

Full Length Research Paper

Effect of humic substances on the quality of essential oils of medicinal plants

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Environmental factors cause changes in growth of medicinal plants as well as the quantity and quality of their essential oil composition. Among those, the factors of soil; especially the roles of fertilizer nutrients (nitrogen, phosphate and potash) possess a remarkable importance. In the present research, we utilized SAPROPEL packed by a Russian Firm and humic acid, extracted from the soil of the north-Iranian forest, on *Artemisia herba-alba* and *Semenovia suffruticosa* plants which had been collected from the heights of Taftan Mountain. Addition of extracted humic acid and SAPROPEL to the roots of plants in early growing stage resulted in increasing the percentage of their composition. About the plant *A. herba-alba* as its root was exposed to humic acid, the percentage of oxygenated Terpenoid from the whole essential oil became 81.39% that in comparison with blank sample, an increase of 24.29% was observed. When the root got SAPROPEL rather than humic acid, we observed only 21.99% increase in the percentage of composition. As the root of plant *S. suffruticosa* received extracted humic acid, the percentage of oxygenated Terpenoid increased to 5.98%. While by adding the SAPROPEL, the increase was only 0.48%. Comparison of the percentage of essential oil composition, after addition of humic substances to their roots, in primary stage of growth depends on factors such as physiological structure, the kind of the growing place soil, climate and the amount of sun-shine.

Key words: Medicinal plant, humic substances, root, essential oil, quality of essential oil.

INTRODUCTION

Soil is one of the important components of basic resources, so that it is considered as main bed of plant cultivation and also, as a unique environment for all kinds of lives (Mogimi, 2007). Presently, there is more ecological movement and fear of water and soil contamination by chemicals from usage in soil than ever. Because their productive effects on physical, chemical and biological properties of soil organic materials are considered as important elements in soil fertility.

Humic substances are criteria of soil fertilization, because in addition to supplying the plants needed nitrogen; they provide the best suitable perimeter for plants growing. Without organic materials especially humic substances, soil is not more than a dead perimeter for plants growing. Fertility of soil can be influential over

medicinal plants bushes as well as their branch-marking strength (Rechinger, 1987; Liu et al., 2007). Humic substances are plant growth stimulating agents that have been applied in agricultural in recent years. However, detailed mechanisms of how these materials work in plants are still not well understood, due to complex humic substances structures in nature. There are many reports of humic substances role in promoting plant biomass, stimulation of root, shoot, and flowering growth, and even direct effects on crop productivity and increases in crop yields. The humic substances consist of humic acids, fulvic acids and humin. They can be extracted from many natural sources such as peat, soil, and Leonardite ore. Municipal yard wastes, sewage sludge and composts also could be sources of these materials. Application of the humics in golf course and sports turf management has recently been rediscovered. Due to major environmental concerns, it is becoming a popular practice to use these materials as soil amendments.

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They not only increase fertilizer efficiency and promote plant growth, but can reduce the potential of groundwater contamination. Plant growth research involving humic substances at the university of Minnesota in the Departments of horticulture and soil, water and Climate has centered on growth chamber, greenhouse, and golf green experiments. Both basic and practical research components are underway to investigate the 'how and why' of humic substances interactions with plant and soil ecosystems (Chen, 2004; Clapp et al., 1998).

Uptake of organic macromolecules, such as direct effects include those changes in plant metabolism that occur following the uptake of organic macromolecules, such as humic acids and fulvic acids. Once these compounds enter plant cells several biochemical changes occur in membranes and various cytoplasm components of plant cells (Hayes, 1985). Safflower (*Carthamus tinctorius* L.) has a high nutrient value and an alternative production of the crop is being tried. Furthermore, because safflower is resistant to arid conditions, it would be adaptable to the climatic and land conditions of the Thrace Region and Edirne.

In general, there is little organic matter (1%) in the soil of both Turkey and the Thrace Region. For this reason, the yield and the quality of the plant are decreasing. To solve this problem, the use of humic acid has become widespread among the producers in recent years (Lu et al., 2000). In this research, we want to study of effect of humic substances on the quality of essential oils of medicinal plants in environmental conditions.

EXPERIMENTAL

Plant material

The plant was collected, from Taftan Mountain located 30 km away from Khash city of Baluchestan region in June 2009. Plant identification was carried out by Dr. Mozaffarian Botanist in the Research Institute of Forests and Rangelands in Tehran-Iran (Mozaffarian, 2007).

For preparing a laboratory sample, we dried it under the shadow of sun light and made it powder by a grinder (20 days). The oil yield for the sample conducted with four conditions is shown in Tables 1 and 2.

Other materials

SAPROPEL-solvent water (pH=6.35), Pelosilt blank-humic acid 30%, from HUMIN TECH Germany Company, HCl, NaOH, KOH, KCl, HF (for extraction of humic acid), all of them have been taken from Merck Germany Company.

Instrument

Extraction of humic acid was performed agitator (ELM 1400 rpm, Germany). IR spectrum of the preparation sample by (disk) Fourier transform-infrared (IR-FT) apparatus Model 460 plus, made in JASCO Company (Japan). For analysis of essential oil, gas chromatography/mass spectrometry (GC/MS) apparatus (HP

Agilent Technology, made, USA).

Extraction of humic acid

We extract humic acid from the soil of Nahakhoran Forest of Gorgan in north of Iran, according to International Humic substances society (IHSS) protocol (Davies et al., 2001; Stevenson, 1994) and then purify it. Since, SAPROPEL has not more than 30% humic acid therefore our extracted humic acid has stronger effect.

Sprinkling of humic substances

We solve the SAPROPEL of a 20 g package in 400 ml water then sprinkle them along with 3 L water on the base of the root, and a little amount on plant leaves in an area about half acre, in the month of March when the plants wake up from winter sleep and start to grow. In this area it does not receive SAPROPEL by some of the plant and they are considered as blank samples.

Oil isolation

Aerial parts of plants were shade dried (20 days) at room temperature with ventilation, minced and immediately hydro distilled (50 g) for 3 h using a modified Clevenger-Type apparatus. The oil was extracted from the distillate water and dried over anhydrous sodium sulfate. After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator at 30°C and the pure oil kept at 4°C in the dark. Essential oil decomposition was done by GC/MS technique (Bilia et al., 2002).

Gas chromatography/mass spectrometry (GC/MS) analysis

The analysis of the essential oils were performed using a Hewlett-Packard 6890 Net work GC system, equipped with a 60 m* 0.25 mm id, 0.25 µm HP-5MS capillary column, and a HP 5973 mass selective detector. Helium was the carrier gas at 1 ml/min. The injector and MS transfer line temperature were at 250 and 260°C respectively. Column temperature was set at 40°C for 1 min, then programmed from 40 to 250°C at a rate of 3°C/min, and finally held isothermally for 20 min.

For GC/MS detection an electron ionization system was used with ionization energy of 70 eV. The linear retention indices for all the compounds were calculated by using retention times a solution containing the homologous series of C₈-C₂₆ n- alkenes that were injected after the oil at the same chromatographic conditions according to Van Den Dool and Kratz (1963) method.

Identification of compounds

Compounds were identified by comparing the retention indices of the peaks on the HP-5 MS column relative to n-alkanes with literature values and comparison of the WILEY library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (7,8). Relative percentages of the separated compounds were calculated from the total ion chromatograms by computerized integration (Adams, 1995).

RESULTS AND DISCUSSION

The chemical analysis of the soil used to grow the specie has a carbon rate of 0.27% for *A. herba-alba*. The result

Table 1. composition of essential oil from the plant *A. Herba. alba*.

Compound	RI	Compound in essential oil from blank plant sample (%)	Compound in essential oil from plant with SAPROPEL at root (%)	Compound in essential oil from plant with SAPROPEL at leaves (%)	Compound in essential oil from plant with humic acid at root (%)
Methyl Cyclohexane	618	0.10	0.09	0.11	0.07
3-Methyl-2-butenal	664	0.01	0.02	0.26	0.17
2-Hexenal	747	0.06	0.15	0.21	0.11
2,4-Hexadienal	755	-	0.06	0.19	0.05
Isoamyl acetate	790	0.34	-	0.54	0.17
4-Methyl-2-pentenoic acid methyl ester	836	0.09	-	0.04	-
2-Methyl-5-isopropenylfuran	846	0.09	-	0.27	-
Alpha-Thujene	848	0.11	0.05	0.03	0.05
α -pinene	854	0.24	0.26	0.14	0.66
Camphene	866	0.11	0.58	1.44	1.62
Cis-3-Hexenyl hexanoate	888	0.17	-	-	-
Sabinene	891	0.28	0.15	0.07	0.15
Beta-pinene	894	0.25	0.08	0.09	0.21
1,3,3-trimethyl-2-oxabicyclo[2.2.2]oct-ene	905	0.35	-	-	-
Herboxide second isomer	908	-	-	0.11	-
Beta-Myrcene	910	0.11	0.12	0.11	0.09
Phellandrene	921	0.07	-	-	0.14
Delta-3-Carene	928	-	-	-	1.28
2-Methyl-2-propanoic acid pentyl ester	931	2.05	0.54	3.68	0.49
α -Terpinene	933	1.67	0.70	0.34	0.70
1,8 Cineole	948	38.43	30.45	27.68	37.69
Cis-ocimene	955	0.07	0.06	-	-
Geranyl acetate	965	-	-	=	0.06
2,2,9-Trimethyl-cis-1,6-Dioxaspiro[4,4]nonane	966	-	0.70	1.28	0.79
beta-ocimene Y	967	0.73	-	-	0.07
γ -Terpinene	973	2.18	0.98	0.40	0.87
Trans-Sabinene hydrate	977	0.88	0.23	-	0.40
Linalool oxide	981	-	-	0.11	-
3-Methyl resacetophenone	996	0.30	-	-	0.36
Alpha-Terpinolene	1000	0.57	0.27	0.11	0.27
Filifone	1002	0.05	0.89	0.67	0.23
Rosefuran	1004	-	-	0.37	-
Cis-Sabinene hydrate	1008	0.78	0.35	-	0.37

Table 1 Contd.

Linalool	1010	0.78	-	0.95	0.24
Isophorene	1013	-	0.32	-	-
Iso-Amyl isovalerate	1016	1.81	-	-	2.45
Chrysanthenone	1019	0.55	18.90	6.99	2.38
n-Hexyl isobutyrate	1022	0.47	-	-	-
3-(1-butyl)-4-methylthiophene	1025	0.42	-	-	-
Methyl 2,4-hexadienoate	1029	0.31	-	-	-
Comphor	1038	0.78	10.77	13.33	18.05
Pinocarvone	1049	0.15	0.23	-	0.22
2,6-Dimethyl-3,7-octadiene-2,6-diol	1054	0.41	-	-	-
Delta-Terpineol	1062	0.68	-	-	-
Borneol	1063	-	1.81	1.54	1.91
4-Terpineol	1075	4.23	3.11	1.27	2.67
Myrtenal	1078	0.16	-	-	-
Beta-Fenchyl alcohol	1084	-	1.91	0.67	1.09
α -Terpineol	1086	1.94	-	0.07	-
p-Allylanisole	1087	0.36	-	0.09	-
Cis-Piperitone	1089	-	0.17	-	0.83
Verbenone	1090	-	0.20	0.21	-
1-Methoxy-2-(1-methylethenyl)-benzene	1094	0.30	-	-	-
m-Methyl cumene	1095	-	-	0.54	-
Cis-3-Methyl-6-(1-methylethyl-2-cyclohexen-1-ol	1100	0.88	-	-	-
Trans-Carvol	1109	-	-	-	0.18
Bornyl formate	1114	-	-	-	0.12
Safranal	1115	-	0.65	-	-
Car-3-en-2-one	1117	-	-	0.90	0.37
Pulegone	1119	0.75	-	-	0.34
Carvone	1120	-	0.35	0.30	0.35
Z-Citral	1123	-	0.31	0.26	0.12
Piperitone	1129	-	-	0.09	0.24
4-Ethyl-2-methoxy-6-methyl pyrimidine	1143	0.49	-	-	-
Geraniol	1145	0.67	0.39	1.88	0.24
E-Citral	1149	-	-	0.40	0.38
p-Allyphenol	1150	0.84	-	-	-
Bornyl acetate	1169	-	0.41	0.41	0.43
5,5-Dimethyl-1-ethyl-1,3-cyclopentadiene	1178	-	2.24	1.20	-

Table 1 Contd.

2-Acetyl-4-methylphenol	1182	-	-	0.40	1.87
Eugenol	1236	1.74	1.51	1.19	5.59
Methyl cinnamate	1260	3.08	-	0.78	-
Cis-Jasmone	1271	9.36	4.88	0.51	-
3,5-Heptadienal,2-ethylidene-6-methyl	1283	-	2.23	-	3.16
Citrol	1285	-	-	9.89	0.99
Methyleugeuol	1299	6.01	6.46	3.95	3.16
Trans-caryophyllene	1320	0.38	0.37	1.26	0.99
Aromadendrene	1333	-	-	-	0.24
Alpha-Caryophyllene	1342	-	-	-	0.29
Trans- β -Farnesene	1349	1.95	-	2.25	-
E-Ocimenone	1351	-	0.73	-	1.21
Homoadamantane	1356	-	2.33	0.68	0.41
Germacrene D	1360	0.45	0.35	-	-
Bicylogermacrene	1370	0.17	0.48	0.78	0.50
5-Methoxy-2,3,4 trimethyl phenol	1381	0.55	-	-	-
3-Methoxy-2,5 ,6 trimethyl phenol	1382	-	0.31	1.32	0.38
5-Methoxy-2,5 ,6 trim ethyl phenol	1385	0.19	-	-	-
Delta-cadinene	1388	-	-	0.38	-
Phenyl ethyl tiglate	1432	-	-	1.52	-
2,Methyl-2-butenic acid-2-phenylethyl ester	1433	1.86	-	-	-
caryophyllene oxide	1436	-	2.44	2.35	3.14
Alpha-Atlantone	1489	0.78	-	-	-
Methyl Jasmonate	1496	0.63	-	-	-
Methyl epijasmonate	1505	-	-	-	1.19
Alpha-Bisabolol	1540	-	-	1.22	-
2,6-Dimethyl-2,6-octadiene-1,8- diol diacetate	1569	1.98	-	-	-
Oil Yield%(g/g)		1.52	1.27	1.1	1.24
Number of compounds		60	46	56	58

shows that soil slope, regarding amount of humus is poor. Amount of metal ions Mn, Zn and Fe is less than those of forest soil but Ca, Mg and Si are more than those of forest soil. *A. herba-alba* plant was collected at flowering stage (Figures 1 and 2). The essential oils were extracted with the

yields of 1.52 (blank sample), 1.27 (the plant sample, the root of which was added, SAPROPEL,), 1.24 (the sample added to its root, the extracted humic acid) and 1.10% w/w (when the solution of SAPROPEL was added to the leaf), respectively. The percent of Terpenoid, whether

oxygenated or hydrocarbon, increased from 66.05 to 83.17%, and 78.64 to 88.43%, respectively (Table 1). The percentage of composition in the essential oil of plants, *A. herba-alba*, changes in different forms. The different ways of humic substances application on the initial growth stages

Table 2. Composition of essential oil from the plant of *Semenovia suffruticosa*.

Name of compound	RI	Compound in essential oil from blank plant sample (%)	Compound in essential oil from plant with SAPROPEL at root (%)	Compound in essential oil from plant with SAPROPEL at leaves (%)	Compound in essential oil from plant with humic acid at root (%)
Methyl Cyclohexane	618	0.06	0.08	0.07	0.07
3-Methyl-2-butenal	665	-	0.02	0.03	-
2-Hexenal	747	0.01	-	0.01	-
3-Hexen-1-ol	763	0.01	-	0.01	-
Isobutyl isobutyrate	829	0.02	0.02	0.03	0.03
Alpha-Thujene	848	0.14	0.11	0.11	0.08
Alpha-pinene	855	1.38	1.12	0.90	0.57
Camphene	866	0.03	0.08	0.02	0.05
3-Methyl 1-Hexyn-3-ol	885	0.31	0.13	0.30	0.13
Sabinene	891	0.76	0.63	0.35	0.35
Beta-pinene	894	0.17	0.15	0.13	0.12
3-Octanone	895	0.04	0.09	0.06	0.06
2-Ethyl-3-methylcyclopentene	909	0.01	-	-	-
Beta-Myrcene	910	0.42	0.44	0.29	0.27
Isobutyl isovalerate	922	1.18	1.70	1.16	1.60
m-Methylanisole	924	0.06	0.04	0.04	0.04
Alpha-Terpinene	933	0.05	0.03	-	0.03
Cymene	936	2.72	1.59	0.72	0.46
1,8 Cineole	942	0.49	0.61	0.53	0.88
l-Limonene	944	0.26	0.33	0.29	0.30
Cis-Ocimene	960	21.43	20.94	15.00	13.24
n-Butyl isovalerate	961	0.23	0.24	-	0.21
β -Ocimene Y	966	1.06	1.16	1.02	0.85
γ -Terpinene	974	7.24	4.12	1.04	0.60
Trans-sabinene hydrate	977	0.06	0.07	0.05	0.06
P-Cresol	993	0.89	0.61	1.28	1.06
cis-sabinene hydrate	997	-	-	-	0.06
α -Terpinolene	1000	1.85	0.89	3.80	2.39
Rosefuran	1003	-	0.03	-	0.05
3,7-Dimethyl-1,6-octadien-3-ol	1007	-	0.12	-	0.09
Linalool	1010	3.18	7.27	-	5.77
Isopenlyl isovalerate	1015	-	-	1.01	-

Table 2 Contd.

Appel oil	1016	2.97	2.52	-	2.23
n-Amyl isovalarate	1019	2.39	-	-	-
Amyl isovalarate	1020	-	-	2.93	-
Iso-Amyl isovalarate	1021	-	2.33	-	2.28
3-Methyl-butanic acid 3-methyl-3-butyl ester	1022	-	0.15	-	-
Valeric acid 4-pentenyl ester	1023	0.12	-	0.12	-
Allocimene	1038	0.34	0.31	0.25	0.22
Amyl valerate	1048	0.06	0.06	0.05	0.05
Methacrylamide	1049	0.19	0.24	0.18	0.22
5,6-Decanedione	1052	0.18	-	0.26	0.16
Ethyldimethylthiophene	1065	3.80	2.54	4.14	2.31
1,8-Metntheadien-4-ol	1071	0.09	0.03	0.13	0.16
4-Terpineol	1073	0.21	0.11	-	0.13
p-Cymen-8-ol	1075	-	0.02	0.34	0.29
Alpha-Terpineol	1083	0.24	0.25	0.18	0.44
p-Ally anisole	1086	0.16	0.11	-	0.11
Cis-2,6-Dimethyl-3,5,7-octatriene-2-ol	1095	0.31	0.29	0.27	0.23
Trans-2,6-Dimethyl-3,5,7-octatriene-2-ol	1101	0.23	0.25	0.24	0.19
Hexyln-valerate	1105	0.42	0.46	0.35	0.92
Octyl acetate	1109	0.24	0.43	0.35	0.71
Nerol	1121	-	0.12	-	-
Dill ether	1123	-	-	-	0.11
Cis-3- Hexylisovalarate	1130	0.68	0.74	0.46	0.31
Hexylisovalarate	1139	2.21	2.32	2.38	1.98
4-Hydroxy-2-methylacetophenone	1146	-	-	0.46	-
p-Acetyl anisole	1148	0.53	0.51	-	0.47
Bornyl acetate	1168	-	0.15	-	0.09
2-Methoxy-4-vinylphenol	1184	-	0.03	-	0.06
n-octyl propionate	1190	0.19	0.10	0.22	0.19
Eugenol	1232	0.16	-	-	-
Bicycloelemene	1236	-	0.21	-	0.16
n-octyl isobutyrate	1252	9.00	4.54	10.89	7.32
Neryl acetate	1258	0.08	0.22	0.12	0.25
4-Methylindole	1269	-	0.03	0.21	-
Cis-Jasmone	1281	-	0.04	0.06	-
Benzyl isovalerate	1284	0.99	1.37	0.30	0.94

Table 2 Contd.

Alpha-Copaene	1288	-	-	0.33	-
Methyleugenol	1295	1.12	2.52	1.35	2.10
Octyl butyrate	1297	0.14	0.04	-	0.21
Beta-Cubebene	1301	-	-	0.17	-
Beta-Elemene	1302	-	0.06	-	0.07
Di-epi-alpha-Cedrene	1315	-	-	0.08	-
Trans-caryophyllene	1320	0.09	0.20	-	-
6-amyI- α -pyrone	1336	3.77	1.62	9.47	9.09
N-octyl-2-methyl butyrate	1340	1.38	8.12	17.59	12.33
Lavandulyl acetate	1343	0.28	-	-	-
Trans- β -Farnesene	1350	0.26	0.23	0.30	0.28
Geranyl butyrate	1352	1.74	5.38	-	5.48
santalol	1354	1.07	0.35	1.17	1.35
Germacrene D	1361	1.01	0.92	-	0.28
Gamma-Curcumene	1364	-	-	0.62	-
Neryl-2-methyl propianoate	1367	1.04	2.60	2.8 9	2.27
Bicyclogermacrene	1372	2.03	3.02	-	2.07
Citrol	1381	-	-	-	0.12
Beta- Bisabolene	1382	-	1.39	-	0.21
Delta-Cadinene	1388	0.25	0.33	0.71	0.23
Trans-gamma- Bisabolene	1396	-	0.09	-	0.12
Cis-alpha-Bisabolene	1406	0.38	0.30	-	0.24
Germacrene B	1415	-	0.53	-	0.78
Nerolidol	1426	0.45	-	1.29	-
Farnesol	1428	-	0.65	-	0.39
Spathulenol	1432	0.35	0.98	-	0.74
Isovaleric acid 3-phenylpropyl ester	1463	0.28	0.17	-	0.29
Geranyl isovalarate	1453	4.63	1.06	2.23	0.43
Cyclodecene	1485	-	0.18	-	-
β -Eudesmol	1512	0.19	0.07	0.33	0.67
Alpha-Fenchene	1526	0.45	0.28	-	0.27
Cinnamyl isovalarate	1535	4.60	2.00	3.59	3.57
α -Bisabolol	1548	4.56	3.23	4.73	4.17
Oil Yield%(g/g)		0.44	0.5 4	0.40	0.60
Number of compounds		74	82	64	79

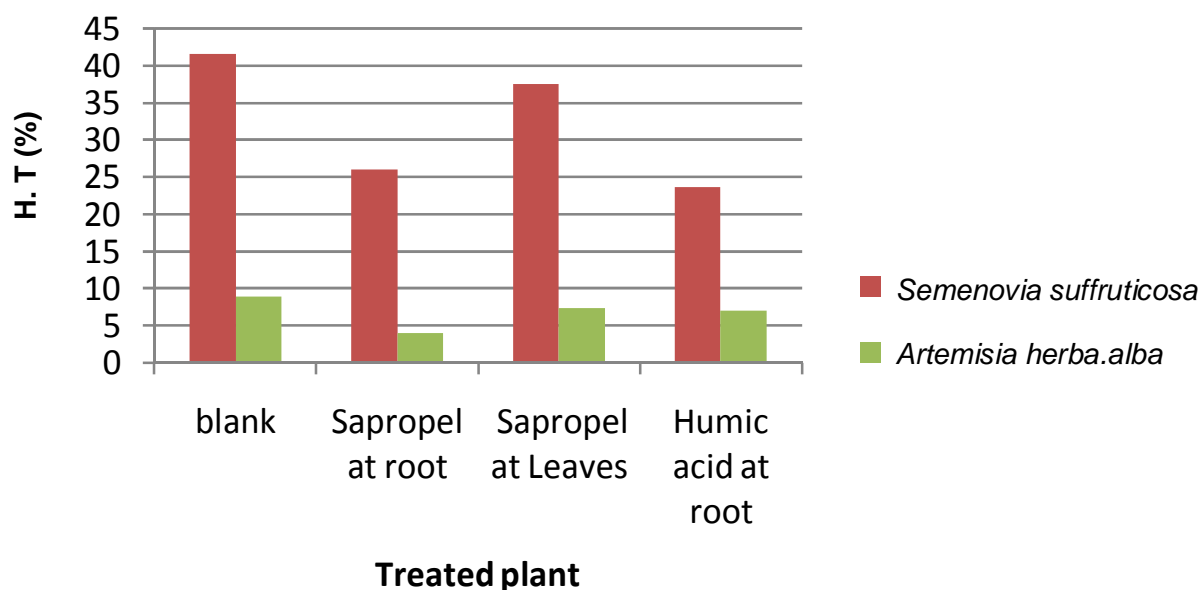


Figure 1. The variation of Terpenoid hydrocarbon in the essential oils from *A. herba-alba* and *Semenovia suffruticosa*.

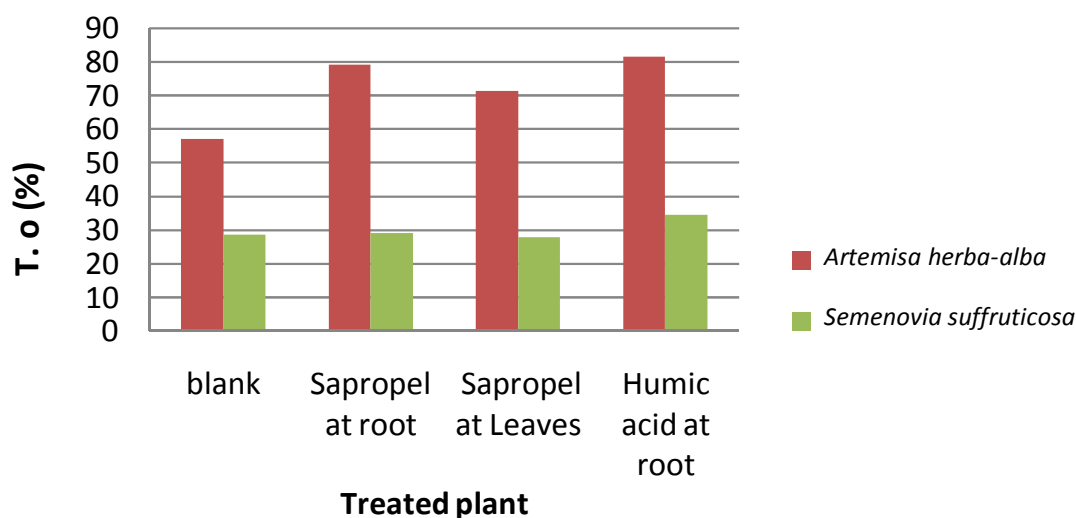


Figure 2. The variation of Oxygenated Terpenoid in the essential oils from *A. herba-alba* and *Semenovia suffruticosa*.

of plant, according to Table 1 are as follows: when SAPROPEL solution was sprayed on the leaf, the percentage of composition alpha-pinene decreased in the essential oil of plant, but increased in other two situations, comparing to its percentage in the essential oil of blank sample. Compounds camphene, Filifone, chrysanthenone and camphor, have experienced a rise in all three states in relation to their percentage in the essential oil of blank sample. The percentage of compound Eugenol experienced a fall in the essential oil

of the plant's sample whose root and leaf were exposed to SAPROPEL as compared to those of the blank sample, whereas it increased when only the root of the plant was exposed to the extracted humic acid.

The percentage of compounds beta-pinene, 4-Terpineol, gamma-Terpinene and cis-Jasmone decreased in all three states according to essential oil of blank sample. Compound Geranial decreased in two states, comparing to its percentage in the essential oil of blank sample, while it increased at the state, in which

Table 3. Percentage of Terpenoid hydrocarbon and Oxygenated Terpenoid in Essential oils from *A. herba-alba* and *Semenovia suffruticosa*.

Treated plant	Essential <i>Semenovia</i> Terpenoid hydrocarbon	Oil of <i>Suffruticosa</i> oxygenated Terpenoid	Essential <i>Artemisia</i> Terpenoid hydrocarbon	Oil of <i>Herba- alba</i> oxygenated Terpenoid
Terpenoid in essential oil from plant with humic acid at root (%)	23.67	34.67	7.04	81.39
Terpenoid in essential oil from plant with SAPROPEL at root (%)	26.08	29.13	4.08	79.09
Terpenoid in essential oil from plant with SAPROPEL at leaves (%)	37.46	28.00	7.41	71.23
Terpenoid in essential oil from blank plant sample (%)	41.49	28.63	8.95	57.10

SAPROPEL solution was sprayed on the leaf of plant. When we sprayed the solution of SAPROPEL on the leaf, composition Citrol, phenyl ethyl tiglate appeared only in the essential oil of leaf sample with a high percentage. Compound 3,5-Heptadienal-2-ethylidene-6-methyl appeared with a percentage of 2.23 when the root of plant was exposed to SAPROPEL. Compounds Delta-3-carene, E- Dcimenone and methyl epijasmonate appeared, when drawn Humic acid was sprayed on the root of plant, Compound trans-beta-Farnesene exists in the essential oil of blank sample with a percentage of 1.95, but it appears when extracted humic acid and SAPROPEL are sprayed on the leaf of plant at early stage of growth, whereas it appears in the essential oil at the time when the leaf is exposed only to SAPROPEL. Compounds, has experienced an increase in the essential oil of the sample whose root was exposed to extracted humic acid, comparing to that of existing in the essential oil of blank sample, but it decreased in the states in which SAPROPEL was sprayed on the leaf and root.

The percentage of oxygenated Terpenoid compounds increased in the essential oil of two samples (when humic acid and solution of SAPROPEL on the root of plant), regarding to the percentage of their existence in the essential oil of blank sample. The percentage of Terpenoid hydrocarbon compounds decreased in three states, comparing to blank sample.

The percentage of oxygenated Terpenoid compounds in the essential oil of blank sample with a percentage of 57.10, but it increased in the essential oil of the sample whose root had received extracted humic acid. *Semenovia suffruticos* plant was collected at flowering stage. The essential oils were extracted with the yields of 0.44 (blank sample), 0.54 (the plant sample, the root of which was added SAPROPEL), 0.60 (the sample added to its root, the extracted Humic acid) and 0.40% w/w (when the solution of SAPROPEL was added to the leaf), respectively. The percentage of Terpenoid whether oxygenated or hydrocarbon have increased from 70.12, 55.21, 58.30 and 65.46%, respectively (Table 3).

The percentage of Ethyldimethy, alpha-Bisabolol, n-octyl isobutyratean increased, comparing with its amount in the essential oil of blank sample but it declined in other

two states. When the root of plant was given SAPROPEL, comparing with its amount in the essential oil of blank sample, but it declined in other two states according to Table 2. The quantity of compositions p-Cresol, alpha-Terpinolene, 6-amyl-alpha-pyrone and beta-Eudesmol decreased, the essential oil of sample, when SAPROPEL was sprayed on the leaf of plant at initial stage of growth, while in two other states, it experienced an increase, in comparison with the percentage of these compositions in the essential oil of blank sample. composition l-Limonene, 1,8-Cineole, isobutyl isovalerate, Methyleugenol, N-octyl-2-methyl butyrate and Neryl-2-methyl propionate have experienced a rise in all three states and composition alpha-pinene, Sabinene, alpha-thujene, Cymene, cis-ocimene, gamma-Terpinene decreased in all three in relation to their percentage in the essential oil of blank sample. Compound iso-Amyl isovalerate will be produced, when we spray the humic acid on the root of plant.

When humic acid solution has been poured in the soil around the root of plant, it showed a higher level of growth. Also, the percentages of composing constituents oxygenated Terpenoid compounds increased in comparison with others. This plant has strong anti-microbial properties. When SAPROPEL solution was sprinkled on the root soil, the plant experienced a healthy growth, too. Its percent of oxygenated Terpenoid compounds increased in relation to blank sample. Table 3 shows that after addition of humic substances to the root, the percentage of oxygenated Terpenoid in essential oil compositions in this plant was more than that of the blank plants which increased considerably. So, their anti-microbial property increases. To sprinkle the solution of humic substances on the Leaves of plants has resulted in decreasing the percentage of oxygenated Terpenoid in their essential oil composition, because humic substances were absorbed by leaves and caused the chlorophyll to decrease, and as a result, the rate of plant growth became slow. It was observed that the percentage of some compositions increased while that of some others decreased. When compared with that of the blank samples, some compositions were totally removed and displaced by new compositions (Liu et al., 2007;

Masoudi et al., 2005).

Conclusion

Addition of humic substances to the root of studied plants has resulted in growth of plant organs such as leaves. Addition of humic substances (extracted humic acid and SAPROPEL) causes biologic activity and decrease of flowering stage in plants. Optimal time for collecting the organs of medicinal plant is the period of flowering in which the essential oil of plant organs has a great deal of effective substances.

The reasons behind this fact, is a better " complex – making" property of humic acid than SAPROPEL that results in assimilation of heavy metals such as Pb and Cd ions, so that the quality of essential oil composition improves (Davies et al., 2001).

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REFERENCES

Adams RP (1995). Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publ. Corp, Carol Stream, IL, USA.

- Bilia AR, Flamini G, Taglioli V, Morelli I, Vincieri FF (2002). GC/MS analysis of essential oil of some commercial Fennel teas. Food Chem., 76(3): 307-310.
- Chen Y, Clapp CE, Magen H (2004). Mechanisms of plant growth stimulation by humic substances: The role of organo-iron complexes. Soil Sci. Plant Nutr., 50: 1089-1095.
- Clapp CE, Liu R, Cline VW, Chen Y, Hayes MHB (1998). Humic substances for enhancing turfgrass growth. The Royal Society of Chemistry, Cambridge, 227-233.
- Davies G, Ghabbour E, Steelink C (2001). Humic Acids: Marvelous products of soil chemistry. J. Chem. Edu., 78(12): 1609-1613.
- Hayes MHB (1985). Studies on humic substances. J. Sci. Food Agric., 36: 272-274.
- Liu K, Rossi PG, Ferrari B (2007). Composition irregular terpenoids chemical variability and Antimicrobacterial Corsica Jordan et Fourr, Photochemistry, 68: 1698-1705.
- Lu XQ, Hanna JV, Johnson WD (2000). The Effects of Humic acid fertilizer on safflower production. J. Appl. Geochem., 15: 1090-10331.
- Masoudi S, Monfared A, Rustaiyan A, Chalabian F (2005). Composition and Antibacterial Activity of the Essential oils of *Semenovia dichotoma* (Boiss). J. Essent. oil Res., 1-6.
- Mogimi J (2007). Introducing some of pasture species in Iran, Arvan publishing, Iran., 94: 102.
- Mozaffarian V (2007). A Dictionary of Iranian Plant Names. Farhang Moaser Tehran, 57: 501.
- Rechinger KH (1987). *Semenvia, Johreniopsis*, Bunium, Smyrnum. In: Flora Ironical Umbellifera. 241 Edits Austria., 162: 456-486.
- Stevenson FJ (1994). Humus Chemistry, John Wiley, New York.
- van Den DH, Kratz PD (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr., 11: 463-471.