Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2549
DOI URL: http://dx.doi.org/10.21474/IJAR01/2549
ISSN NO. 2320-5407

RESEARCH ARTICLE

EFFECT OF DIFFERENT ABIOTIC STRESS ON ESSENTIAL OIL YIELD FROM AERIAL PART OF CYMBOPOGON FLEXUOSUS (NEES EX STEUD) WATS.

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Manuscript Info

Manuscript History

Received: 27 October 2016
Final Accepted: 25 November 2016
Published: December 2016

Key words:-
Cymbopogon flexuosus, GA, ZN, sUV-B, Essential oil, GC-MS analysis.

Abstract

In nature, the plants are exposed to variety of stress which is broadly categorized as biotic and abiotic stress. During the present study, the effect of abiotic stress such as Gibberellic acid, Zinc nitrate and supplemental Ultraviolet-B (sUV-B) radiation on essential oil yield and composition has been studied in the aerial parts of *Cymbopogon flexuosus* (Nees ex Steud) Wats. The plants were exposed to GA and ZN radiation (2mM, 3mM and 4mM) and sUV-B radiation at different intervals of time (0.5h, 1.5h and 3h) and the effect of sUV-B stress for enhanced essential oil yield has been investigated. The oils obtained from different stress treated plants were analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS). The oils showed high citral content (isomer of geranial and neral) in sUV-B treated plants (sUV-B–1.5h), the compound extensively used in perfumery and pharmaceutical industry. A considerable increase in total oil content and citral percentage was seen in sUV-B treated plants when compared to other stress treatments and Control plants. The abiotic stress altering molecular, biochemical, physiological and morphological levels result in variation in essential oil production.

Introduction:-

Cymbopogon flexuosus (Nees ex Steud) Wats is a major aromatic grass belonging to the family Poaceae. The plant is commonly known as East Indian lemongrass/lemongrass/Cochin grass/Malabar grass. The wild grass is distributed in southern parts of India particularly in the coast of Malabar regions (Stapf, 1906). The lemongrass is native to the Indian subcontinent and distributed in Sri Lanka, Burma and Thailand. The plant yield is one of the top ten major essential oils in the World (Lawrence, 1985). The lemongrass is considered as an important member of the tropical C4 grass and mainly grown for their citral content. This grass is also extensively used as culinary herb and serves as an important ingredient in the herbal teas. The grass yields essential oil containing wide array of aroma chemicals. The quality of the essential oil is determined by the percentage of citral present in it (Jigisha and Parikh, 2011). The citral content in lemongrass oil higher than 75% is considered as high quality product (Guenther, 1950; Schaneberg and Khan 2002). Besides citral, the essential oil contains geranyl acetate, linalool, limonene, caryophyllene, pinene 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-Ionones are used as raw material for flavor, cosmetic and perfume industries; β-ionones are used in the synthesis of Vitamin-A (Pinder, 1960). The essential oil is used for treating different

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ailments and act as spasmolytic, analgesic, anti-inflammatory, antioxidant, antimicrobial, antipyretic, antinociceptive, anticancer, diuretic and transquilizing agents and used in treating digestive disorders, inflammation, diabetes, nervous disorders and fever, including various health problems (Kumari R et al., 2009; Prasad C et al., 2011; Quintans L et al., 2012).

The plant stress is defined as "changes in physiology that occur when the species is exposed to extraordinary unfavorable conditions that need not represent threat to life but will induce an alarm response" (Larcher, 1987). The balance between tolerance and sensitivity determines the positive and negative effect of stress (IlseKranner et al., 2010).

Gibberllic acid (GA) enhances metabolic activity within the plant pathway leading to production of secondary metabolites (Ohlsson and Bjork, 1988). Zinc nitrate (Zn) plays an important role in several plant metabolic processes; it activates enzymes and is involved in protein synthesis and carbohydrate, nucleic acid and lipid metabolism (Pahlsson, 1989). UV radiation (UV-B) is another important abiotic factor in plants life. The understanding of the underlying mechanism of plant UV-B response lags behind compared to light response mediated photoreceptors (Bobby A Brown et al., 2005). The tolerance exhibited by plants is attributed to effective absorption of UV-B by a wide range of secondary metabolites and UV-B is known to stimulate the biosynthetic pathway consisting of more than a dozen genes. During the present investigation, the *C. flexuosus* plants subjected to different stress treatments were studied for variation in content and composition of essential oil.

Materials and Methods:-

Stress induction:-

The *C. flexuosus* plants were subjected to different abiotic stress treatments such as GA, ZN and sUV-B and pot scale studies were performed to evaluate their effect. The foliar application at the concentration of 2mM, 3mM and 4mM were sprayed on GA and ZN treated plants (10 no. each) separately. Spraying was specifically done during evening hours (4-5 PM). The treatment was administered three times successively at an interval of 10 days for each treatment. After one month of treatment the plants were harvested for extraction of essential oil. The plants (10 no. each) were exposed daily to supplemental UV-B radiation for different intervals of time (0.5 h, 1.5 h, 3.0 h) for 10 days. The experiment was carried out in the growth chamber fitted with UV fluorescent tubes (TL40W/12 RS UV-B Medical, Philips) with an output of 312 nm. The lamps were placed at a distance of 45cm above the plants. Cellulose diacetate and polyester films of 0.13mm thickness were used to filter the transmission of wavelength below 290nm. The plants received +1.8 KJ/m^2 UV-B radiation and the Control plants were exposed to direct sunlight.

Histological studies:-

The Hand-cut leaf sections of *C. flexuosus* were prepared from fresh leaves and incubated for 30 min at room temperature in 1% (w/v) pararosaniline chloride and 4% sodium bisulphite in 0±25 NHCl (Schiff's reagent, Sigma Chemical Co.). The sections were then washed three times (10 min) with a freshly prepared solution of 0±5% (w}v) sodium metabisulphite in 0±1% HCl and examined under a Zeiss Standard 18 light microscope, using both transmitted light and epifluorescence (blue or UV excitation).

Essential oil studies:-

Extraction:-

The aerial parts of the plant were cut into small pieces (25mm length) and air dried at room temperature for three days. Fresh and dry weight of the herbage was recorded and subjected to hydrodistillation using Clevenger's apparatus for 3 hours. The essential oils thus obtained were stored under anhydrous sodium sulphate and kept in dark bottles at 4ºC for further analysis.

Analysis:-

The essential oils were analyzed during GC-MS technique. 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×0.25mm×0.25µm). The temperature program was 70ºC (6 min) rising to 260ºC at a rate of 60º/min. Injector and detector temperature was 260ºC. 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:-

The *C. flexuosus* plants subjected to stress treatments such as GA, ZN and sUV-B was studied for their effect on essential oil in the aerial part. The sUV-B treated plants produced high citral content (such as 81.80, 84.79 and 68.34) when compared to other stress treated plants and also Control plants (64.98).

Histological studies of the leaves of *C. flexuosus* showed the presence of essential oil cells in the adaxial side of mesophyll located inbetween the vascular bundles. In the sections from sUV-B treated plants, more number of oil cells were found having dense oil content, giving purple red color, indicating the presence of citral. The comparison of leaf sections of the three different treatments offered are shown in Fig 1.

The essential oils extracted were analyzed from the stress treated plants showed drastic variation in content and composition of essential oil. In Control plants studied, the fresh weight and dry weight of the herbage was found to be 281.18 and 102.32g respectively. In comparison, the GA treated plants of fresh weight varied from 381.08 to 424.54g along with the dry weight from 137.77 to 152.83g. In ZN treatment, the fresh weight and dry weight ranged from 363.07 to 429.09g and 121.47 to 157.17g respectively. The sUV-B treated *C. flexuosus* showed fresh weight of 161.59 to 237.63g and dry weight between 64.75 to 146.79g. The hydrodistillation of *C. flexuosus* yielded essential oil of yellow colored viscous liquid having strong lemon odor. The essential oil yield varied from 0.2 to 0.8% for different types of stress. In different cultivars of *C. flexuosus* the oil yield varied from 0.7 to 1.0%. The essential oil yields were analyzed by GC-MS method. In GA treated plants the oil yield ranged from 0.65 to 0.70%. In ZN treated plants it showed from 0.86 to 1.15% and sUV-B treated plants ranged from 0.76 to 1.68%. The fresh weight and dry weight of the herbage along with essential oil yield as studied in Control and stress induced plants are given in Table 1. The chemical composition of essential oils from GA and ZN treated plants (2mM, 3mM and 4mM) are listed in Table 2-7. The chemical composition of essential oils from sUV-B treated plants (0.5h, 1.5h and 3h) is shown in Table 8-10. The variation in citral content for different stress offered is given in Table 11. The GC-MS analysis of essential oils obtained from GA, ZN and sUV-B treated plants are shown in Fig 2, 3 and 4. The important aromatic compounds such as geranial, neral, nerol, â-caryophyllene, geranyl acetate and linalool showed variation in percentages for different types of stress treatments and is shown in Table 12.

The predominant compounds identified in the *C. flexuosus* essential oil for stress treatments presently studied are citral, nerol, farnesol, limonene oxide, linalool, nonanone, geranyl acetate, neryl acetate, caryophyllene, humulene, selinene, cadinene, isogeraniol, verbenol, carveol, pinene oxide and junipene. There was considerable variation observed in the essential oil concentrations for different types of stress administered.

Discussion:-

The abiotic stress induces signaling cascade and activate defense genes leading to physiological stress besides offering defense reaction (Arun Kumar Shanker, 2011). The stress signals confer the plants with ability to tolerate unfavorable conditions through gene expression, protein modification and primary/secondary metabolite composition (Dalcorso et al., 2010). The abiotic stress in Cymbopogon species showing variation in essential oil composition has been studied (Sangwan et al., 2001, Silva et al., 2005). The economic significance and high demand for lemongrass oil need to be met by large scale cultivation of Cymbopogon grass. The GA treatment offered expressed higher yield of *C. citratus* essential oil, besides enhancing plant growth and development (Figueiredo et al., 2006).

During the present investigation, the effect of different stress factors (GA, ZN and sUV-B) on *C. flexuosus* were studied which showed enhanced biosynthesis of essential oil in the aerial parts. The study revealed the significance of sUV-B stress where the increase in the citral concentration over GA and ZN was observed. This increase is due to higher concentrations of reduction equivalents for sUV-B stress leading to enhanced synthesis of highly reduced compounds like terpenoids, phenols or alkaloids (Dirk Selmar, 2008). The histological investigations presently made using leaf sections showed high oil producing cells for sUV-B treatment, which was supported by GC-MS report. The enhancement of Z-citral for sUV-B treatment, an important component for imparting essential oil quality for has been reported (Kumari et al., 2009). The present study showed noteworthy results for stress treatment (GA, ZN and sUV-B) which showed variation in essential oil yield, citral content and its composition. The sUV-B treated plants showed essential oil yield ranging from 0.76 to 1.65 and citral content 81.80% (0.5h), 84.79% (1.5h) and 68.34% (3 h). UV mediated enhancement is speculated to have been controlled by regulatory blur of compensatory molecular and physiological interactions (Marcel A K Jansen et al, 2012). To understand the molecular mechanism involved

for enhanced *C. flexuosus* essential oil for sUV-B treatment require further probing at molecular level.

Table 1:- Fresh weight, dry weight and essential oil yield for different stress treatment.

Sl. No	Treatment	Herbage Fresh wt (g)	Herbage Dry wt (g)	Essential oil Yield (%)
1.	Control	281.18	102.32	1.27
2.	GA-2mM	424.54	152.83	0.65
3.	GA-3mM	383.55	138.89	0.66
4.	GA-4mM	381.08	137.77	0.70
5.	Zn-2mM	423.68	148.53	0.86
6.	Zn-3mM	429.09	157.17	0.95
7.	Zn-4mM	363.07	121.47	1.15
8.	UV-0.5h	161.59	84.19	1.55
9.	UV-1.5h	222.45	68.39	1.65
10.	UV-3h	234.16	138.95	0.76

Table 2:- Chemical composition of essential oil (GA treatment-2.0 mM)

Sl. No.	Compound	Area %
1.	14-hexadiene,5-methyl-3-(1- methylidene)-	0.09
2.	β-Myrcene	0.10
3.	Ocimene	0.12
4.	α-Pinene oxide	0.8
5.	1,6-Octadiene, 2,6-dimethyl-	0.34
6.	β-Ocimene	4.67
7.	1-Octyn-3-ol	0.87
8.	Allo-ocimene	0.42
9.	Myrcenol	4.89
10.	Linalool	38.62
11.	Trans-chrysanthemal	21.36
12.	3,6,6-Trimethyl-cyclohex-2-enol	0.05
13.	Citronellal	7.33
14.	(-)-Isopinocampheol	0.10
15.	1-Pentanol,5-cyclopropylidene-	0.10
16.	3-undecyne	0.12
17.	3-carvomenthenone	0.01
18.	(Z)-linalool oxide (furanoid)	0.16
19.	Neral	0.09
20.	Geranial	0.02
21.	β-Vatirenene	0.05
22.	Citronellol	0.26
23.	Dextro-carvone	0.05
24.	Cycloisolongifolene	0.06
25.	Trans-(-)-Carveol	0.36
26.	cis-Carveol	0.25
27.	Nerol	0.34
28.	Methyl n-nonyl ketone	0.45
29.	Oxiranmethanol,3-methyl-3(4-mathyl-3-pentenyl)	0.35
30.	Bicyclopentylone	0.05
31.	Geranic acid	0.05
32.	Geranyl acetate	0.03

Purple-Isomers of citral, **Red**-Aromatic compounds, **Green**-altered compound

Table 3:- Chemical composition of essential oil (GA treatment-3.0 mM)

Sl. No.	Compound	Area %
1.	Geranyl vinyl ether	0.08
2.	Nerol	0.08
3.	Geraniolformate	0.08
4.	Farnesol	0.10
5.	3-Ethyl-1,5-octadiene	0.33
6.	Verbenol	4.99
7.	trans-Verbenone	0.91
8.	Limonene oxide	0.50
9.	Carane, 4,5-epoxy-, (E)-	4.99
10.	E-Citral	39.76
11.	Z-Citral	23.36
12.	Adrenalone	0.01
13.	Tetrahydrophthalimidine	7.69
14.	2-Undecanone	0.02
15.	2-Nonanone	0.04
16.	Piperazine	0.02
17.	Dodecadien-1-ol	0.02
18.	Geranyl acetate	0.10
19.	Neryl acetate	0.08
20.	Valeranone	0.01
21.	Nerolidyl acetate	0.01
22.	trans-Caryophyllene	0.24
23.	à-Humulene	0.03
24.	á-Selinene	0.03
25.	ã-Muurolene	0.26
26.	ç-Cadinene	0.26
27.	à-Amorphene	0.26
28.	(-)-Caryophyllene oxide	0.27
29.	Aromadendrenepoxide	0.27
30.	Azulenol	0.02
31.	Cubenol	0.02
32.	ç-Linolenic acid, methyl ester	0.04
33.	Campherenone	0.02
34.	Neophytadiene	0.02
35.	Phytol	0.02
36.	â-Doradecin	0.01
37.	Urs-12-en-28-al	0.01
38.	Ethyl geranate	0.04

Purple-Isomers of citral, **Red**-Aromatic compounds, **Green**-altered compound

Table 4:- Chemical composition of essential oil (GA treatment-4.0 mM)

Sl. No.	Compound	Area %
1.	1-Undecanol	0.02
2.	Maleic acid	0.04
3.	Neryl propionate	0.07
4.	Linalyl acetate	0.07
5.	Geranylpropanoate, (E)-	0.07
6.	Linalyl propionate	0.07
7.	Nonadienol, 4,8-dimethyl- (fragrance)	0.35
8.	ç-Isogeraniol	0.35
9.	Limonene oxide	1.08
10.	Carane, 4,5-epoxy-, trans	0.97

11.	Verbenol	6.56
12.	E-Citral	41.09
13.	Z-Citral	26.96
14.	Trans-(+)-Carveol	0.06
15.	Cobaltocene	0.02
16.	3-tert-Butylmalemide	7.32
17.	Isopropylamine	7.32
18.	2-Undecanone	0.03
19.	Geranyl acetate	0.05
20.	Neryl acetate	0.10
21.	Geraniol ester	0.01
22.	(-)-á-Elemene	0.01
23.	â-Caryophyllene	0.30
24.	à-Humulene	0.03
25.	á-Selinene	0.03
26.	à-Caryophyllene alcohol	0.01
27.	Longiborneol	0.01
28.	Aeruginol	0.01
29.	Junipene	0.01
30.	ã-Cadinene	0.25
31.	Isolongifolan-8-ol	0.02
32.	(-)-Caryophyllene oxide	0.32
33.	Aromadendrenepoxide	0.32
34.	Humulene oxide	0.06
35.	Ledene	0.02
36.	Nerolidyl acetate	0.02
37.	Nerolidol	0.02
38.	Longipinocarveol, trans-	0.02
39.	Neophytadiene	0.01
40.	Phytol	0.01

Purple-Isomers of citral, **Red** - Aromatic compounds, **Green -** altered compound

Table 5:- Chemical composition of essential oil (ZN treatment-2.0 mM)

Sl. No.	Compound	Area %
1.	2,3-Pinanediol	0.12
2.	Linalool	0.15
3.	à-Terpinolene	0.15
4.	3,7-Dimethylocta-2E,6-dienal	0.13
5.	Phenol, 4-(2-aminoethyl)-	0.48
6.	Verbenol	2.78
7.	Limonene oxide	1.25
8.	E-Citral	40.02
9.	Z-Citral	23.68
10.	cis-Isolimonenol	0.09
11.	cis-Carveol	0.09
12.	4-Pentenylcyclopentadiene	6.33
13.	2-Undecanone	0.01
14.	17-Octadecynoic acid	0.05
15.	Geranyl acetate	0.16
16.	Neryl acetate	0.09
17.	Geraniolformate	0.09
18.	trans-Caryophyllene	0.21
19.	ã-Cadinene	0.66
20.	1H-3a,7-Methanoazulene	0.02

21.	á-Chamigrene	0.02
22.	Junipene	0.02
23.	Patchoulene	0.02
24.	(-)-Caryophyllene oxide	0.35
25.	(+)-(R)-à-Ionol	0.06
26.	ã-linolenic acid methyl ester	0.03
27.	á-Cedren-9-à-ol	0.02
28.	Uvidin A	0.04
29.	Ethyl geranate	0.04
30.	ë-Damascone	0.03
31.	Quercetin	0.03

Purple-Isomers of citral, **Red**-Aromatic compounds, **Green**-altered compound

Table 6:- Chemical composition of essential oil (ZN treatment 3.0 mM)

Sl. No.	Compound	Area %
1.	2,3-Pinanediol	0.12
2.	Linalool	0.15
3.	à-Terpinolene	0.15
4.	3,7-Dimethylocta-2E,6-dienal	0.13
5.	Phenol, 4-(2-aminoethyl)-	0.48
6.	Verbenol	2.78
7.	Limonene oxide	1.25
8.	E-Citral	40.02
9.	Z-Citral	23.68
10.	cis-Isolimonenol	0.09
11.	cis-Carveol	0.09
12.	4-Pentenylcyclopentadiene	6.33
13.	2-Undecanone	0.01
14.	17-Octadecynoic acid	0.05
15.	Geranyl acetate	0.16
16.	Neryl acetate	0.09
17.	Geraniolformate	0.09
18.	trans-Caryophyllene	0.21
19.	ã-Cadinene	0.66
20.	1H-3a,7-Methanoazulene	0.02
21.	á-Chamigrene	0.02
22.	Junipene	0.02
23.	Patchoulene	0.02
24.	(-)-Caryophyllene oxide	0.35
25.	(+)-(R)-à-Ionol	0.06
26.	ã-linolenic acid methyl ester	0.03
27.	á-Cedren-9-à-ol	0.02
28.	Uvidin A	0.04
29.	Ethyl geranate	0.04
30.	ë-Damascone	0.03
31.	Quercetin	0.03

Purple-Isomers of citral, **Red**-Aromatic compounds, **Green**-altered compound

Table 7:- Chemical composition of essential oil (ZN treatment 4 mM).

Sl. No.	Compound	Area %
1.	6-Methyl-5-hepten-2-one	0.17
2.	3-Octanone, 2-methyl- (CAS)	0.11
3.	4-Nonanone (CAS)	0.11
4.	Geranyltiglate	0.14
5.	Linalyl acetate	0.14
6.	Neryl acetate	0.14
7.	Linalool	0.14
8.	Citronellyltiglate	0.02
9.	3-Ethyl-1,5-octadiene	0.47
10.	2,6-Octadiene, 4,5-dimethyl	0.47
11.	ç-Isogeraniol	0.47
12.	E-Citral	31.48
13.	Z-Citral	20.84
14.	Verbenol	3.41
15.	cis-Limonene oxide	2.51
16.	Cyclohexene carboxaldehyde	1.61
17.	Carvyl acetate	0.16
18.	2-Undecanone	0.02
19.	trans-Carveol	0.25
20.	Geranyl acetate	0.25
21.	â-Caryophyllene	0.43
22.	à-Humulene	0.03
23.	ã-Cadinene	0.30
24.	Clovene	0.02
25.	Junipene	0.02
26.	(-)-Caryophyllene oxide	0.27
27.	Farnesol	0.27
28.	Ledene (CAS)	0.27
29.	Humulene oxide	0.03
30.	5-Isocedranol	0.03

Purple-Isomers of citral, **Red**-Aromatic compounds, **Green**-altered compound

Table 8:- Chemical composition of essential oil (UV treatment 0.5h)

Sl. No.	Compound	Area %
1.	Exo-2-Hydroxycineole	0.07
2.	6-Methyl-5-hepten-2-one	0.07
3.	4-Octanone, 7-methyl- (CAS)	0.09
4.	2,3-Octanedione (CAS)	0.09
5.	4-Nonanone	0.09
6.	Linalool	0.18
7.	Nerol	0.18
8.	1,5-Heptadiene, 3,4-dimethyl-	0.52
9.	Farnesol	0.50
10.	1,7-Nonadien-4-ol, 4,8-dimethyl-	0.52
11.	Verbenol	0.96
12.	Verbenone	2.84
13.	Limonene oxide, trans-	0.96
14.	Carane, 4,5-epoxy-, trans	1.72
15.	à-Pinene oxide	0.07
16.	Trans – Carveol	0.07
17.	E-Citral	48.11
18.	Z-Citral	33.69

19.	Cis – Carane	14.03
20.	Catechol	14.03
21.	2-Undecanone (CAS)	0.02
22.	Geraniol acetate	0.15
23.	Neryl acetate	0.15
24.	Geranyl acetate	0.20
25.	trans-Caryophyllene	0.20
26.	RT: 17.71	
There is no library search data to show the results for.		
27.	ã -Cadinene (CAS)	0.58
28.	à-Amorphene	0.29
29.	Caryophyllene oxide	0.32
30.	Farnesol	0.32
31.	Menthol	0.03
32.	Octadecatrienoic acid	0.03
33.	Phytol	0.06
34.	Neophytadiene	0.03
35.	trans-Crotonophenone	0.04

Purple-Isomers of citral, **Red**-Aromatic compounds, **Green**-altered compound

Table 9:- Chemical composition of essential oil (UV treatment 1.5 h)

Sl. No.	Compound	Area %
1.	Linalyl acetate	0.07
2.	2,6-Nonadienal, 3,7-dimethyl-	0.01
3.	(+)-9-O-Demethylhomolycorine	0.33
4.	Geranyl nitrile	0.33
5.	Ethanone	0.54
6.	Z-limonene-1,2-epoxide	0.54
7.	E-Citral	52.81
8.	Z-Citral	31.98
9.	trans-Verbenone	0.97
10.	Verbenol	5.68
11.	cis-Carvyl acetate	0.08
12.	cis-Carveol	0.04
13.	trans-Resveratrol	0.02
14.	Mefenorex	6.64
15.	trans-carvyl acetate	6.64
16.	2-Undecanone	0.02
17.	Rhombifolin	0.02
18.	Geranyl acetate	0.06
19.	Neryl acetate	0.04
20.	trans-Caryophyllene	0.09
21.	trans-ã-cadinene	0.29
22.	à-Amorphene	0.13
23.	Torreyol	0.02
24.	Spathulanol	0.02
25.	Cubenol	0.02
26.	Isolongifolan-8-ol	0.02
27.	Caryophyllene oxide	0.31
28.	Longifolenaldehyde	0.21
29.	Humulene oxide	0.04
30.	(+)-(R)-à-Ionol	0.02
31.	5-Isocedranol	0.02
32.	Farnesyl acetate	0.02

33.	Alloaromadendrenoxide-(1)	0.02
34.	Isoaromadendrene epoxide	0.02
35.	Longipinocarveol, trans-	0.02
36.	Neophytadiene	0.01
37.	Linoleic acid methylsilyl ester	0.01
38.	Urs-12-en-28-al	0.01
39.	Ethyl geranate	0.01

Purple-Isomers of citral, **Red**-Aromatic compounds, **Green**-altered compound

Table 10:- Chemical composition of essential oil (UV treatment 3 h)

Sl. No.	Compound	Area %
1.	6-Methyl-5-hepten-2-one	0.17
2.	4-Nonanone	0.14
3.	Linalool	0.19
4.	Demethylhomolycorine	0.39
5.	1,7-Nonadien-4-ol, 4,8-dimethyl-	0.33
6.	Verbenol	4.63
7.	Cis-Limonene Oxide	0.97
8.	Carane, 4,5-epoxy-, trans	1.65
9.	à-Pinene oxide	0.10
10.	E-Citral	38.49
11.	Z-Citral	29.85
12.	2-Undecanone	0.01
13.	Borneol	0.02
14.	Geraniolformate	0.09
15.	Neryl acetate	0.09
16.	Methanoazulen	0.02
17.	trans-Caryophyllene	0.24
18.	à-Santalol (CAS)	0.02
19.	á-Selinene	0.02
20.	à-Humulene	0.02
21.	ã-Murolene	0.30
22.	à-Amorphene	0.30
23.	ç-Cadinene (CAS)	0.30
24.	(-)-Caryophyllene oxide	0.31
25.	Humulene oxide	0.02
26.	Octadecadienoic acid	0.01
27.	Cedrane, 8-propoxy-	0.01
28.	à-Cedrol	0.01

Purple-Isomers of citral, **Red**-Aromatic compounds, **Green**-altered compound

Table 11:- Variation in percentage of Citral for different stress treatments

Sl. No	Treatment	Citral content (%)
1.	Control	64.98
2.	GA-2mM	54.66
3.	GA-3mM	63.12
4.	GA-4mM	68.05
5.	ZN-2mM	68.37
6.	ZN-3mM	63.70
7.	ZN-4mM	52.32
9.	**UV-0.5h**	**81.80**
11.	**UV-1.5h**	**84.79**
14.	UV-3h	68.34

Table 12:- Variation in percentage of aromatic compounds for different stress treatments .

Compound	Control	GA (2mM)	GA (3mM)	GA (4mM)	Zn (2mM)	Zn (3mM)	Zn (4mM)	UV (0.5h)	UV (1.5h)	UV (3 h)
Geranial	35.13	38.02	39.12	34.91	16.24	15.96	12.03	48.11	52.81	38.49
Neral	29.85	27.80	28.93	28.21	15.42	17.74	10.29	33.69	31.98	29.85
Nerol	2.85	2.67	2.26	1.40	-	-	-	1.52	1.57	2.55
β-caryo phyllene	1.63	0.10	0.12	0.02	0.18	0.21	0.20	1.44	0.17	0.20
Geranyl acetate	0.22	0.05	0.08	0.05	0.02	0.09	0.25	0.06	0.09	0.15
Linalool	0.16	0.12	0.16	0.12	0.12	0.15	-	0.14	0.19	0.18

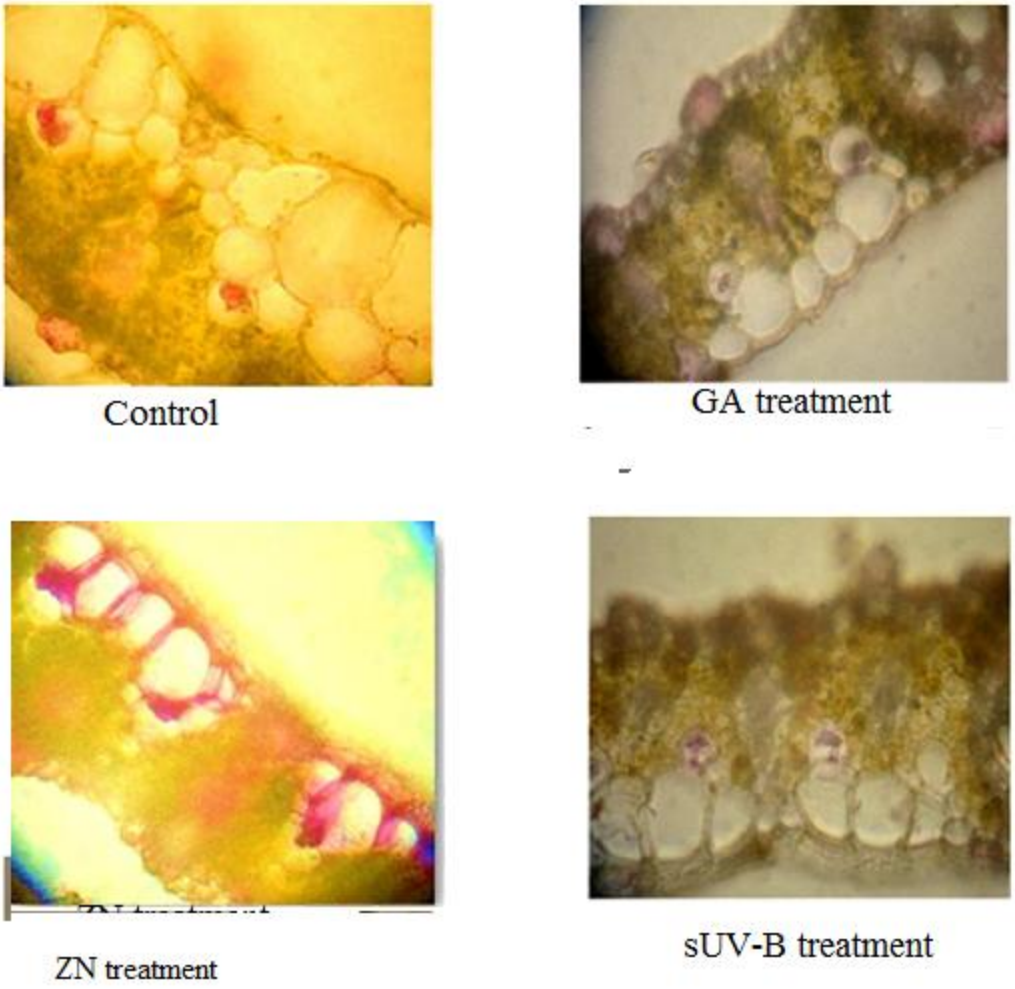

Fig 1:- Leaf sections showing essential oil synthesis for different stress treatments.

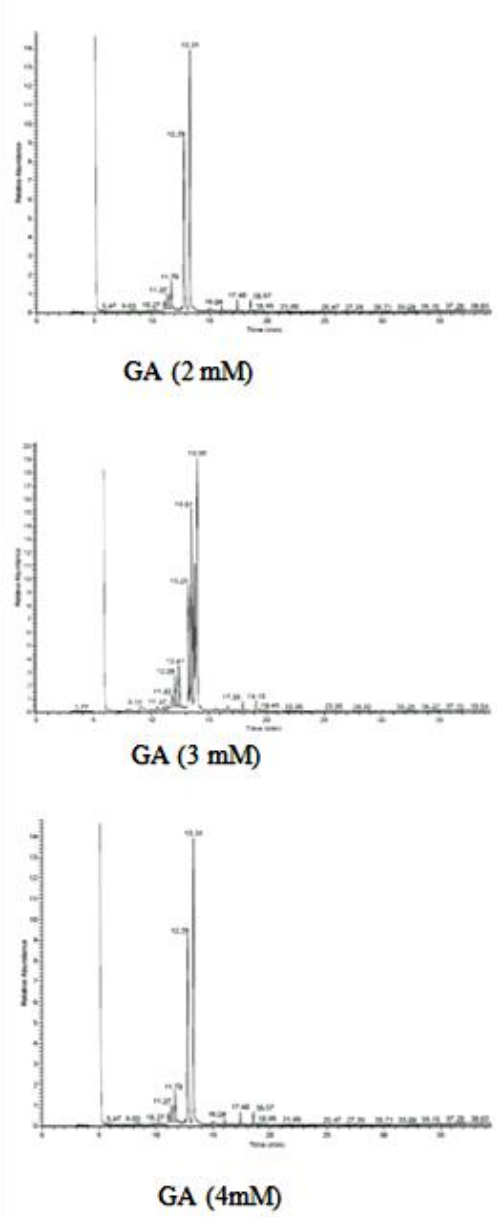

Fig 2: GC-MS analysis of essential oil for different GA treatments.

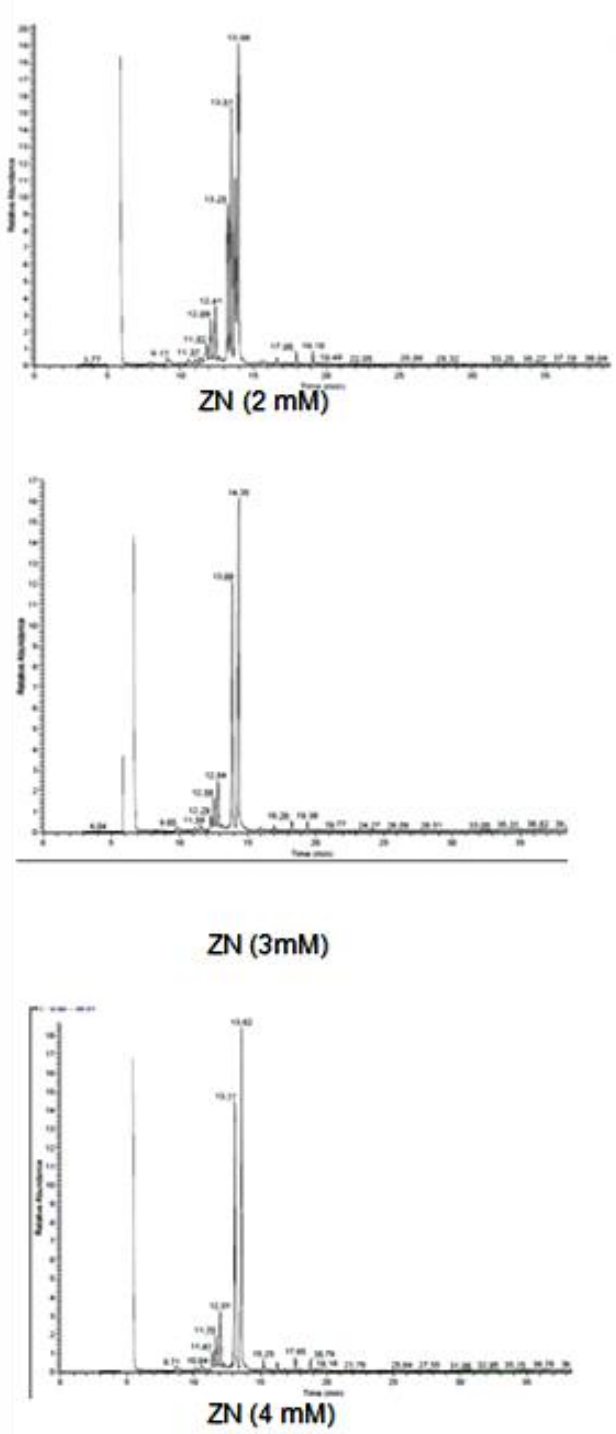

Fig 3:- GC-MS analysis of essential oil for different ZN treatments.

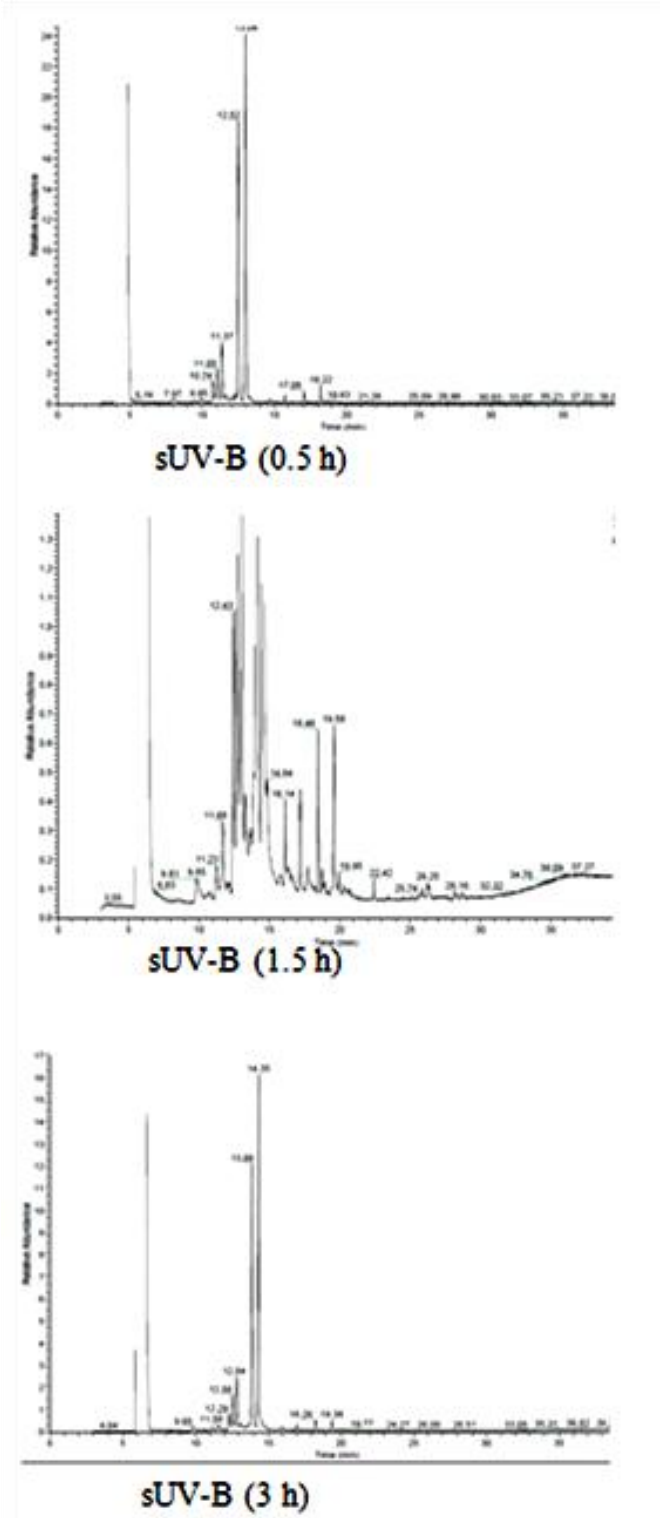

Fig 4:- GC-MS analysis of essential oil for different sUV-B treatments.

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