene oxide	0.71	0.32	0.26	0.32
6	Trans-Caryophyllene	0.34	_	_	0.20
7	Geranyl acetate	0.44	0.16	0.06	0.15
8	Cis-Verbenol	1.77	_	0.72	0.96
9	Trans-verbenol	0.96	0.67	0.72	1.72
10	$^{\gamma}$ Cadinene	0.45	0.33	0.39	_
11	4-Nonanone	0.11	0.08	_	_
12	Cis-carveol		0.03	0.04	0.07
13	Trans-carveol	0.07	_	_	0.07
14	2-Undecanone	0.03	_	_	0.02
15	Neryl acetate	0.22	_	_	_
16	Linalool	0.16	0.10	0.14	0.18
17	Nerol	2.85	3.17	1.52	2.55
18	à Humulene	_	0.04	_	_
19	6-methyl-5-hepten-2-one	0.05	_	_	0.07
20	α-pinene oxide	0.02	_	_	0.07

Table IV. Important essential oil compounds from sub-aerial part of control and UV treated plants.

Sl no	Compound Name	Peak Area (%)			
		Control	UV(15 min)	UV(30 min)	UV(1hr)
1	Z –citral	8.12	14.73	0.49	_
2	E –citral	22.35	_	_	1.67
3	ς-elemene	1.04	0.07	0.03	0.03
4	ς-cadinene	1.88	0.02	_	_
5	Germacrene B	_	0.99	0.03	0.09
6	Junipene	1.36	52.70	_	_
7	Juniper camphor	1.12	_	_	_
8	á Eudesmol	17.82	0.04	0.03	0.03
9	á Cadinol	_	0.53	_	0.14
10	à Bisabolene	_	0.04	_	0.03
11	à-Humulene	_	_	_	0.03
12	Azulene	_	0.32	_	0.09
13	Nerol	_	0.12	_	1.67
14	Limonene	_	_	0.74	0.19

15	Phellandrene	_	_	_	_
16	Germacrene C	_	_	_	0.14
17	Caryophyllene	_	0.26	0.12	0.14
18	Trans-Caryophyllene	_	_	0.12	0.14
19	Caryophylleneoxide	_	0.04	0.15	_
20	Farnesol	_	0.29	0.15	1.67

Discussion

The wild species of*C. flexuosus* studied earlier (Vinutha M and TharaSaraswathi KJ, 2013) were investigated presently for essential oil content and composition by administering UV treatment. The aerial part of sUV-B treated plants for 30 min showed increase inessential oil yieldand citral percentage compared to control. In sub-aerial part, exposure for 1 hour had marginally increased the essential oil content.Isomers of citral (geranial or neral), high concentrations of neral (14.73%) and Junipene (52.70%) were found in sub-aerial part of sUV-B treated plants.

The increase in essential oil yield and its components can be explained as lowlevels of UV-B exposure is found to induce secondary metabolite genes through central component of light signalling [Constitutive Photomorphogenesis 1 (COP1)] and UV Resistance Locus8 (UVR8) protein. (Heijde and Ulm, 2012; Favory et al., 2009; Brown et al., 2005).UVR8, a photoreceptor (Rizzini et al., 2011; Christie et al., 2012; Wu et al., 2012) plays a significant role in inducing secondary metabolite genes. Interaction of UVR8 with COP1 in UV-B dependant manner activates genes for enzymes involved in flavonoid biosynthesis, DNA repair and also genes involved in protection against UV-B damage. Genes of MAPK cascade is known to provide tolerance to plants against UV radiation. (Nawkar GM et al., 2013).

The enhancement of secondary metabolite compoundsdue to UV-B treatment has been found in many plants such as*C. citratus*where Z-citralhad increased by two fold. (Kumari et al., 2009a,b,c). Similarly linalool, 1,8-cineole, germacrene and eugenolincreased by three fold in *Ocimumbasilicum* L.(Nitz& Schnitzler, 2004). Catharanthine and vindolineincreased by three fold and 12 fold respectively in*Catharanthusroseus*L..

In earlier studies on *C. citratus,* exposure to UV treatment for 3 hours had shown increase in essential oil content (Kumari R et al., 2009). SEM observations in *C. citratus* exposed to lower doses of sUV-B (+1.8KJ/m^2d^1) showed thick waxy deposition on adaxial surface of leaves which could account for more production of essential oil in the plant. (Kumari R and Agarwal SB 2010). A positive effect of UV-B on essential oil production in *Menthaspicata*(Karousou R et al., 1998), *Menthapiperita*(Maffei M and Scannerini S, 2000), and*Ocimumbasilicum* (Johnson CB et al., 1999; Nitz GM and Schnitzler WH 2004)has been recorded. The light acting via phytochrome controlling enhancement of monoterpene levels in *Saturejadouglasii*has beenstudied (Peer WA and Langenheim JH, 1998). The beneficial effect of low doses (hormesis) of UV could be attributed as an adaptive response resulting in enhanced fitness of plants (Calabrase and Baldwin, 2001). At low doses of UV-B lesser amounts of cyclobutanepyramidine dimers (CPDs) are produced stimulating the protective response (Batschauer et al., 1996; Kim et al., 1998; Frohnmeyer et al., 1999) specially the biosynthesis of flavanoids and UV-B absorbing phenolic compounds (Li et al., 1993; Landry et al., 1995; Bieza and Lois 2001). UV-B is found to stimulate more than a dozen of genes involved in biosynthetic pathway of plants (Frohnmeyer H and Staiger D, 2003). sUV-B induced stimulation of growth in sub-aerial parts of plant species in fen ecosystem has been recorded.(Zaller et al., 2004).

During the present study on wild species of *C. flexuous*, sUV-B treatment for 30 min has enhanced the production of essential oil which is rich in geranial content (48.11%). The UV-B treatment showed increase in citral content (81.80%) in aerial parts of the plant compared to control (65%). Citral percentage higher than 75% in the essential oils of *Cymbopogon* species is considered as having high quality. UV treatment in the sub-aerial part of the plant showed slight increase in the essential oil content with isomers of citralbeing produced separately.

The compounds with high significance in essential oils (aerial and sub-aerial) of control and UV-B treated plants are limonene and limonene oxide used in cosmetics, perfumery, flavouring and in food industries. Caryophyllene, caryophyllene oxide and trans-caryophyllene used in the production of soaps, detergents and shampoos.β-Caryophylleneused as a dietary cannabinoid. Geranyl acetate has wide application in perfumery. Cis-verbenol has anti-ischemic, anti-inflammatory activity (Choi IYetal., 2010) highly valued as food flavouring agent and acts as a pheromone. Trans-verbenol is antioxidant in nature. 4-Nonanone is commonly used in the production of other chemicals. Cis and trans-carveol are used in cosmetics and flavoring industries. Cadinene has antioxidant property (Kundu A et al., 2013). 2-undecanone has application in the production of soaps, detergents, creams, lotions, perfumes, flavoring, as insect repellent, as a solvent in the production of vinyl resins, nitrocellulose, synthetic rubber and in the production of synthetic essential oils. Neryl acetate is used in flavors and perfumery to impart floral and fruity aromas. Linalool has application in the production of soaps, shampoos, detergents and lotions. It acts as a chemical intermediate in the synthesis of vitamin-E and has insecticidal property. Humulene has anti-inflammatory property. 6-methyl-5-hepten-2-one is the raw material in the synthesis of synthetic spices, baked goods, meat, sugar, beverage and in the production of other chemicals. Pinene oxide has anti-inflammatory and antimicrobialproperty and is used to treat an allergic condition called eczema. Elemene has medicinal properties. Cadinene is used in fragrance industry. It is an antioxidant and a pheromone. Germacrene is the precursor for sesquiterpene synthesis. Eudesmol exhibits anti-tumor activity (Ma EL et al., 2008). Cadinol has antifungal and hepatopotective property. Bisabolene is a natural sweetener, used in the biosynthesis of other compounds and in soaps. Azulene is used in ointments, cosmetics, shaving creams, has anti-inflammatory and antibacterial property. Phellandrenes has application in fragrance industry.Farnesol is used in perfumery and is also a natural pesticide and a pheromone. Junipene also called as longifolene is a raw material in flavor and fragrance industry, synthetic resins, synthetic perfumes, flotation oils and synthetic organic chemicals and can be used to make Isolongifolene andIsolongifolanone.

The present study highlights the effect of sUV-B on essential oil yield and variation in composition of essential oil from aerial and sub-aerial parts of *C. flexuosus*.

Conclusion

sUV-B stress induction in*C. flexuous* had led to the increased production of essential oil with higher citral content in aerial part compared to control.There was no significant variation observed in essential oil percentage of sUV-B treated sub-aerial parts.One of the isomers of citrali.e, geranial or neralwere obtained by UV treatment along with enhanced production ofjunipene by 50 fold compared to control. Further field studies are required to confirm the quality and quantity of the essential oil from control and UV-B stress induced plants.

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