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Humic Substances Effect and Climatic Tensions on the Growth and Essential Oil Quality of the Cultivated Aloysia.triphylla (Iran)

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ABSTRACT

Aloysia. triphylla (L, H, er.) Britton (Family Verbenaceae) is applied in nutritional and pharmacological purposes. Soil factors and climatic tensions are essential in the growth of the herbs. A. triphylla plants were planted in the University greenhouse (Zahedan) in March. Humic substances solutions were sprinkled onto the soil surrounding the root of the plant. This way of planting simultaneously was repeated in the Zahedan outskirts and Zarand area. In late June, the leaves of planted samples were collected and prepared for senescing through the hydro distillation method. After collecting leaves and be sprinkled with humic substances solution onto the root surrounding soil of the same plant, the leaves were picked up again in late September for oil extraction. Oil yields (w/w%) of the greenhouse's June samples were 0.45 and 0.48 for the blank and humic samples respectively. By analyzing oils, using GC & GC-MS techniques, 47 and 46 compounds were identified from the blank sample and humic sample oils, constituting 93.21% and 91.58% respectively. The higher percent of which: limonene, 1, 8-cineole, Z-citral, E-citral, bicylogermacrene, trans-caryophyllene, spanthenol compounds. Oxygenated terpenoids percentage in the humic sample oils varies between 43.70 and 60.16 of which 43.70 belongs to the oil of Zarand September humic sample (Sapropel) and 60.16 to the oil of the greenhouse's June humic sample (Sapropel). Oxygenated terpenoid compounds in September samples have decreased a fewer than those of June samples. These changes result from humic substances and climatic tensions. As a result, the flowering period is shortened and the oil quality has been optimized.

Keywords: Cultivated A. triphylla plant; Humic substances; Climatic tensions; Growth stage; Essential oil quality

INTRODUCTION

Quince is a native plant of wet and hot regions of southern America which is a tropical area. The plant is sensitive to cold and loses its leaves at a temperature below 0 ^{0}C. Since its stem is resistant to freezing the temperatures down to around -10 ^{0}C, it can be grown under different environmental conditions (Bremness, 1998; Gil *et al.*, 2007).

Soil fertility depends on the intensity of organic materials. By chelating necessary elements, humic acid, and fulvic acid, increase the absorption of metal ions, soil fertility and productivity in the plants (Hayes, 1985). Humic substances are plant growth, stimulating agents that have been applied in agriculture in recent years. However, due to complex structures of humic substances in nature, detailed mechanisms of how these materials work in plants are still not well understood (Hayes ,1985).

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FIGURE1: Image. *Aloysia. Triphylla* (L' Her) Britton plant

There are many reports on the role of humic substances in promoting plant biomass, stimulation of root, shoot, and flowering growth, and even direct effects on crop productivity and increases crop yields (Sardashti *et al*.,2012). They can be extracted from many natural sources such as peat, soil, and linarite ore. Municipal yard wastes, sewage sludge, and composts also could be sources of these materials. The application of humic substances in golf courses and sports turf management has recently been highlighted. Due to major environmental concerns, it is becoming a popular practice to use these materials as soil amendments (Chen *et al*.,2004). Humic substances not only increase fertilizer efficiency and promote plant growth but also can reduce potential groundwater contamination. Plant growth research involving humic substances has focused on growth chambers, greenhouse, and golf green experiments. Both basic and practical research components are underway to investigate the 'how and why' of the humic substances interacting with plant and soil ecosystems (Liu and Cooper,2000).

Lotosh (1991) worked, on the applications of the humic substance in soil fertility. In his research, the effect of humate ammonia on plants growth and soil optimization was studied in detail (Lotosh ,1991). Additives and humic substances can promote soil fertility, azotic fertilizers have a remarkable effect on leaf and stem creating and shooting of plants (Lu *et al.*,2000). The term of quality in medicinal plants can be attributed to either the ratio of storage organs of effective substances to either other parts of a plant or the rate of heavy metals such as Cadmium, Lead, Zinc and other element affecting the growth of that plant (Kabata-Pendias and Pendias, 1992).

The foliar application of organic fertilizers can supply nutrients more rapidly than methods involving root uptake which made the local growers use foliar fertilizers to supplement soil applied nutrients to compensate for decreased root activity. These products have numerous agronomic applications, including the supply of plant nutrients, control of pests and diseases, and in management of soil health. Its effects may be attributed to many factors, including the natural source and concentration of humic substances, soil pH, and plant species (McCarthy *et al*.,2015).

The price of these plants from their natural and wild habitats is very low. The cultivation and planting of medicinal plants and their follow-up industrial processing should be conducted by relevant experts as it helps to protect the land from destruction, promoting the uniformity and quality of final products (Hüsnü and Baser ,1999; Omid Baigi ,2005).

De Figueiredo *et al* (2004), conducted research on essential oil compounds, extracted from leaves *Aloysia triphylla* collected in Sao Paulo (Brazil). The compounds with the highest percentage are: geranial (29.54%), neral (27.01 %), limonene (15.93 %), geranyl acetate (4.0 %), geraniol (3.96 %) (De Figueredo *et al*.,2004).

Argyropoulou against (2007) about the chemical composition of the essential oil from the leaf of *Lippia citriodora* HBK at two developmental stages which were collected from plants growing in the gardens of the Agriculture University of Athens, that showed the main compounds included: geranial (38.7 %), neral (24.5 %) and limonene (5.8 %) for essential oil during the vegetative stage (May), geranial (6.0 %), germacrene-D(1%) ,α-curcumin (3.1 %), bicylogermacrene (2.4 %), β-caryophyllene (1.8 %), (Z)-β-ocimene (1.3 %) and geranyl acetate (1.1 %) for essential oil during the vegetative stage (Argyropoulou *et al*.,2007).

Oliva *et al* (2010), investigated the antimicrobial activity of *A. triphylla* oils from different regions of Argentina. The essential oils shared common compounds but presented differences in the quantity and quality of the rest of them. The essential oil from La Paz showed the highest citral/ limonene relation and the best antimicrobial activity. This essential oil containing: geranial (29.20%), neral (20.0%), limonene (2.90%). The compounds of essential oil from region Las Vinas with the highest percentage are geranial (3.30%), neral (12.40%), limonene (21.30%) (Oliva *et al*.,2010).

In 2013, Parodi *et al*, extracted the essential oil from leaves *Aloysia triphylla*, collected the local market of Santa Maria (RS, Brazil) .by means of the SFC method, by pressurized CO_2 extraction at 30, 50 and 70ºC, and 100, 150, and 200 bars, and analyzed by GC-FID and GC-MS techniques. The compounds with highest percentage for 50 ^{0}C and 150 bars are: Z-citral (12.60%), E-citral (16.30%), geranyl acetate (2.70%) and β-caryophyllene (6.50%) (Parodi *et al*.,2013).

Given the required conditions for planting this plant, it has been successfully planted under greenhouse and environmental conditions. In general, among different environmental factors affecting the growth of medicinal plants as well as the quantity and quality of effective substances (e.g. essential oils and alkaloids), soil factors are of considerable importance (Santos-Gomes *et al*.,2005). The purpose of this paper is to analyze the composition of the essential oil from leaves of the cultivated *A.triphylla* in the greenhouse condition, Zahedan outskirts and Zarand area (Iran), as well as the effect of soil factors, humic substances and climatic tensions on plant growth and quality of the essential oil in the composition at the different developmental stages, using GC &GC-MS and FAAS techniques were used for comparison to check whether the methods give similar results concerning the main compounds.

Materials and Methods

Instrument

Extraction of humic acid was performed by agitator (ELM 1400 rpm, Germany). FTIR 460 plus, Jasco Company, made in Japan). Clevenger- type apparatus for essential oil extraction. To analyze the essential oil, by GC-MS apparatus was used (HP Agilent Technology, made in the USA). The amounts of metal ions (i.e. mineral analysis) were determined by the Flame atomic absorption apparatus (model VGA 77, Varian Company, made in Australia). The amounts of nitrogen in aerial parts plant and growing place soil by (Kjeltce method). The soil humus was measured using the Engrain method, and the soil pH was determined using a digital pH meter CD620 with the glacial combined electrode, calomel reference electrode (Zag Shimi, Iran). All reagents used were of the analytical grade with the highest purity available.

Planting methods

Plant samples of *Aloysia. triphylla* (L' H' er.) Britton was planted in a greenhouse belonging to the University of Sistan and Baluchestan (Zahedan) in early March 2010. Simultaneously, another sampling was planted as a blank sample, one meter away from the subject plant. This way of planting simultaneously was repeated in the Zahedan outskirts and Zarand area. Humic substances (e.g. Sapropel and Sodium humate prepared by Russian company ORISTAN) and extracted humic acid from

Naharkhoran forest soil of Gorgan in the form of the solution, were sprinkled onto the soil around the plant root. Simultaneously, two other saplings were planted as the blank samples, one meter away from the subject samples. Such arrangements for actual and the blank samples were repeated at two more sites including Zahedan outskirts and Khanok_Zarand area. After collecting the leaves in late June, the stems were cut to smaller sizes and the humic solution was again sprinkled on the soil around the roots. Plant identification was carried out by Dr Mozaffarian Botanist in the Research Institute of Forests and Rangelands (Tehran-Iran) (Mozaffarian ,2007).

Humic substances

Humic substances, the most valuable components of sapropel, are a complex mixture of high-molecular natural organic compounds with bioactive properties. Humates have a stimulating, immunomodulatory, anti-inflammatory, and antibacterial effect, i.e. they can sorb and bind toxic elements. Another important quality of sapropel deposits is sustainability and naturalness. Sapropel is a natural organic substance formed by the deposition of dying plants and microorganisms with limited access to oxygen, which is always found on the bottom of freshwater bodies. New types of fertilizers based on peat and sapropel were developed and can be used as solid and liquid forms of organic and organic mineral fertilizers and preparations for different crop cultivation. It was shown that the use of SAPROAgro and SAPROElixir fertilizers increases the productivity of agricultural plants by 9-16 %. Sodium humate feed additive, it's humic acids sodium salt obtained after humic acid reacts with NaOH, which is soluble in water. Have shiny flake, shiny crystal and powder type.

Humic acid extraction

The soil samples were dried in shadow, ground and sieved so that the final particles became 1.0 mm. Humic acid was extracted from the Nahakhoran forest soil of Gorgan (north of Iran) according to the IHSS (International Humic Substances Society) protocol and then it was purified (Davies *et al*., 2001; Stevenson,1994). The efficiency of extracted humic acid was 1% (w/w) dry weight. Since they were then sieved to particles with 75-150µm sizes. Sapropel has not more than 30% humic acid implying that our extracted humic acid has a stronger effect.

To sprinkle humic substances

A 20±0.01g pack of Sapropel was solved in 400±0.1 ml water. The new mix was then sprinkled with 3 L water on the base of the plant root and much less on plant leaves in an area of about half an acre. This should be performed over March when the plants wake up from winter sleep and start to grow. As previously mentioned, next to the actual samples, some blank samples with no Sapropel**,** treatment were considered.

Sampling

Collected leaves from the plants grown in late June and early September were exposed to extraction. At the same time, the growing place soil. The leaves were freeze-dried in the shade at the ambient temperature and stored in double-layer paper bags at room temperature (Cumhu et al.,2016; Ratti,2012), protected from the direct light, until further analysis. They were then sieved to particles with 0.5 mm sizes.

Essential oil isolation

The essential oils were extracted by mixing 50±0.01g fresh leaves of *A. triphylla* with 500±0.1 ml of distilled water in a 2L-balloon at the temperature of 100 ^{0}C and at normal pressure for 3 h, using a Clevenger- type apparatus based on the recirculation of water according to the method recommended in the European Pharmacopoeia (Council of Europe,1997). The appearance characteristic of essential oils is greenish-yellow color and tang. The obtained essential oil was collected in n-hexane-solvent and more water in the essential oil was extracted by the sodium sulfate. Then the essential oil was maintained in the small metal container in the refrigerator. Finally, essential oils were analyzed using GC&GC-MS techniques (Sardashti and Kordi Tamandani,2021; Sardashti,2017).

GC-MS analysis

The essential oils were analyzed using a Hewlett-Packard 5890 II GC equipped with INNOWAX and an HP-5MS capillary column (60 m x 0.25 mm x 0.25 µm film thickness) and a mass spectrometer HP 5972 as the detector. The helium gas was carrier a gas flow rate of 1 ml/min. The temperature was gradually increased column and from 60 ^{0}C to 260 ^{0}C with a rate of 3 ^{0}C/ min for the polar column. For GC-MS detection, an Electron Ionization System was used with an ionization energy of 70 eV. The injector and MS transfer line temperatures were set at 220 ^{0}C and 290 ^{0} C, respectively. One microliter of the sample was manually injected with splitless mode. A solution containing the homologous series of C_8-C_{26} n- alkenes was added to the oil at the same chromatographic conditions according to the Van Den Dool method (Van Den Dool and Kratz,1963). n- Alkenes were used as reference points in the calculation of the Kovats indices. Tentative identification of the compounds was based on the comparison of their relative retention time and with the mass spectral database library data of the GC-MS system and the literature data (Adams,2001).

Statistical analysis

The measurements were done in triplicates to test their reproducibility. All results are presented as mean ±S.E. Statistical analyses were performed by Student's t-test. The values of $P<0.05$ were considered statistically significant.

Results and Discussion

The plant leaves were collected in two phases; once in June (during growth) and once in September (during flowering) and their essential oils were extracted using hydrodistillation method. The essential oil samples in different growth conditions were analyzed using the GC & GC-MS techniques. The relative error for measurement of oil yields in different growth conditions was 0.11 %.

Results

Results of essential oil of June's samples

According to table 1, the oil yields (w/w%) in June's samples varies between 0.35 and 0.48 of which 0.35 belongs to Zahedan' s humic sample and 0.48 to the greenhouse's humic sample. In the oil of the greenhouse's June blank sample, forty-seven constituents embracing 93.21% of the total essential oil were recognized and Unknown constituents (1.59 %). In the oil of the greenhouse's June humic sample, forty-six constituents embracing 91.58% of the total essential oil were identified and Unknown constituents (2.30%). Forty-eight constituents embracing 90.43% of total oil were identified in Zahedan's June humic sample.

In the essential oil of June's samples in different conditions exist 32 similar compounds with high percentage. According to table 1, the main compounds include : (22.73 % ≥ E-citral ≥20.23%),(18.95% ≥ Z-citral ≥17.34 %) , (6.15% ≥ 1, 8-cineole ≥1.71%) , (6.05% ≥spathulenol ≥ (3.96 %), (6.04% ≥ limonene ≥1.69%) , (5.78% ≥ α-cubebene≥ 3.91%), (5.65% ≥ trans-caryophyllene≥ 3.93 %), (4.55 % ≥curcumene≥0.82%),(3.94%≥ bicylogermacrene ≥ 2.77 %), (2.76 % ≥cis-gerainol≥1.27 %), (2.32 % ≥ β-pinene ≥ 1.17%) , (2.10 % ≥ β-fenchyl alcohol ≥1.73%), (2.10 % ≥ β-fenchyl alcohol ≥1.73%), (1.75 % ≥ farnesol ≥ 1.25%) ,(1.21% ≥ epi-(+) bicycle Sesquiphellandrene≥ 1.05 %) , (1.30% ≥ linalool ≥0.35 %), (1.20 ≥ sabinene ≥ 0.33%) and (0.65% ≥ α- pinene ≥0.17 %) compounds.

Table 1. Chemical composition of essential oils of *A. triphylla* collected in June

Compound	RI	Greenhouse blank sample essential oil	Greenhouse humic sample(Sapropel) essential oil	Zahedan blank sample essential oil	Zahedan humic sample (Sapropel) essential oil	Zarand blank sample essential oil	Zarand humic sample (Sapropel) essential oil
hexanal	703	0.02	0.01	0.02	-	0.02	0.02
2,4-dimethyl heptane	746	0.02	0.02	-	-	-	-
2-hexanl	753	0.10	0.10	0.16	0.12	0.16	0.13
santolina triene	833	0.01	-	-	-	-	-
α-thujene	852	0.07	0.04	0.02	0.03	0.03	0.04
α-pinene	858	0.46	0.34	0.17	0.23	0.23	0.65
camphene	870	0.12	0.02	0.03	0.03	-	0.12
sabinene	898	1.20	0.94	0.33	0.56	0.49	0.49
β-pinene	904	2.32	1.71	1.17	1.40	2.26	2.18
1-octene-3-ol	910	0.39	0.33	0.8 3	0.52	0.65	0.58
β-myrcene	917	0.39	0.25	0.11	0.14	0.16	0.17
isobutyl valerate	925	-	-	-	-	0.05	-
δ-3-carene	935	-	-	-	-	-	0.91
o-cymene	942	-	-	-	-	0.32	-
1,8-cineole	954	6.15	3.44	1.97	2.70	1.71	2.32
limonene	958	6.04	5.44	1.69	3.40	2.48	2.77
cis-ocimene	961	-	-	0.04	0.08	0.38	-
β-ocimene Y	970	0.20	-	0.36	0.91	0.65	0.70
2,6-dimethyl hept-5-l-al	974	0.04	0.05	-	-	-	-
cis-2,6-dimethyl – 2,6-octadiene	979	2.14	1.4 2	-	-	-	-
γ-terpinene	984	0.06	-	-	-	-	-
cis-sabinene hydrate	989	0.62	0.54	0.56	0.52	0.54	0.55
α-terpinolene	1004	0.03	0.21	0.23	0.22	0.18	0.18
δ-fenchene	1006	0.22	0.28	-	-	-	-
rosefuren	1009	0.18	0.17	0.20	0.14	0.30	0.26
β-terpineol	1011	-	0.11	-	-	-	-
linalool	1017	1.30	0.52	0.63	0.35	0.62	0.65
chrysanthenone	1022	0.15	-	-	-	-	-
trans-p-mentha-2,8-dienol	1032	0.10	0.10	-	-	-	-
thujone	1037	-	-	0.29	0.47	0.25	0.31
β-thujone	1039	0.66	0.47	-	-	-	-
trans-chrysanthemal	1048	0.67	0.84	0.62	0.68	0.60	0.58
citronella	1056	0.36	0.33	0.20	0.27	0.34	0.28
Unknown	1066	-	0.88	-	-	-	
pulegone	1069	-	-	0.93	0.86	0.91	0.89
rosefuren epoxide	1075	0.62	0.80	0.81	0.67	0.92	0.98
Unknown	1084	1.25	1.42	-	-	-	-

ethenyl cyclooctane	1086	-	-	1.37	1.30	1.34	1.3 1
β-fenchyl alcohol	1097	2.10	1.95	1.89	1.78	1.73	1.89
Z-citral	1144	17.34	18.95	18.83	17.56	18.29	18.16
piperitone	1148	0.21	0.32	0.18	0.25	0.22	0.16
E-citral	1176	20.23	22.25	22.73	21.10	21.57	21.42
bicycloelemene	1246	0.17	0.17	0.15	0.07	0.22	0.22
cis-geraniol	1292	1.95	2.05	1.27	1.41	2.04	2.76
α-copaene	1295	-	-	0.49	0.67	-	-
β-bourbonene	1301	-	-	0.37	0.48	-	-
3,5-heptadinal,2-ethylidene-6-methyl	1313	-	-	0.20	0.21	-	-
trans-caryophyllene	1326	5.02	5.65	3.93	5.17	5.08	4.80
germacrene D	1331	-	-	0.06	0.10	0.10	0.09
α-caryophyllene	1346	0.47	0.50	0.42	0.47	0.41	0.59
aromadendrene	1351	0.70	0.69	0.71	0.72	0.68	0.71
geranyl acetate	1357	0.52	0.61	0.47	0.60	0.48	0.50
α-cubebene	1368	5.75	5.22	3.91	4.90	5.78	5.46
curcumene	1370	0.82	1.54	4.55	2.99	2.68	2.53
bicyclogermacrene	1379	3.03	2.77	3.10	3.20	3.94	3.68
cedr-8-ene	1386	-	1.70	2.72	3.09	2.89	3.01
zingiberene	1388	-	-	0.38	-	0.41	0.38
calarene	1389	2.06	0.38	-	-	-	-
δ-cadinene	1418	0.32	0.34	0.38	0.41	0.35	0.40
farnesol	1437	1.45	1.3 8	1.25	1.39	1.75	1.69
spathulenol	1454	3.96	4.74	5.94	6.05	3.98	5.30
allospathulenol	1467	-	-	-	-	1.56	-
isospathulenol	1506	-	-	0.98	0.78	0.25	0.48
epi-(+) bicycle sesquiphellandrene	1533	1.05	1.16	1.15	1.21	1.19	1.17
α-cadinol	1545	-	-	-	-	0.13	0.34
bicyclo [4.4.o] dec-1-ene,2-isopropoyl-5-methyl-9-methylene	1579	-	-	0.31	0.24	0.27	0.23
Unknown	1591	0.34	-	-	-	-	-
4,6- bis(4-Methylpent-3-en-1-yl)- a-methylcyclohexa-1,3-diene -carb	1862	-	0.28	-	-	-	-
phytol	1886	0.11	0.13	-	-	-	-
Hydrocarbon monoterpenes		11.12	9.68	4.24	7.05	6.17	8.25
Oxygen-containing monoterpenes		53.79	53.91	50.80	48.63	49.59	50.71
Total monoterpenes		64.91	63.59	55.04	55.68	55.76	58.96

Hydrocarbon sesquiterpenes	19.39	20.12	22.31	23.46	23.73	22.82
Oxygen-containing sesquiterpenes	5.41	6.12	8.17	8.23	7.67	7.81
Total sesquiterpenes	24.80	26.24	30.48	31.69	31.40	30.63
Oxygenated diterpene	0.11	0.13	-	-	-	-
Other oxygen-containing compounds	1.17	1.30	1.86	1.53	1.82	1.71
Other compound hydrocarbons	2.22	0.32	1.68	1.53	1.63	1.54
Unknown compounds	1.59	2.30	0	0	0	0
Hydrocarbon terpenoids	30.51	29.80	26.55	30.51	29.90	31.07
Oxygenated terpenoids	59.31	60.16	58.97	56.86	57.26	58.52
Total terpenoids	89.82	89.96	85.52	87.37	87.16	89.59
Total without Unknown compounds	93.21	91.58	89.06	90.43	90.61	92.84
Total	94.80	93.88	89.06	90.43	90.61	92.48
Oil yields (w/w%-dry basis)	0.45	0.48	0.35	0.35	0.38	0.40
Number of compounds	49	48	48	46	48	46
Number of compounds without Unknown compounds	47	46	48	46	48	46

Retention index relative to n-alkanes C_8-C_{26} on the HP-5Ms capillary column

Results of essential oil of September's samples

According to table 2, the oil yields (w/w%) in September' s samples varies between 0.21 and 0.49 of which 0.21 belongs to Zahedan's blank sample and 0.49 to Zarand' s humic sample. In the essential oil of the greenhouse's September blank sample, thirty-nine constituents embracing 84.53% of the total essential oil were recognized. In the oil of the greenhouse's September humic sample (Sapropel), forty-two constituents embracing 85.84% of the total essential oil were identified and forty-one constituents embracing 59.11% of the total oil were identified in Zahedan's September blank sample.In the essential oil of September's samples in different conditions exist 31 similar compounds with high percentage. According to table 2 September's results in different conditions were reported for (21.79% ≥ E-citral ≥14.79%), (17.51% ≥ Z-citral ≥ 12.06 %), (7.12% ≥ limonene ≥1.86 %) , (7.0 %≥ bicylogermacrene ≥ 2.42%), (6.76% ≥ germacrene D ≥ 2.84%),(6.23% ≥spanthenol ≥ (0.90%), (5.21 % ≥ trans-caryophyllene≥ 2.87%) , (3.83% ≥curcumene≥1.1 5%), (3.72% ≥ nerolidol ≥ 1.45%), (2.45% ≥ β-ocimene-y ≥0.33%),(2.15 % ≥ 1, 8-cineole ≥1.08 %) , (1.88 % ≥ geranyl acetate ≥ 0.92 %),(1.57% ≥ linalyl propionate ≥ 1.02%), (1.49 % ≥ 2,5-Octadiene≥0.62 %) ,(1.20 % ≥ epi-(+) bicycle sesquiphellandrene≥0.75.%) (0.55% ≥ linalool≥0.36%), and (0.23 % ≥ alpha-pinene≥ 0.12%) Compounds.

Table 2. Chemical composition of essential oils of *A. triphylla* collected in September

Compound	RI	Greenhouse blank sample essential oil	Greenhouse humic sample (Sapropel) essential oil	Greenhouse humic sample (extracted humic acid) essential oil	Zahedan blank sample essential oil	Zarand blank sample essential oil	Zarand humic sample (Sapropel) essential oil
α-thujene	693	0.03	-	0.02	-	0.02	-
α-pinene	742	0.23	0.21	0.14	0.1 6	0.12	0.17
sabinene	767	0.71	0.6 0	0.35	0.37	0.29	0.37
6-methyl-5-hepten-2-one	788	2.49	2.26	-	0.70	3.97	3.63
1-octen-3-ol	809	0.52	0.43	2.29	0.49	0.88	0.87
β-myrcene	850	0.24	0.17	0.10	0.09	0.10	0.11
3-octanal	871	0.03	0.03	0.04	0.03	0.05	0.04
allocimene	875	-	0.03	-	-	-	-
1,8-cineole	892	1.6 3	1.10	1.88	1.08	2.15	2.06
limonene	913	7.12	5.17	2.25	1.98	1.86	2.15
cis-ocimene	934	-	0.11	-	-	-	-
β-ocimene X	943	0.17	-	-	-	-	-
β-ocimene Y	955	2.45	1.63	1.08	0.33	0.65	0.72
cis-β -terpineol	965	0.44	0.42	0.40	0.30	0.63	0.58
rosefuren	976	0.26	0.21	0.36	0.38	0.53	0.47
linalool	995	0.37	0.40	0.38	0.36	0.55	0.45
citronella	1015	0.33	0.25	0.29	-	0.34	0.35
rosefuren epoxide	1034	0.41	0.47	0.48	0.65	0.71	0.76
2,5 –octadiene	1054	1.49	0.99	1.04	0.63	0.62	0.80
linalyl propionate	1073	1.28	1.44	1.42	1.02	1.35	1.57
Z-citral	1092	15.06	17.51	15.19	12.06	12.80	12.55
piperitone	1112	0.10	0.11	0.11	0.07	0.13	-
E-citral	1132	18.20	21.79	18.35	15.15	15.27	14.79
santolina triene	1157	0.33	0.30	0.44	0.1 5	0.34	0.19
α-murolene	1186	0.04	0.05	0.06	0.03	0.05	0.07
geranyl acetate	1216	1.73	1.81	1.77	0.92	1.88	1.33
α-copaene	1229	-	-	-	0.34	-	0.31
β-bourbonene	1237	0.25	0.33	0.23	0.28	0.37	0.33
Zingiberene	1254	0.47	0.52	0.56	0.48	0.63	0.55
trans-caryophyllene	1270	5.16	4.64	5.21	2.87	4.13	3.41
nonyl benzene	1286	-	-	-	0.08	-	-
β-cubebene	1303	-	-	-	0.06	-	-
β-sesquiphellandrene	1319	-	-	-	0.07	-	-
α-humulene	1336	0.42	0.38	0.4 2	0.28	0.37	0.32
aromadendrene	1352	0.68	0.68	0.72	0.56	0.78	0.66

β-bisabolene	1362	-	-	0.43	-	-	-
geranyl isobutyrate	1368	-	-	-	0.38	-	-
β-farnesene	1370	0.41	-	-	-	-	-
germacrene D	1385	6.45	6.06	6.76	2.84	5.11	4.24
curcumene	1401	1.15	1.25	1.46	3.83	3.51	3.33
bicyclogermacren e	1406	5.61	5.54	7.00	2.42	5.35	4.41
Diep-α- cedren 1	1418	2.11	2.31	-	-	-	-
α-cedrene	1434	-	-	3.01	1.46	2.74	2.30
tau-muurolol	1450	-	-	-	0.32	-	-
γ-cadinene	1456	-	0.24	-	-	-	-
δ-cadinene	1467	0.27	0.38	0.39	0.24	0.30	0.27
nerolidol	1491	1.56	1.45	3.72	0.86	2.37	1.97
caryophyllene oxide	1520	-	2.13	-	-	-	-
spathulenol	1549	2.32	0.90	2.08	4.28	6.23	5.90
alpha-cedrol	1579	-	0.14	-	-	-	-
isospathulenol	1591	1.19	0.33	0.40	0.35	0.59	0.53
epi-bicyclosesqui phellandrene	1608	0.79	0.92	0.79	0.75	1.20	1.04
phytol	1669	-	0.13	0.25	-	0.35	0.32
Hydrocarbon monoterpenes		11.34	8.23	4.40	3.08	3.40	3.98
Oxygen-containing monoterpenes		38.12	43.60	41.72	30.32	34.28	34.89
Total monoterpenes		49.46	51.83	46.12	33.40	37.68	38.87
Hydrocarbon sesquiterpenes		23.85	23.30	26.60	16.62	24.54	21.24
Oxygen-containting sesquiterpenes		4.97	4.95	6.20	5.81	9.19	8.40
Total sesquiterpenes		28.82	28.25	32.80	22.43	33.73	30.04
Oxygenated diterpenes		-	0.13	0.25	-	0.35	0.32
Other oxygen-containing compounds		4.76	4.64	2.84	2.65	7.20	6.87
Other hydrocarbon compounds		1.49	0.99	1.04	0.63	0.62	0.80
Hydrocarbon terpenoids		35.19	31.53	31.0	19.70	27.94	25.22
Oxygenated terpenoids		43.09	48.68	48.17	36.13	43.82	43.70
Total terpenoids		78.28	80.21	79.17	55.83	71.76	68.92
Total		84.53	85.84	83.05	59.11	79.58	76.59
Oil yield (w/w%-dry basis)		0.42	0.46	0.47	0.21	0.44	0.49
Number of compounds		39	42	38	41	38	37

Retention index relative to n-alkanes C_8-C_{26} on the HP-5Ms capillary column

Discussion

Discussion 1-Essential oil of June's samples

Limonene compound's percentage in the essential oil of June's humic samples varies between 2.77 and 5.44 of which 2.77 belongs to Khanok-Zarand' s environmental condition (12% increase compared to its percentage in their blank sample) and 5.44 to the greenhouse condition (9.9% decrease compared to its percentage in their blank sample) .1, 8-cineole compound's percentage in June' s humic samples varies between 2.32 and 3.44, of which 2.32 belongs to Zarand' s environmental condition (36% increase compared to its percentage in their blank sample) and 3.44 to the greenhouse condition (44% decrease compared to its percentage in their blank sample). Z-citral compound' s percentage varies between 17.56 and 18.95 in the essential oil of June' s humic samples, of which 17.56 belongs to Zahedan's humic sample (6.7% decrease compared to its percentage in their blank sample) and 18.95 to the greenhouse humic sample (9.3% increase compared to its percentage in their blank sample). E-citral compound' s percentage varies between 21.10 and 22.25 in the essential oil of June' s humic samples, of which 21.10 belongs to Zahedan, s humic sample (7.2% decrease compared to its percentage in their blank sample) and 22.25 to the greenhouse humic sample (10% increase compared to its percentage in their blank sample) (Table 1). The percentage of total monoterpenes in the essential oil of June's humic samples varies between 55.68 and 63.59, of which 55.68 belongs to the essential oil of humic sample of Zahedan's environmental condition (1.20% increase compared to its percentage in their blank sample) and 63.59 to the essential oil of humic sample of the greenhouse condition (Sapropel) (2 % decrease compared to its percentage in their blank sample). Total sesquiterpenes percentage in the essential oil of June's humic samples varies between 26.24 and 31.69 of which 26.24 belongs to the essential oil of greenhouse humic sample (5.80 % increase compared to its percentage in their blank sample) and 31.69 to the essential oil of Zarand's humic sample (4 % increase compared to its percentage in their blank sample). Hydrocarbon terpenoids percentage in the essential oil of June's humic samples varies between 29.80 and 31.07 of which 29.80 belongs to the essential oil of greenhouse humic sample (2.30 % decrease compared to its percentage in their blank sample) and 31.07 to the essential oil of Zarand's humic sample (3.90% increase compared to its percentage in their blank sample).

The percentage of oxygenated terpenoids in the essential oil of June's humic samples varies between 56.86 and 60.16, of which 56.86 belongs to the essential oil of humic sample of Zahedan's environmental condition (3.60% decrease compared to its percentage in their blank sample) and 60.16 to the essential oil of humic sample of the greenhouse condition (1.4% increase compared to its percentage in their blank sample).

The percentage of the total terpenoids in the essential oil of June's humic samples varies between 87.37 and 89.96, of which 87.37 belongs to the essential oil of Zahedan's humic sample (2.2 % increase compared to its percentage in their blank sample) and 89.96 to the essential oil of the greenhouse humic sample (0.16 % increase compared to its percentage in their blank sample) (Table 1). It is noteworthy that in all these researches, the essential oils contain different and similar compounds with varied percentages, which are a result of climate conditions and soil type.

Discussion 1 -Essential oil of September's samples

The percentage of limonene compound in the essential oil of September's humic samples varies between 2.15 and 5. 17, of which 2.15 belongs to Zarand's environmental condition (16% increase compared to its percentage in their blank sample) and 5.17 to the greenhouse condition (Sapropel) (27% decrease compared to its percentage in their blank sample) while the percentage of this compound is 2.25 in greenhouse condition (humic acid sample) (68% decrease compared to its percentage in their blank sample) .1, 8- cineole compound's percentage varies between 1.10 and 2.06 in September's humic samples, of which 1.10% belongs to the greenhouse condition (Sapropel) (33% decrease compared to its percentage in their blank sample) while its percentage of the greenhouse condition (at the beginning of growth, extracted humic acid was sprinkled on the soil surrounding its root) is 1.88% (15% decrease compared to its percentage in their blank sample) and 2.06% belongs to Zarand's environmental condition (4.20% increase compared to its percentage in their blank sample) . Z-citral compound' s percentage varies between 12.55 and 17.51 in the essential oil of September' s humic samples, of which 12.55 belongs to Khanok_Zarand' s environmental condition (2% increase compared to its percentage in their blank sample) and 17.51 to the greenhouse condition(16% increase compared to its percentage in their blank sample) while its percentage is 15.19 in the greenhouse humic acid sample (0.86% increase compared to its percentage in their blank sample). Z-citral and E-citral compounds' a percentage have increased in the essential oil of the greenhouse's September humic samples compared to their blank samples. The percentage of Total monoterpenes in the essential oil of September, s humic samples varies between 38.87 and 51.83, of which 38.87 belongs to the essential oil of the humic sample of Zarand 's environmental condition (3.20 % increase compared to its percentage in their blank sample) and 51.83 to the essential oil of humic sample (Sapropel) of the greenhouse condition (4.80 % increase compared to its percentage in their blank sample). while its percentage is 46.12 in the essential oil of greenhouse's humic acid sample (6.80 % decrease compared to its percentage in their blank sample). Total sesquiterpenes percentage in the essential oil of September's humic samples varies between 28.25 and 30.04 of which 28.25 belongs to the essential oil of greenhouse humic sample (Sapropel) (2 % decrease compared to its percentage in their blank sample) and 30.04 to the essential oil of Zarand's humic sample (11 % decrease compared to its percentage in their blank sample). while its percentage is 32.80 in the essential oil of greenhouse's humic acid sample (14 % increase compared to its percentage in their blank sample). Hydrocarbon terpenoids percentage in the essential oil of September's humic samples varies between 25.22 and 31.53, of which 25.22 belongs to the essential oil of Zarand's environmental condition(9.70 % decrease compared to its percentage in their blank sample) and 31.53 to the essential oil of the greenhouse's sample (Sapropel) (10 % decrease compared to its percentage in their blank sample) while its percentage is 31.0 in the essential oil of greenhouse's humic acid sample (12% decrease compared to its percentage in their blank sample). The percentage of oxygenated terpenoid compounds in the essential oil of September's samples varies between 43.70 and 48.68, of which 43.70 belongs to the essential oil of Zarand 's humic sample (0.27 % decrease compared to its percentage in their blank sample) and 48.86 to the essential oil of the greenhouse humic sample (Sapropel) (13% increase compared to its percentage in their blank sample) while its percentage is 48.17 in the greenhouse humic acid sample(12% increase compared to its percentage in their blank sample). The percentage of the total terpenoids in the essential oil of September's humic samples varies between 68.92 and 80.21, of which 68.92 belongs to the essential oil of Zarand's humic sample(4 % decrease compared to its percentage in their blank sample) and 80.21 to the essential oil of the greenhouse's humic sample (Sapropel) (2.50 % increase compared to its percentage in their blank sample) while its percentage is 79.17 in the essential oil of the greenhouse's humic acid sample (1.10 % increase compared to its percentage in their blank sample) (Table 2). The quantitative and qualitative differences in the volatile constituents can be considered as the chemotaxonomic significance and it may be attributed to their different ecological and geographical origin factors (Ozguven and Tansi,1998; Azimi *et al*.,2014)**.**

DISCUSSION 2

According to tables 1 and 2, the efficiency of the extracted essential oil of June's and September's samples indicate an increase in the efficiency of the extracted essential oil of humic samples compared to their blank samples. The essential oil samples do not have the same composition of the compounds, some disappeared and some new compounds are formed.

The main compounds (mean percentages) of α-pinene, β-myrcene, sabinene hydrate, limonene,1,8-cineole, cis-geraniol, α -caryophyllene, aromadendrene, δ-cadinene, spathulenol, indicates an increase in the essential oil of June, s environment condition humic samples compared to its mean percentages of the essential oil of blank samples. Linyl propionate,2,5-Octadiene, rosefuren epoxide, Z-citral, compounds, mean percentages indicate an increase in the essential oil of September, s humic samples compared to its mean percentages of the essential oil of blank samples. The main compounds that are active in pharmaceuticals in June sample essential oils are found which have the highest amount. Therefore, the best time collection of plant is June.

The percentage of total terpenoids in June's samples is more than 85 %. Terpenoids' results increase in a better quality of the essential oil in June humic samples; particularly the increase in oxygenated terpenoids increases anti-bacterial properties (Duarte *et al*.,2005; Duschatzky *et al*.,2004; López *et al*.,2004; Ohno *et al*.,2003; Sartoratto *et al*.,2004). Therefore, the essential oils of June's samples enjoy a better quality compared to the essential oils of September's samples (Argyropoulou *et al*.,2007). These changes are depicted clearly in tables 1 and 2.

Table 3. Decomposition of soil blank samples and growing place soils from three conditions

Type of soil	**Humic substances (±0.01% w/w)**
Soil blank sample (June greenhouse condition)	0.19
Growing place soil (June greenhouse condition)	0.27
Soil blank sample (September greenhouse condition)	0.19
Growing place soil (Sapropel) (September greenhouse condition)	0.29
Growing place soil (extracted humic acid) (September greenhouse condition)	0.35
Growing place soil with sodium humate a root (September greenhouse condition)	0.26
Soil blank sample (June Zahedan, s environmental condition)	0.21
Growing place soil (Sapropel) (June Zahedan, s environmental condition)	0.28
Soil blank sample (September Zahedan, s environmental condition)	0.20
Growing place soil with sodium humate a root (September Zahedan, s environmental condition)	0.27

Soil blank sample (June Zarand, s environmental condition)	0.23
Growing place soil (Sapropel) (June Zarand, s environmental condition)	0.30
Soil blank sample (September Zarand, s environmental condition)	0.24
Growing place soil (Sapropel) (September Zarand, s environmental condition)	0.32

Humic sample = growing place soil= Sapropel a root

According to table 3, humus' percentage is 0.19 (w/w) in the soil of the greenhouse's blank sample in June and it is the same in September but humus' percentage in the soil surrounding the greenhouse's Sapropel a root has been measured 0.27 in June and 0.29 in September. When the extracted humic acid was sprinkled on the soil surrounding the greenhouse's root in June after sampling instead of Sapropel, humus' percentage is measured 0.35 w/w in September. This result indicates the strong effect of humic acid. Humus' percentage is 0.23(w/w) in the soil of June's blank sample of Zarand's environmental condition and 0.30% (w/w) in the soil surrounding the root of its humic sample, and its percentage (w/w) is 0.24(w/w) in September's blank sample and 0.32 in its humic sample. Humus' percentage is 0.21(w/w) in the soil of June's blank sample of Zahedan's environmental condition and 0.28 (w/w) in its humic sample and its percentage is 0.20 (w/w) in September's blank sample and 0.27% (w/w) in its humic sample. All the measures were done using "Methods in Determining Soil Organic Carbon Sequestration Rates" (Olson *et al.*,2014). The reason for changes in humus' percentage of samples of Zahedan and Zarand environmental conditions is related to changes in temperature, humidity, and rainfall. Soil's pH amount varies between 8 and 8.47 in June for the blank samples and between 7.90 and 8.10 for humic samples. These pH changes in soil conform completely to humus percentage in the soil of humic and blank samples

Table 4. Identification of the amounts of nitrogen in leaves of the plant and growing place soils in two phases of growth

Sample	**Nitrogen(w/w%) in June**	**Nitrogen(w/w%) in September**
plant leaf of the blank sample (Greenhouse condition)	-	1.780
plant leaf sample with sodium humate a root (Greenhouse ondition)	-	3.040
Growing place soil with sodium humate a root (Greenhouse condition)	0.027	-
Growing place soil with extracted humic acid a root (Greenhouse condition)		
Growing place soil without Sapropel (Greenhouse condition)	0.029	0.020
Growing place soil with Sapropel a root (Greenhouse ondition)	0.027	0.019
Growing place soil without Sapropel (Zahedan's nvironmental condition)	0.036	0.016
Growing place soil with Sapropel a root (Zahedan's nvironmental condition)	0.021	0.015

Growing place soil without Sapropel a root (Zarand, s environmental condition)	0.039	0.032
Growing place soil with Sapropel a root (Zarand, s nvironmental condition)	0.025	0.025

According to table 4, nitrogen's percentage (w/w) varies between 0.029 and 0.039 in the soil of June's blank samples while its percentage(w/w) in the soil surrounding the root of humic samples varies between 0.021and 0.027. Nitrogen's percentage (w/w) in September's blank soil samples varies between 0.016 and 0.032. Nitrogen's percentage(w/w) in the soil surrounding the root of humic samples varies between 0.015 and 0.025, of which 0.015 belongs to Zahedan's environmental condition and 0.025 to Zarand's environmental condition. Nitrogen's percentage(w/w) is 0.019 in the soil of the greenhouse humic sample (Sapropel) and 0.022 in the humic acid sample. Nitrogen's percentage (w/w) has been measured 1.78 in the blank sample plant leaf and 3.04 in the plant leaf sample of its with sodium humate a root of the September greenhouse condition that indicates of increase in nitrogen's percentage(w/w) after sprinkling humic substance solution on the soil surrounding the root. Soil's pH measurement of plant's growth in the greenhouse condition (growing place soil) varies between 7.90 and 8.40 of which 7.90 belongs to the essential oil of humic sample of June and 8.40 of the September. Growing place soil, s pH of Zarand,s environmental condition varies between 8.1 and 7.90 of which 8.10 belongs to the essential oil of humic sample of June and 7.90 of the September. Soil's pH measurement of plant's growth in the Zarand,s environmental condition (growing place soil) varies between 8.0 and 8.10 of which 8.0 belongs to the essential oil of humic sample of June and 8.10 of the September. pH decrease at the end of growth indicates the reduction of heavy metal ions in plant's leaves. An increase in the amount of soil's humus after sprinkling humic substances to the soil surrounding the root, according to table 3, is another reason for the elimination of heavy metal ions and better plant growth. Measurement of the amount of humus in the growing place soil and mineral analysis of this soil and plant leaves conforms completely to increase in the length of stems and leaves in all planting environments.

Table 5. Identification of the amounts of mineral elements in the blank soils and growing place soils from three conditions (after collection of leaves in June)

Metal ion	The number of metal ions in growing place soil of greenhouse condition (Dry mg/kg)	The number of metal ions in a blank soil sample of greenhouse condition (Dry mg/kg)	The amount of metal ions in (Dry mg/kg) in growing place soil of Zahedan, s environmental condition	The amount of metal ions in a blank soil sample (Dry mg/kg) of Zahedan, s environmental condition	The number of metal ions in growing place soil (Dry mg/kg) of Zarand, s environmental condition	The amount of metal ions in a blank soil sample (Dry mg/kg) of Zarand, s environmental condition
Ag	0.80±0.02	1.12±0.02	0.62±0.02	0.72±0.02	0.72±0.02	0.80±0.02
Cd	0.75±0.01	1.00±0.01	0.90±0.01	1.00±0.01	1.02±0.01	1.57±0.01
Co	2.80±0.03	3.40±0.03	2.82±0.03	3.42±0.03	3.15±0.03	4.72±0.03
Cr	0.97±0.02	1.67±0.02	1.05±0.02	1.40±0.02	1.30±0.02	1.35±0.02
Cu	0.57±0.02	0.80±0.02	0.55±0.02	0.65±0.02	0.75±0.02	0.80±0.02

Mn	161.70±0.07	230.82±0.07	144.15±0.07	144.17±0.07	120.55±0.07	195.67±0.07
Ni	4.45 ± 0.03	5.45± 0.03	4.72± 0.03	5.22± 0.03	5.10± 0.03	5.77± 0.03
Pb	5.05±0.01	4.24±0.01	3.77±0.01	5.10±0.01	4.51±0.01	4.75±0.01
Fe	123.80 ±0.05	147.88± 0.05	123.78± 0.05	124.08± 0.05	123.88± 0.05	124.48± 0.05
Zn	66.33±0.04	20.70±0.04	24.32±0.04	34.65±0.04	33.54±0.04	30.83±0.04

Humic sample = growing place soil= Sapropel a root

According to table 5, mineral analysis of plant's leaves and the growing place soil indicate the chelating property of humic substances deactivates heavy metal ions such as Lead and Cadmium that decrease the quality of effective substances of medicinal plants at the very beginning of plant's growth. Although the populations of *Aloysia. triphylla* were planted in the same ecological and agronomical conditions, they had significant differences in terms of phytochemical traits, which probably could be due to the genetic factors (Nikkhah *et al*.,2007; Moradi *et al*.,2011; Habibi *et al*.,2011).

Table 6. Comparison longer of stems and leaves in find growing stages

Sampling	**Max long of stem(±0.1cm)**	**Max long of leaf (±0.1cm)**
plant blank sample (June greenhouse condition)	108	9
plant humic sample (June greenhouse condition)	116	10
plant blank sample (September greenhouse condition)	113	10
plant humic sample (September greenhouse condition)	122	10.50
Plant extracted humic acid sample (September greenhouse condition)	125	10.50
plant blank sample (June Zahedan, s environmental condition)	68	6.50
plant humic sample (June Zahedan, s environmental condition)	75	8
plant blank sample (September Zahedan, s environmental condition)	53	5.50
plant blank sample (June Zarand, s environmental condition)	56	10.50
plant humic sample (June Zarand, s environmental condition)	69	11
plant blank sample (September Zarand, s environmental condition)	51	8
plant humic sample (September Zarand, s environmental condition)	64	9.50

Humic sample= growing place soil =Sapropel a root

According to table 6, the stem length of the greenhouse plant sample, to its root extracted humic acid was sprinkled in late June, reached 125 cm in late September that shows a 10.62% increase compared to stem length of its blank sample; it also shows a 15.74% increase compared to stem length of its blank sample in late June. Using humic substances as a soil fertilizer for plant's growth results in an increase in the length of leaves and stems, it also increases the efficiency of the extracted essential oil. Nitrification is sensitive to hot temperatures and mostly happens at 5 to 40 °C. As seen in Table 3, the increase of Sapropel results in a rise in soil humus. Microbes like acetolactic which increases soil nitrogen are considerably sensitive to the amount of soil Carbone. The amount of nitrogen in the greenhouse soil to which Sapropel was sprinkled has decreased during leaf collection in late September, however, its amount in the plant’s leaves has increased 70.78% compared to its blank sample in late September. The decrease in the amount of nitrogen in the soil and its increase in the plant's leaves are routine. An increase in the fertility of the growing place soil and good growth of humic plant samples (that is, increase in the length of stems and leaves) relative to their blank samples are the results of humic substances' influence Zahedan's June samples, considering it's suitable the climatic condition for the plant's growth, showed more growth than Khanok_ Zarand’s environmental condition but its September samples grew less than Khanok’s environmental condition. Zarand’s environmental condition quality oil samples than are the Zahedan’s environmental condition oil samples due to the difference in air temperature and humidity and can be found in soil humus (Tables 1, 2, 3, 4,7,8). The greenhouse's September plant samples grew better than the greenhouse's June's samples since the climatic condition is fixed in the greenhouse.

Table 7. Study of Average Moisture, Scale of Rainfall and Average temperature of Air in length growth stage plant in Zahedan's environmental condition

Year	Month	Average temperature of Air(0C)	Average Moisture(%)	Scale of Rainfall (mm)
2010	March	18.50	35	9.40
2010	April	20.70	26	0.00
2010	May	24.90	29	12.10
2010	June	27.60	16	0.00
2010	July	30.20	13	0.00
2010	August	26.70	15	0.00
2010	September	23.70	15	0.00

According to table 7, the temperature in Zahedan's environmental condition varies between 18.50 and 27.60 degrees Celsius from March to June while humidity percentage varies between 16 and 35 and Rainfall was zero mm in June and April. Temperature varies from 20.30 to 23.70 degrees Celsius in the Zahedan’s environmental condition from July to September while humidity percentage varies between 13 and 15 and rainfall was zero (mm) in all months from July to September. At a constant temperature, the amount of nitrogen increases exponentially in the surface layer, with increasing soil moisture. As the humidity is constant, the temperature of the air increases, the nitrogen in the surface layer decreases exponentially.

Table 8. Study of Average of Moisture, Scale of Rainfall and Average of temperature of Air in length growth stage plant in Khanok _Zarand, s environmental condition

Year	Month	Average temperature of Air (^{0}C)	Average of Moisture(%)	Scale of Rainfall (mm)
2010	March	15.10	35	8.00
2010	April	17.20	30	0.60
2010	May	21.80	30	2.70
2010	June	26.30	18	0.00
2010	July	29.90	15	0.00
2010	August	25.00	18.30	0.00
2010	September	23.40	26	0.00

According to table 8, temperature (^{0}C) varies between 15.10 to 26.30 in Zarand's environmental condition from March to June while humidity percentage varies between 18 and 35 and rainfall reaches 8 mm in March and zero(mm) in June. In Zarand's environmental condition, temperature (^{0}C) varies between 29.90 and 23.40 from July to September while humidity percentage varies between 15 and 26 and rainfall was zero (mm) in all months from July to September. Zarand quality oil samples than are the Zahedan oil samples due to the difference in air temperature and humidity and can be found in soil humus.

Conclusion

To produce high-quality plants, a controlled environment called a greenhouse needs to be followed by favourable environmental conditions such as relative humidity of air and soil moisture, etc. By controlling damaging factors such as wind and storms related to weather climatic tensions. The use of natural fertilizers, such as humic substances, is increasing Crop tolerance to drought (dehydration), salt, is cold and disease and pests (Langenheim,1994). Humic substances do not present a hazard to plants and humans, and they also help to clean the environment. With the increase of humic substances into the growing place soil-plant root, a good amount of nitrogen in a lot of plants, especially in the leaves is increasing and also reduce the heavy metal ions have also been used to prevent plant growth. By sprinkling humic substances in the soil surrounding the plant's root, the flowering period is shortened and terpenoid compounds percentage in the plant's essential oil (humic samples) increases. The essential oils of the *A. trip*le plant have very important compounds (α-pinene, β-pinene,1,8-cineole, β-ocimene Y, limonene, linalool, Z-citral, E-citral, cis-geraniol, geranyl acetate, β-fenchyl alcohol,trans-caryophyllene,α-cubebene,bicylogermacrene, cur cumene, spathulenol, etc.) in terms of pharmaceutical properties, therefore it is of great importance in the pharmaceutical industry. The essential oil of the plant to the *A. tripylla* grown with humic substances (June) under the environmental conditions of Zahedan and Zarand has a high quality due to the number of total terpenoids and oxygenated terpenoids. So, using humic substances and controlling climatic tensions in the air can affect the quality of the essential oil. The genus of *Aloysia.triphylla* has a wide distributional range and the chemical composition of the essential oils varies with the geographical location of collection site, climate and other ecological factors (Cabo *et al.*, 1982; Putievsky and Basker,1977).

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