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Investigation of the ingredients of *Eryngium caeruleum* essential oils and its changes under the influence of mycorrhizal symbiosis and the use of Azotobacter

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ABSTRACT

Eryngium caeruleum belongs to Apiaceae family and is a perennial plant.Fresh leaves of this plant are used as vegetable in many parts of the country such as Mazandaran, Gilan and Golestan. In order to determine the ingredients of essential oil of *Eryngium caeruleum* under the effect of mycorrhizal symbiosis and the use of Azotobacter, a research as a factorial experiment in a randomized complete block design with 5 replications was conducted in the fall of 1392 in the city of Sari. The elements which were used include *Glomus Mosse* Fungus, *Azotobacter chroococcum* and control (no use of bio fertilizer). The ingredients of essential oil were identified and quantified using a gas chromatograph device and a Gas chromatograph connected to a mass spectrograph. The results of this study led to the identification of compounds in *Eryngium caeruleum* plant which the highest percentages in the control for D-Limonene the 1.17%, cyclohexane 15.75% and naphthalene 49.37% and in the use of Azotobacter, Falcarinol 78.52%, morpholine 5.04% and naphthalene 3.33% and in mycorrhizal symbiosis, 70.33% naphthalene, methyl phenyl 3.1%, and Benz aldehyde 2.23%.

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INTRODUCTION

The use of herbs for illness treatment has a history as old as human life. In recent years the use of medicinal plants has increased due to fewer complications and costs and compatibility of patients to them and compared to the known side effects of synthetic medicines. There are nearly eight thousand species of plants in Iran that can have medicinal effects [12]. *Eryngium caeruleum* is a perennial plant which normally grows in spring and turns to sky blue after flowering. It is distributed in northern Iran, from Ramsar to Galugah, and in central heights of Alborz in northern slopes. *Eryngium caeruleum* is native to north of Iran. *Eryngium caeruleum* plant is prickly and in Apiaceae family. *Eryngium caeruleum* is one of the most important species of boghang genera in north of Iran which is used as vegetable [3]. *Eryngium caeruleum* plant also has medicinal properties (diuretic, appetizer, pain killer and a powerful antioxidant) [11]. *Eryngium caeruleum* is multiplied by seed and root. Oil is extracted from root, stem, leaf and inflorescence. Due to high use of this plant in north of Iran, for the first time,this study aims to investigate the extraction and identification of ingredients of *Eryngium caeruleum* plant`s essential oil under the effect of mycorrhizal symbiosis and Azotobacter use.

MATERIALS AND METHODS

The study was carried out in the fall of 1392 in the city of Sari with 53 degrees 62 minutes east longitude position, 36 degrees 46 minutes north latitude and height of 3.17 m above sea level and with a mild temperature. This research was done as a factorial experiment in a randomized complete block design with 5 replications. The investigated factors included the use of Glomus moseae fungus (2 tons in a hectare) and Azotobacter (Azotobacter chroococcum) (0.5 Liter in a hectare) and the control was no use of bio fertilizers. Moreover, for

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all plots, vermicompost (5 tons in hectare) with animal origin was applied. Each plot was 1.5 × 1.5 and in each plot, 6 rows were planted. Planting operations took place in November and preserving practices such as thinning operations (4-leaf stage) and fighting with weeds were also carried out. In this study, aerial parts and leaves were collected in spring 1393. Then they were put into paper bags. Collected leaves were dried in normal conditions and away from sun for ten days. After crushing the leaves, after weighing 20 g of sample with distilled water, oil extraction was done by Clevenger apparatus for 2 hours. After removing the water by sodium sulfate, then percentage, amount and performance of extract were determined. The components of Essential oils were determined and identified using GC / MS device

Identification of the components of essential oil:

The GC / MS. 5973 gas chromatograph connected to a mass spectrometer, equipped with HPS Column with 30 meters length and internal diameter of 250 mm and a Stationary phase thickness equal to 25/0 mm was used. Oven temperature was increased from 45 ° C to 250 ° C with a speed of 5 ° C per minute and then it reached to 280 ° C at a rate of 20 ° C min. Helium gas with ionization energy of 70 eV was used. Obtained Spectra were identified by the comparison with Mass spectra of Standard components. The relative percentage of each constituent of the composition according to the area under its curve in chromatogram spectrum was obtained [4].

RESULTS AND DISCUSSION

After the extraction operation, the percentages of oil yields were determined using the following equation [1]. 100 × (dry weight / weight of essential oil) =% of essential oils

Weighted yield of obtained essential oil was calculated from 1.2. Essential oil was analyzed by GC / MS in order to identify its components. More than 40 compounds of these plants were identified.

The percentages of extracted compounds are as follows:

Table 1: Identified compounds existing in *Eryngium caeruleum* essential oil in control group

Row	Composition	Percent	Row	Composition	Percent
1	D-Limonene	1.17	12	Octane	0.52
2	Cyclohexanone	0.25	13	Naphthalenecarboxylic acid	0.69
3	Cyclohexanol	0.20	41	Furan	0.30
4	Hexadecane	1.3	15	Pyridine	0.56
5	Dodecane	1.11	16	Longifolene	0.43
6	Copaene	0.30	17	Naphthalene	49.37
7	Benzaldehyde	0.22	18	Benzene	0.61
8	Hexadecane	1.3	19	Octadecane	0.22
9	Azete	2.62	20	4-Methyl-4-phenyl	0.83
10	Cyclohexene	15.75	21	Phthalic acid	0.50
11	Phenol	4.32	22	Carbonic acid	0.55

Table 2: Identified compounds existing in *Eryngium caeruleum* essential oil in Azotobacter group

Row	Composition	Percent	Row	Composition	Percent
1	Benzaldehyde	1.53	4	Falcarinol	78.52
2	Ammonia	0.34	5	Morpholine	5.04
3	Naphthalene	3.33	6	Ethanediimidic acid	0.38

Table 3: Identified compounds existing in *Eryngium caeruleum* essential oil in Mycorrhiza group

Row	Composition	Percent	Row	Composition	Percent
1	Octanal	0.75	13	Hentriacontane	0.18
2	Decane, 3,6-dimethyl	0.12	14	Octadecane	0.33
3	Nonanal	0.18	15	Falcarinol	1.3
4	Cyclohexanone	0.20	16	2,1-Benzenedicarboxylic acid	0.31
5	Cyclohexanol	0.15	17	Hexadecane	0.82
6	Nonadecane	0.35	18	2,1-Methyl-4-phenyl	3.1
7	Benzaldehyde	2.23	19	Benzene	0.68
8	Cyclohexene, 1-methyl	0.54	20	1-Octadecene	0.20
9	Phenol	0.42	21	Phytol	0.32
10	Cyclohexene	0.32	22	Dodecane	0.17
11	Naphthalene	70.33	23	Heptadecane	0.44
12	Pyridine	0.69	24	cyclohexane	0.66

As listed in table 1, the highest percentages of essential oil were for D-limonene 1.17% cyclohexane 15.75%, and naphthalene 49.37%. In another research, Semnani et al (1381) showed that the main components of essential oil are in Chvchakh limonene (1/52%), beta -Szkvyy Flandren (8.1%), alpha -Pnyn (5.5%) and delta-2-Karen (5.3%). the highest percentages in the control for D-Limonene the 1.17% , cyclohexane 15.75%

and naphthalene 49.37% and in the use of Azotobacter, Falcarinol 78.52%, morpholine 5.04% and naphthalene 3.33% and in mycorrhizal symbiosis, 70.33% naphthalene, methyl phenyl 3.1%, and Benz aldehyde 2.23%. but in another research by Semnani et al (1381), Limonene (52.1%), beta Sezkuee Flandren (8.1%), alpha -Pnyn (5.5%) and delta-2-Karen (5.3%). According to extracted compounds, naphthalene in mycorrhizal symbiosis was 70.33% which was 3.33 % and 49.37 % higher than control and Azotobacter respectively. And the compound of Falcarinol in Azotobacter use has assigned 78.52 % to itself which decreased 1.3 % in Mycorrhizal symbiosis. Also Benz aldehyde compound has assigned 1.53 % to itself in Azotobacter use which in mycorrhizal symbiosis it has assigned 2.23 %to itself and has increased.

Darzi et al [5] with the study of the effect of biofertilizers on fennel essential oil compounds reported that application of mycorrhiza and vermicompost and integrating them with an increase in the rate of Anatole has caused the decrease of limonene amount in the essential oil of this plant. [8] with the study of the effect of phosphate solubilizing bacteria *Bacillus polymyxa* on the medicinal plant of Lemongrass, showed that this bacterium with an increase of geraniol in essence, reduces the number of some compounds in the essential oil.In another study, the maximum essential oil yield per hectare and also Chamazulene of German chamomile, were respectively seen in the treatment of PSB and Nitroxin (Combination of Azotobacter and Azospirillum) [7]. The use of Bacillus bacteria on basil plant showed that with the use of bacterium, the amount of eugenol and alpha terpineol existing in essential oil will increase 10 and 2 times respectively [2]. Darzi et al (1387) also reported that the use of biofertilizers improves the amount of Anatole essence and as a result improves the quality of fennel essential oil. He stated that the desired biological fertilizer treatments compared with chemical fertilizer, provide better conditions for the improvement of microbial activities in soil. And in addition to providing desired macro and micro elements for anise, increase the quality of essential oil of this plant. Various studies show that habitat conditions affect the quantity and quality of essential oil of aromatic plants [6]. Growth and performance of plants in ecosystems is affected by various factors such as Species, regional climate, soil type, altitude and geographical location. Each of these factors can have a significant impact on the quantity and quality of plant products. Although the production of secondary metabolites is controlled by genes, but their yields are significantly affected by the environmental conditions including physical and chemical properties of soil and micro or macro nutrients [9]. Due to the identification of medicinal and chemical compounds, the existing compounds in *Eryngium caeruleum* plant can be applied to be used in industrial situations. It can be concluded that applying chemical fertilizers, with affecting microbial populations in soil and creating a food cycle and making them available, increase the absorption of nutrients and in this way increase and change the amounts of compounds and ingredients of *Eryngium caeruleum* plant.

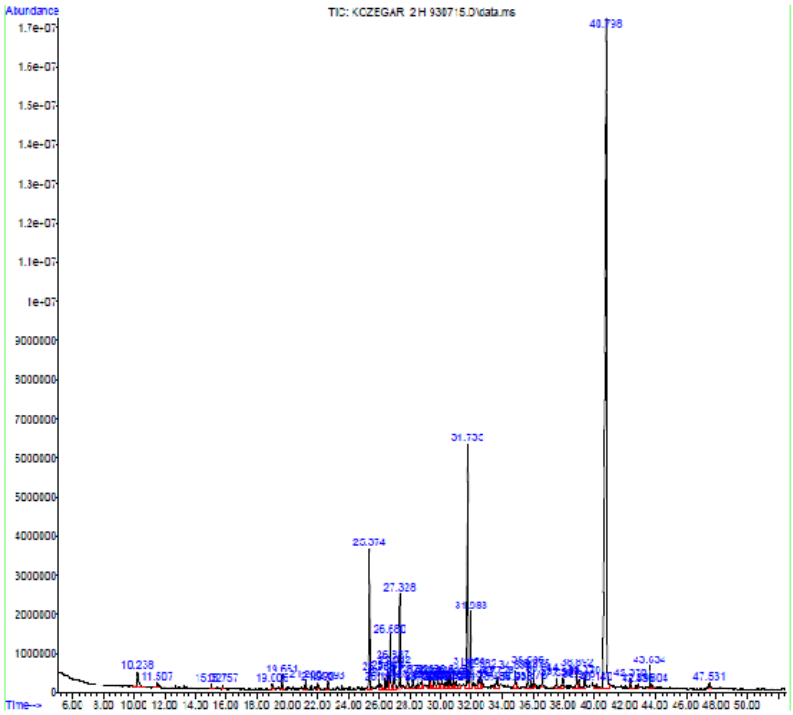

Fig. 1: chromatograph essential oil in control group

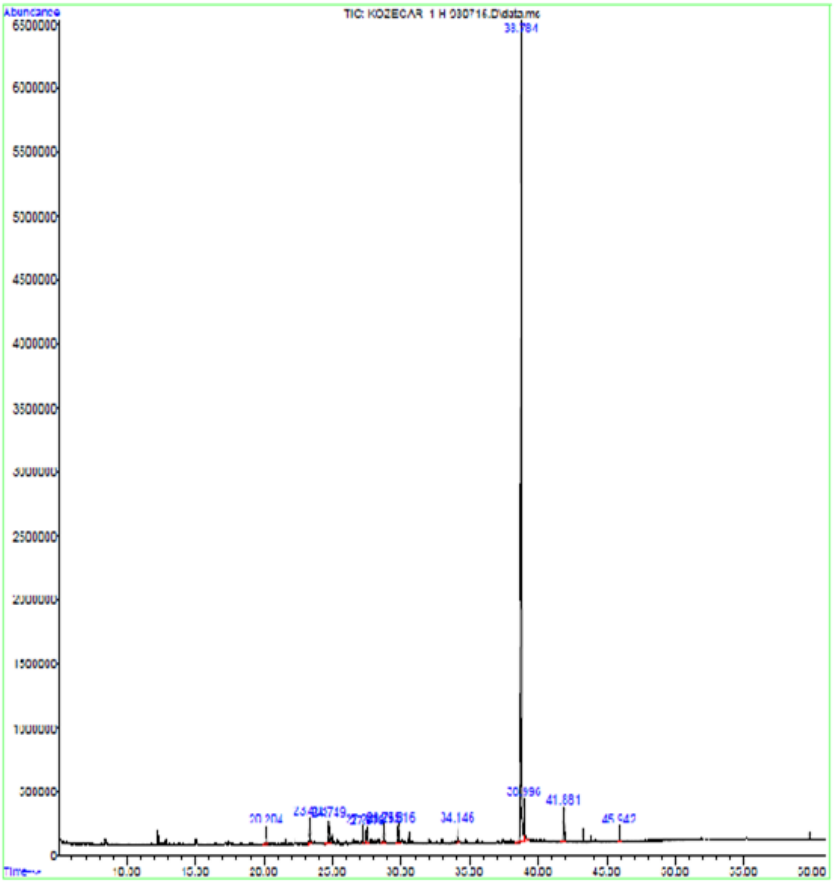

Fig. 2: chromatograph essential oil in Azotobacter group

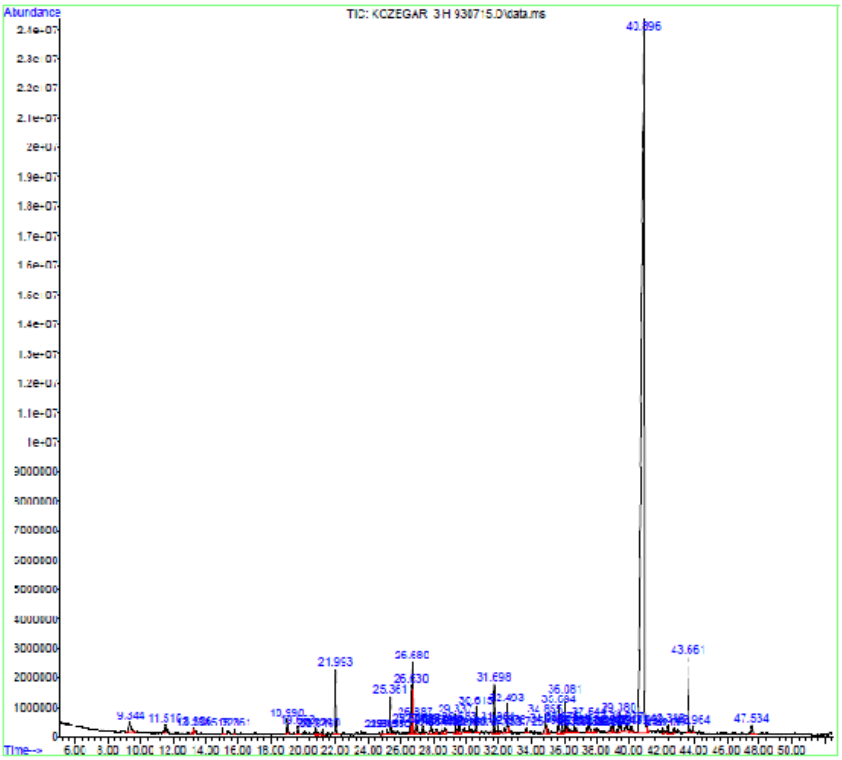

Fig. 3: chromatograph essential oil in Mycorrhiza group

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