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The Influence of Soil Ionic Content on the Essential Oil Composition of Shirazi Thyme in Ecotypes of Yazd Province

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ABSTRACT

The characteristics of the growing area and the soil of the region are among the factors that can affect the amount and type of essential oil composition. The purpose of this research is to study the ionic content of Shirazi thyme plant soil in three different habitats of Yazd province and to know the relationship between the amount and type of essential oil composition with soil content. Initially, Shirazi thyme plants (*Zataria multiflora* Boiss.) were randomly collected from the Manshad, Khezrabad, and Esfandabad habitats in Yazd Province. The plant's essential oils were extracted using hydro distillation with a Clevenger apparatus, and the compounds were identified using GC and GC/MS devices. To evaluate the essential physical and chemical properties of the soil from each habitat, soil samples were taken to a depth of 0-30 cm. Parameters such as pH, calcium, magnesium, chlorine, carbonate and bicarbonate, salinity, phosphorus, and lime percentage were calculated. In the essential oils of the ecotypes studied, totaling 35 different compounds. The assessment of the essential oil components revealed that Carvacrol, p-Cymene, Thymol, Linalool, and γ-Terpinene were the dominant compounds in all ecotypes. The soil in the Manshad region had the highest phosphorus content and the lowest bicarbonate content among the ecotype ($p< 0.05$). Compounds such as 1-Octen-3-ol, 3-Octanone, α-Terpinolene, and Thymol methyl ether showed a positive correlation with phosphorus, while Ledene showed a negative correlation with phosphorus. Overall, soil clay and sand content consistently showed positive correlations with essential oil compounds and no significant correlation between the five main compounds of Shirazi thyme and soil factors.

Keywords: *Zataria multiflora*, Ecotype, Essential oil, Soil content

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INTRODUCTION

The identification of superior medicinal plants is essential for improving community health. Herbal treatments provide an effective alternative and complementary approach to traditional medical practices. The growth, development and distribution of plants across various habitats are influenced by parameters such as climate, soil, and geographical characteristics. These factors significantly impact both the quantity and quality of plant essential oils [1,2]. Understanding the relationship between the quantity and quality of active ingredients and soil conditions is critical to identifying optimal habitats that maximize the yield of these substances [3]. Furthermore, predicting the effects of edaphic factors on species distribution and the prevalence of specific compounds can provide valuable insights into plant diversity and its relationship with soil chemical properties [4,5]. Soil is one of the most critical factors in determining the nutrient content of plants, as it reflects the cumulative effects of environmental conditions over time. In other words, soil characteristics significantly influence plant growth and the concentration of active substances [6,7]. Among these characteristics are the soil's chemical composition, pH levels, moisture content and nutrient availability. Key nutrients such as nitrogen, phosphorus and potassium are essential for the biosynthesis of essential oils. Moreover, soil pH significantly influences nutrient absorption; maintaining an optimal pH can enhance the production of essential oils by facilitating more effective nutrient uptake. Furthermore, the presence of organic matter in the soil contributes to improved soil structure and increased microbial activity, both of which positively impact the synthesis of essential oils. In summary, understanding the interplay between these soil factors is vital for optimizing essential oil production in various plant species.

Shirazi thyme (*Zataria multiflora* Boiss.), which belongs to the Lamiaceae family, derives its name from the Arabic word "zaatar" meaning thyme and is it indigenous to Iran, Afghanistan, and Pakistan [8,9]. This aromatic, perennial shrub grows wild on dry slopes and rocky terrains [1, 8, 10-13] also another study noted a negative and significant relationship between thyme cover with soil acidity and phosphorus content [1]. Whereas its density correlated negatively with soil EC and stone and gravel content [8].

The essential oil of Shirazi thyme is enriched with oxygenated phenolic monoterpenes, with Carvacrol, Thymol, Linalool and p-Cymene being the main chemical components [4,10-15]. Another study on this plant noted that Carvacrol, Thymol, and Linalool were the most significant constituents of the essential oil in the flowering tops [14]. Similarly, another research identified

Carvacrol, Thymol, p-Cymene, γ-Terpinene, and Linalool as the primary components of Shirazi thyme essential oil. Essential oil rich in Thymol exhibited strong antioxidant activity, whereas oil rich in Carvacrol demonstrated potent antibacterial, antifungal, and antitumor properties. So research on Shirazi thyme found Linalool, Thymol, Carvacrol, γ-Terpinene, p-Cymene, Carvacrol methyl ether, Pinene, and Myrcene as major constituents [16].

Considering the significant applications of volatile compounds and essential oils in the pharmaceutical, food and cosmetic industries, the extraction and analysis of these compounds in various medicinal plants have gained considerable importance [17]. Numerous reports have documented the chemical diversity of essential oils from Shirazi thyme collected from various natural habitats in Iran. For instance, number of reports in IRAN consistently identify Thymol, Linalool, and Carvacrol as the main components of Shirazi thyme essential oil [4, 10, 14, 16-18]. The high content of phenolic compounds such as Thymol, Carvacrol, Linalool and monoterpenes like γ-Terpinene and p-Cymene are noted as key components of this essential oil and Another study on the aerial parts of Shirazi thyme collected from Fars Province revealed that α-Pinene was also the most significant components [1, 17, 20- 21].

Although much research has concentrated on the essential oil composition of Shirazi thyme, there is a paucity of information regarding the influence of soil content on its essential oil. Shirazi thyme is a valuable plant threatened by overharvesting, underscoring the significance of considering both its phytochemical properties and soil factors in natural habitats. This study aimed to investigate the impact of soil ionic content on the quantity and composition of Shirazi thyme essential oil in various habitats of Yazd Province and its influence on plant growth.

MATERIAL AND METHODS

To assess the influence of edaphic factors on the quantity and composition of Shirazi thyme essential oil, plant materials were gathered in 2021 from three distinct natural habitats in Yazd Province, each with three replicates. Accordingly, regions with high densities of this plant were randomly selected (Fig. 1)

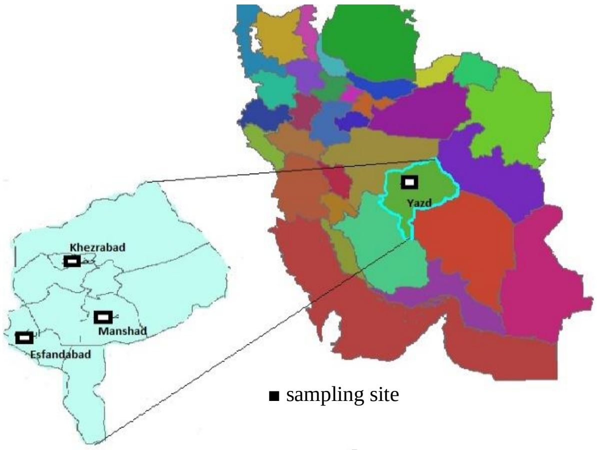

Fig. 1 Geographical map of the study area.

Samples of plant were collected Based on the random systematic method, it was done by dropping 10 transects 200-meter and randomly selecting bases along the transect. Soil samples were taken from depths of 0 to 30 cm near randomly selected thyme plants in each site.

To assess the influence of habitat conditions, each site was visited and sampling locations were recorded using GPS. Field operations were conducted in May across Yazd Province, including the counties of Manshad, Khezrabad and Esfandabad. To evaluate the essential oil content and its components, flowering tops of Shirazi thyme were randomly collected in mid-spring 2021 during the flowering period, with three repetitions. The flowering tops samples were processed in the Medicinal Plants Laboratory at Yazd University. Subsequently, the essential oil yield was measured based on volume-to-weight percentage. The essential oil was then extracted stored in a laboratory refrigerator until GC and GC/MS analysis. Meteorological data for the past five years, including average annual temperature, minimum and maximum temperatures, average annual rainfall, and relative humidity were collected from regional meteorological stations (Table 1).

Table 1 Habitat and meteorological data

Region	Longitude	Latitude	Average rainfall (mm)	Average temperature (°C)	Average relative humidity (%)	Elevation (m)
Manshad	54°18′	31°22′	263.8	13	32.2	2470
Esfandabad	53°23′	30°56′	94.3	18.95	30.2	1485
Khezrabad	53°57′	31°05′	107.15	17.9	29.15	1732

The plants were shaded and air-dried for 10 days. Subsequently, 25 g of dry, ground powder from each batch of Shirazi savory plants was placed in a flask for 3-4 hours to facilitate EO extraction. The extracted EO was then transferred into glass vials for analysis, sealed with paraffin, wrapped in aluminum foil and stored at a temperature of 4 °C analysis. The quantities were calculated on a weight basis and reported as essential oil yield.

The essential oils were analyzed using GC–MS on a Thermoquest–Finnigan Trace GC–MS instrument. The setup included a DB-5 fused silica column (60 m × 0.25 mm I.D., with a 0.25 µm film thickness). Mass spectrometry analysis was conducted with a quadrupole detector, scanning across a mass range of 35–465 amu, at an ionizing voltage of 70 eV and an ionization current of 150 µA.

To evaluate important physical and chemical soil properties. The obtained samples were analyzed in the soil laboratory of Yazd University. Various parameters were measured such as pH, calcium (Ca), magnesium (Mg), chloride (Cl), carbonate and bicarbonate, salinity, phosphorus (P), and lime percentage. The pH of the flower extract was measured using a pH meter. Furthermore, P content was measured using a spectrophotometer. Besides, Sodium (Na) and Potassium (K) levels (mEq/L) were measured via photometric film methods. K and Mg were also determined using titration and complexometric methods with versine and sulfuric acid 0.01 N in each of the samples. Moreover, Soil P (ppm) content was measured using a spectrophotometer. Soil texture determination was performed using a hydrometer (152H-62) or density meter in the laboratory. Soil nitrogen (N) content was evaluated using distillation methods and the Kjeldahl method [7]. In order to analyze the statistical data, the normality of the data was first checked, and in case of non-normality, normalization was done. The data was analyzed with SPSS20 software and the averages were used It was compared using Duncan's multiple range test at the 5 % probability level.

RESULTS

Soil analysis results indicated that the region of Khedrabad exhibited the highest percentages of K, Mg, Ca, Na, Cl, and N compared to other regions ($P < 0.05$). Additionally, the soil P content in the Manshad was the highest rather than other areas ($P < 0.05$). (Table 2).

Furthermore, the essential oil analysis of the three studied ecotypes revealed significant diversity in EO composition types and percentages among different populations of *Zataria multiflora* (tab 3). Accordingly, 31 compounds were identified in Manshad, 28 in the Esfandabad ecotype, and 31 in the Khedrabad ecotype, totaling 35 different compounds were determined in the all ecotypes. different Furthermore (tab 3), our results indicated that Carvacrol, p-Cymene, Thymol, Linalool, and γ-Terpinene were predominant components in all ecotypes, though their quantities varied among different ecotypes. These compounds constitute 62.84% of the total composition in Manshad ecotype, 88.79% in Esfandabad ecotype and 86.55% in Khedrabad ecotype.

Table 2 Edaphic factors in natural plantations of *Zataria multiflora*

Region	Soil Texture	K (meq/ lit)	Mg (mg/kg)	Ca (mg/kg)	pH	Na (mg/kg)	Cl (%)	Nitrogen (N)	Calcium carbonate ($CaCO_3$; %)	OC %	P (mg/kg)	CO_3 (cc)	HCO_3 (cc)
Manshad	Loamy sand	21.1	4	5.5	8.29	326.5	12	0.065	32.45	1.23	56.65	1.35	3.4
Esfandabad	Loamy sand	8.9	2.53	4.03	8.3	160.2	11/7	0.33	52/5	1.3	23.34	1.8	6.06
Khezrabad	Sandy clay loam	26.46	8	10.6	8.25	433	18	0.066	25	1.56	16.7	1.8	5.76

Table 3 Quantitative and qualitative analysis of Shirazi Thyme (*Zataria multiflora*) essential oil

No	Compound	RI	Manshad (%)	Esfandabad (%)	Khezrabad (%)
1	α-Thujene	927	0.165	0.17	0.42
2	α-Pinene	935	0.535	0.45	0.67
3	Camphene	954	0.035	0	0.09
4	β-Pinene	983	0.025	0.103	0.28
5	1Octen$_3$ol	986	0.12	0	0
6	Octanone3	990	0.92	0	0
7	βMyrcene	991	0.985	0.56	0.856
8	α-Phellandrene	1015	0.23	0.146	0.14
9	α-Terpinene	1021	0	0.936	0.8
10	p-Cymene	1030	5.84	5.17	5.88
11	Limonene	1033	0.51	0.47	0.32
12	β-Phellandrene	1036	0	0	0.603
13	1,8-Cineole	1037	0.42	0.47	0.04
14	γ-Terpinene	1061	1.56	3.76	1.46
15	β-Terpinolene	1076	0.51	0.166	0.48
16	α-Terpinolene	1089	0.375	0	0
17	Linalool	1105	5.65	1.8	1.22
18	Trans-Sabinenehydrate	1109	0.58	0.29	0.203
19	Borneol	1182	0	0	0.003
20	α-Terpineol	1189	0.5	0.6	0.7
21	Data MS	1213	0.64	0.49	0.69
22	Dihydrocarvone	1219	0	0	0.153
23	Thymol-methyl ether	1233	1.30	0.5	0
24	Carvacrol methyl ether	1242	1.60	0.8	1.46
25	Cuminol	1295	0	0.016	0.06
26	Thymol	1302	25.87	38.5	11.09
27	Carvacrol	1318	45.5	39.56	66.09
28	Thymol acetate	1351	0.085	1.53	0.09
29	Carvacrol acetate	1369	1.20	0.256	1.27
30	Trans-Caryophyllene	1421	1.23	2.01	1.3
31	Aromandendrene	1439	0.22	0.23	0.27
32	α-Humulen	1456	0.19	0.095	0
33	Ledene	1490	0.07	0.2	0.19
34	Spachulenol	1581	0	0.11	0.29
35	Caryophyllene oxide	1586	3.09	0.11	0.08
	Total	-	99.97	99.03	98.01

Table 4 Plant compounds and soil factors correlation

	Mg	Ca	Cl	HCO_3	P	EC	% OC	% lime	Na	pH	% Clay	Camphene	β-Pinene	1-Octen-3-ol	3-Octanone	β-Phellandrene	α-Terpinolene	Borneol	Dihydrocarvone	Cuminol	Ledene
Camphene	-0.016	0.22	0.41	0.300	-0.58	0.23	0.39	-0.64	0.20	0.10	.95 **	1									
β.Pinene	-0.09	0.04	0.25	0.49	-0.63	0.08	0.33	-0.44	0.13	0.26	.81 *	.93 **	1								
1-Octen-3-ol	-0.19	-0.15	-0.42	-0.58	0.81 *	-0.20	-0.52	0.05	-0.21	-0.06	-0.30	-0.302	-0.39	1							
3-Octanone	-0.1	-0.1	-0.3	-0.72 *	0.9 **	-0.1	-0.5	-0.07	-0.	0.001	-0.29	-0.374	-0.48	.93 *	1						
β-Phellandrene	-0.07	0.16	0.36	0.29	-0.58	0.17	0.34	-0.62	0.157	0.15	0.96 **	0.99 **	0.90 **	-0.29	-0.36	1					
α-Terpinolene	-0.16	-0.16	-0.38	-0.70 *	0.91 **	-0.17	-0.51	-0.05	-0.10	-0.01	-0.29	-0.36	-0.47	0.95 **	0.99 **	-0.35	1				
Borneol	0.9 **	0.9 **	0.8 **	0.16	-0.05	0.9 **	0.7 *	-0.41	.83 *	-.9 **	-0.01	0.11	0.01	-0.16	-0.20	0.049	-0.19	1			
Dihydrocarvone	0.47	0.67	0.76*	0.32	-0.5	0.68	0.72 *	-0.72 *	0.59	-0.38	0.76 *	0.86 **	0.73 *	-0.32	-0.40	0.82 *	-0.39	0.60	1		
Cuminol	0.24	0.51	0.67	0.42	-0.66	0.46	0.47	-0.44	0.32	-0.33	0.67	0.78 *	0.59	-0.42	-0.52	0.75 *	-0.51	0.40	.83 *	1	
Ledene	-0.17	-0.03	0.194	0.5192	-0.74*	-0.06	0.13	0.16	-0.09	0.18	0.29	0.40	0.59	-0.75 *	-0.83 **	0.40	-.83 *	-0.03	0.30139	0.41	1

The mean comparison results of essential oil yields collected from natural habitats showed significant differences ($p<0.05$) among them (fig 2). The highest mean value was associated with the Manshad ecotype (3.78 %), while the lowest one belonged to the Khedrabad ecotype (3.49%).

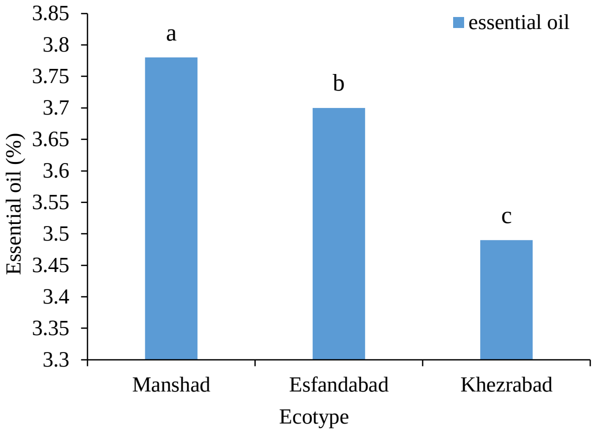

Fig. 2 Comparison of Shirazi Thyme essential oil in different ecotypes

Correlation between Plant Compounds and Soil Factors

Table 4 demonstrates a significant correlation between soil compounds and plant essential oils in at least one instance. The simple correlation coefficients between soil factors and measured plant compound properties showed a significant positive or negative correlation. Compounds such as 1-Octen-3-ol, 3-Octanone, α-Terpinolene, and Thymol methyl ether exhibit positive correlations with P, while Ledene exhibited a negative correlation with this element (table 4). Other compounds namely 3-Octanone, α-Terpinolene, and Thymol methyl ether exhibit negative correlations with bicarbonate. Notably, Borneol showed the highest correlation with soil elements. Overall, the lime content in the soil is negatively correlated with certain compounds while being positively correlated with clay and sand content. Furthermore, no significant correlations were found between the five main compounds of Shirazi Thyme (γ-Terpinene, Linalool, p-Cymene, Thymol, and Carvacrol) and soil factors (Table 4).

To determine the most effective plant compounds affecting the EO of Shirazi thyme, the data of essential oils and soil characteristics were analyzed using the Principal Component Analysis (PCA) method through SPSS20 software across three different habitats. PCA results indicated that there were 8 observations in this study (Fig 4). Accordingly, observations 1 and 2 belonged to the Manshad region, while 3, 4, and 5 observations were associated with the Esfandabad ecotype. Furthermore, observations 6, 7, and 8 belonged to the Khazrabad ecotype. According to this analysis, it can be concluded that in the Manshad ecotype, two observations were different in terms of plant compounds and soil factors, indicating intraspecific variation in this ecotype. However, in the Esfandabad and Khezrabad ecotypes, all three observations were grouped together in terms of both soil factors and plant compounds, suggesting no intraspecific variation in these ecotypes.

Figure 3 shows that in samples 7 and 8 of Khezrabad, the K content of the soil is highly influential, which is the only dominant soil factor in these two regions. Whereas, in sample 6 of Khazrabad, other factors were influential, namely carbonate, Mg, Ca, Na, Cl and CO percentage. It is widely acknowledged that P is an essential nutrient for both vegetative and reproductive growth in plants. Increased application of this element is known to positively impact the number of produced flowers. P also plays numerous other vital cellular functions in plants and influences both primary and secondary metabolites. Therefore, P fertilization in the cultivation of medicinal plants, particularly in regions with low soil P availability, is strongly recommended.

Similarly, it is widely recognized that K fertilizers enhance growth parameters and performance quality. These fertilizers contribute to plant metabolism, carbohydrate synthesis, water movement in the xylem, and an increase in cell elongation.

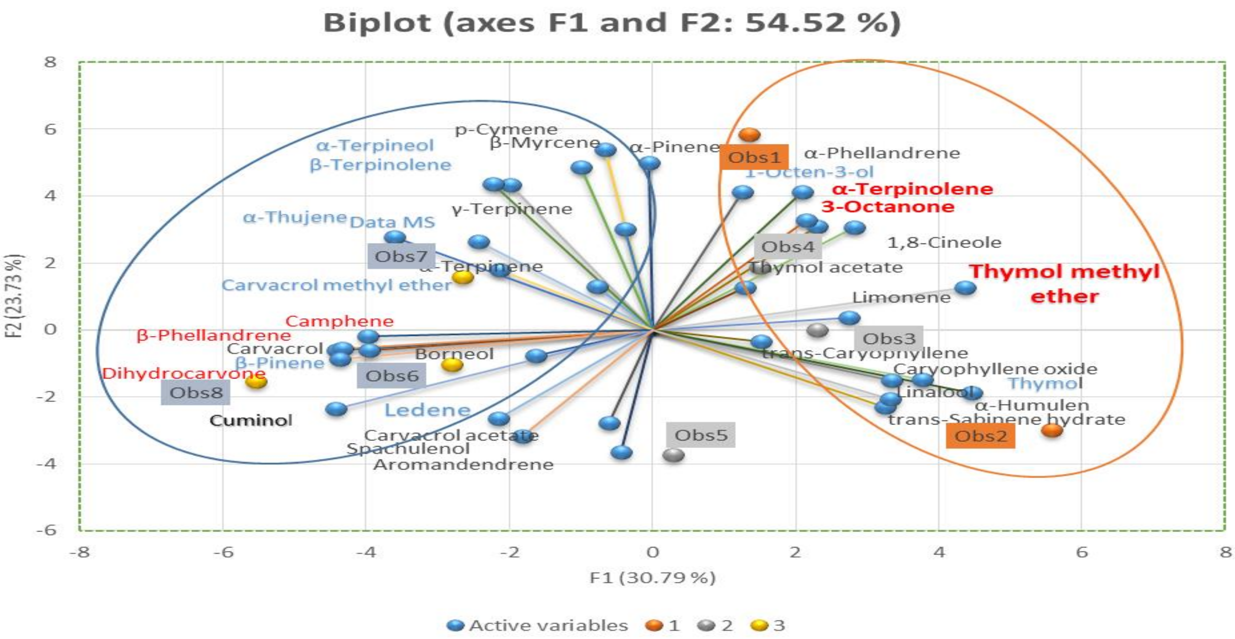

Fig. 3 The results of PCA analysis

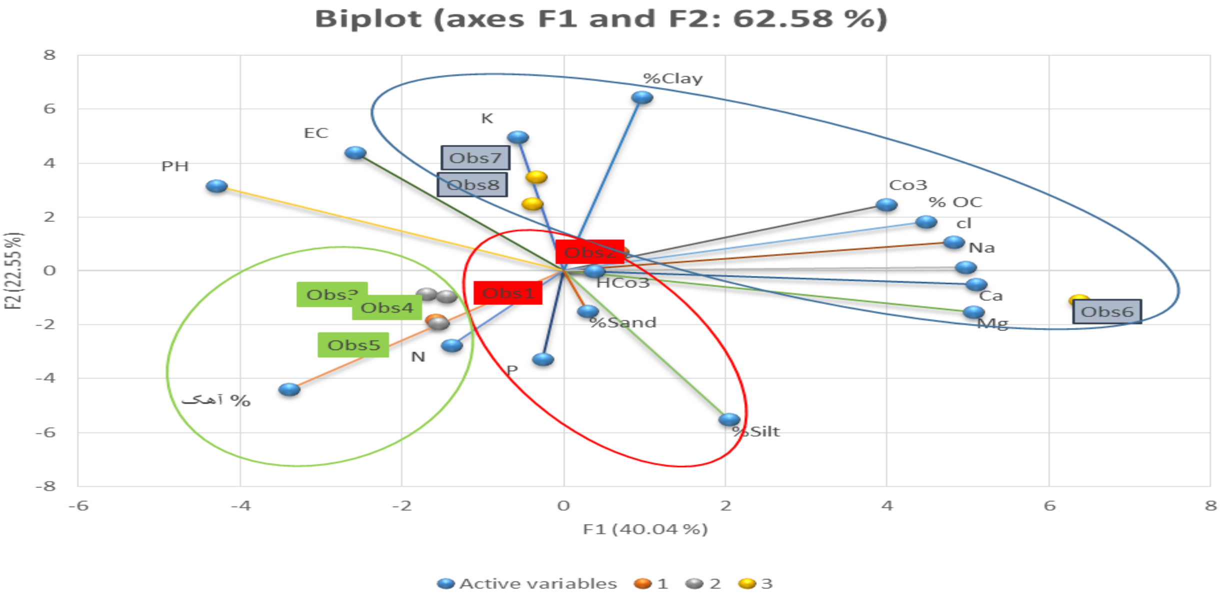

Fig. 4 The results of PCA principal component analysis

DISCUSSION

This study aimed to investigate the effect of soil ion content on the amount of Shirazi thyme essential oil composition. Accordingly, 35 compounds were identified in the present study, including 5 major compounds: thymol, carvacrol, linalool, p-cymene, and linalyl acetate.

The highest average percentage of essential oil was recorded in Manshad ecotype (3.78%), while the lowest value was related to Khezrabad ecotype (3.49%; $P<0.05$). The percentage of carvacrol and thymol in Manshad ecotype There were 37.71%, in Esfandabad, was 78.6% in Manshad and was 99.7% in Khezrabad of total compounds. The research conducted on this species in Iran has identified between 23 and 35 distinct compounds [22, 24, 26-28], with thymol and carvacrol being recognized as the key constituents [11, 14, 22, 24, 26, 27, 28]. However, the subsequent major compounds differ across various sources. In some studies, compounds such as linalool, p-cymene, and γ-terpinene have been identified as prominent, which is consistent with our results [11, 14, 22, 24-28].

Numerous studies have demonstrated that soil chemical properties can significantly influence the produced quantity of essential oil by various plant species. For example, the highest EO yield in the *T. mastichina* species was observed after the soil fertilization with N, P, and K in Portugal [11]. In a study [16] it was found that the P and K levels were positively affect the growth, yield, and quality of *T. vulgaris* L. as a medicinal and herbal plant. The influence of soil parameters and climatic conditions on plant performance and EO content has been demonstrated for many plant species. For example, *Kelussia odoratissima* Mozaff grows in black, mineral-rich soils [29], while *T. pulegioides* grows in areas with high levels of Al, Ca, Fe, K, and Si and low levels of P and Mn [30]. Mexican oregano cultivated in soils with high N and iron (Fe) content, limited available soil water, and elevated soil pH demonstrated a higher EO yield [9]. Overall, it has been established that environmental conditions significantly affect both the quantity and composition of plant EO [31]. Several studies have demonstrated that the higher levels of carvacrol or thymol in various Lamiaceae species are associated with environmental factors. Furthermore, EO content and its compositions can be influenced by edaphic factors and climatic conditions. For instance, soil type affected the chemotype of *Origanum syriacum* [32]. In a study [16] it was found that demonstrated that the amount of EO in *T. vulgare* ssp. is negatively correlated with altitude and positively correlated with soil temperature and air temperature. Furthermore, in another study [33] indicated that there was a positive correlation between the EO percentage and the amount of lime, silt, and clay. On the other hand, a negative correlation was observed between mentioned parameter with the sand quantity. In other words, the EO content was found to be higher in heavy lime soils in comparison to soils with lighter texture and lower lime content. In the present study, the EO percentage was not correlated with soil elements and climatic factors. It has been reported that Shirazi thyme thrives in regions with high concentrations of calcium (Ca), iron (Fe), potassium (K), and aluminum (Al), where the annual rainfall typically ranges from 46.40 to 302.72 mm, and the average annual temperature spans from 14.9 to 28.8 °C [34].

Based on the results of data analysis, it can be concluded that the soil of the Maneshad region in Yazd displayed the highest concentration of (P) and the lowest level of bicarbonate compared to other area. Additionally, in the Khezrabad region, the soil exhibits elevated levels of Ca, Cl, K, Na, and Mg compared to the other two ecotypes. Considering the higher rainfall and altitude in Manshad, it can be inferred that as rainfall and altitude increase, the soil P content rises while the soil bicarbonate content decreases in this region.

According to Results, it can be inferred that compounds such as 1-Octen-3-ol, 3-Octanone, α-Terpinolene, and Thymol-methyl ether, exclusively identified in the EO components of the Manshad region. These compounds exhibited a positive correlation with P and a negative correlation with bicarbonate. Thus, it can be concluded that in this region, as the soil P content increases, the proportion of these compounds in the EO of the region tends to increase. A deficiency or excess of phosphorus can alter the chemical composition of essential oils. Specifically, variations in phosphorus levels may influence the relative proportions of different essential oil constituents, including terpenes and phenolic compounds, and can lead to an increase or decrease in the concentration of certain essential oil components [35]. Conversely, the compound Borneol, found exclusively in the Khezrabad ecotype, demonstrated a positive correlation with Ca, Cl, K, Na, and Mg elements. Therefore, the presence of these compounds may be attributed to the higher levels of P, Ca, and K in the soil of that region.

CONCLUSION

The assessment of EO components across different ecotypes revealed that Carvacrol, p-Cymene, Thymol, Linalool, and γ-Terpinene were predominant EO components in all ecotypes. However, their concentrations varied among different ecotypes. It should also be noted that in the Manshad region, which has higher rainfall and altitude, the EO yield and the percentage of specific compounds such as α-Terpinolene, 3-Octanone, and Thymol methyl ether increase, and the Carvacrol percentage decreased. Our results are consistent with previous research reports [2, 36-37], the elevation of average temperature within the oregano habitat emerges as a significant factor contributing to the EO production enhancement. They also observed that elevated temperatures may stimulate plant EO biosynthesis, a finding which contrasts with the results of the present study. As the concentration of Carvacrol increases in a region, the Thymol content tends to decrease. It is noteworthy that an inverse relationship exists between the levels of Thymol and Carvacrol in ecotypes (Table 3), wherein higher levels of Thymol coincide with lower levels of Carvacrol, and vice versa. This phenomenon is inherently logical as Thymol and Carvacrol are isomers of each other and originate from similar precursors such as p-Cymene and γ-Terpinene [21].

Overall, our findings indicate that the soil in the Manshad region of Yazd exhibited the highest concentration of phosphorus (P) and the lowest level of bicarbonate, whereas the Khezrabad region demonstrated elevated levels of calcium (Ca), chloride (Cl), potassium (K), sodium (Na), and magnesium (Mg). Considering the higher altitude and precipitation in Manshad, it can be inferred that an increase in both altitude and precipitation correlates with higher soil phosphorus content and lower bicarbonate levels in this area. Furthermore, compounds such as 1-Octen-3-ol, Octanone-3, α-Terpinolene, and Thymol-methyl ether, which are exclusively found in the Manshad region, exhibited a positive correlation with P and a negative correlation with carbonate. Additionally, the presence of Borneol, solely observed in the Khazarabad area, exhibited a positive correlation with other elements such as Ca, Cl, K, Na, and Mg. Therefore, the presence of these compounds may be attributed to elevated levels of of P, Ca, or K.

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