INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 15, No. 6, p. 40-52, 2019
http://www.innspub.net

RESEARCH PAPER | OPEN ACCESS

Physiological and anatomical responses of *Teucrium polium* L. *growing* under different habitat conditions at North West Coast and South Sinai

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Article published on December 30, 2019

Key words: Soil analysis, Physiological responses, Anatomical responses, Correlation, *Teucrium polium*, Wadi El-Arbaeen, Wadi El-Obayed

Abstract

The objectives of the study were to identify the physiological behavior in soil and plants, to study the anatomical characters as well as the correlations of soil analysis with chemical estimates and amino acids in *T. polium* plants during spring and autumn seasons in Wadi El-Arbaeen at South Sinai and in Wadi El-Obayed at Marsa Matruh, Egypt. The location, sites and depths in soil properties; locations and seasons in plant chemical compositions and amino acids as well as their interactions were showed significant ($P < 0.05$ or 0.01) effects. The pH values were higher than 7 in the two depths at the three streams during the two locations. The values of water content, EC, Cl^-, Ca^{2+}, Na^+ and K^+ in soil at the two depths in three streams in Wadi El-Obayed were higher than in Wadi El-Arbaeen. As for plant chemical compositions, succulence ratio, total protein and Mg^{2+} concentrations in the spring season were higher than in the autumn season under the two studied Wadis. Most concentrations of plant analysis and amino acids concentrations and leaf anatomical characters of *T. solium* in the Wadi El-Arbaeen were higher than in the Wadi El-Obayed. Proline ranks first in accumulation in *T. polium* at the spring and autumn seasons, which contributed about 25% and 26% of the total amino acids during Wadi El-Arbaeen and Wadi El-Obayed, respectively. Anatomical characters in *T. polium* exhibhited significant differentiation under Wadi El-Arbaeen and Wadi El-Obayed locations. Positive correlation coefficients were found between water content, EC, pH, Na^+, Mg^{2+}, Ca^{2+} and Cl^- in the soil and K^+, Na^+, Mg^{2+}, total protein; total carbohydrate and succulence ratio in the plants under the two Wadis. Most soil analysis were showed positive correlated with most amino acids during the two locations.

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Introduction

Sinai Peninsula is considered as one of the major known sources for ethno-medicinal plants in the Arabian deserts (Ahmed *et al.*, 2016). South Sinai contains 472 plant species including 19 Egyptian endemic species, 115 of medicinal interest, and approximately 170 species used in folk medicine (Fayed & Shaltout, 2004). South Sinai and Marsa Matrouh in Egypt are characterized by ecological uniqueness due to their diversity in terrestrial forms, geological structures and climate, which resulted in diversity of plant species, characterized mainly by the dispersal and dominance of shrubs, sub-shrubs and tree paucity.

Teucrium is a genus of perennial plants, the largest of the Labiatae (Lamiaceae) family in the Mediterranean and Middle East regions, which constitute more than 300 species widespread all around the world (Tutin *et al.*, 1972 and Danihelka *et al.*, 2012) about 100 species cosmopolitan, but especially Mediterranean region one of them is *Teucrium polium* L. (*T. polium*) or Jaadah as it is known in Egypt (Boulos, 2002). Plants belonging to the genus *Teucrium* have evolved in nature through natural hybridization and selection, showing substantial variation in terms of their natural habitats, growth characteristics, and aromas (Tutin *et al.*, 1972). *Teucrium* species are rich in phenolic compounds and used for digestive and respiratory disorders and as diuretic, anti-septic, anti-rheumatic, anti-inflammatory, anti-oxidative, anti-microbial, anti-diabetic, hepatoprotective, hypolipidemic and anti-cancer (Jaradat, 2015). In cardiovascular pathology, particularly in the management of CAD risk factors, *T. polium* showed dose-dependent hypotensive, antidiabetic, antioxidant, and anti-inflammatory effects in animal studies (Mohd Nor *et al.*, 2019). A critical taxonomic revision of the genus *Teucrium* (Lamiaceae) in Egypt is presented. It reveals the presence of five species, including two subspecies and four varieties. Two new nomenclatural combinations are proposed: *T. jordanicum* and *T. jordanicum var. sinaicum*. The latter in addition to *T. polium* subsp. *capitatum* are added for the first time to the flora of the country (Fayed *et al.*, 2015).

Drought and salinity are two major environmental factors determining plant productivity and plant distribution (Bray *et al.*, 2000). In semi-arid climatic conditions, desertification is becoming a serious problem, with a progressive reduction of the vegetation cover coupled with rapid soil erosion. Drought resistance is a complex trait involving several interacting properties and there is increasing interest in studying the physiological behavior of plant species in order to identify and understand drought-resistance mechanisms (Martınez, 2005). The ability of plants to accumulate the inorganic ions in high quantities inside their tissues is the most important mechanism to maintain the plant water potential more negative than the external medium in order to maintain the water uptake (Mile *et al.*, 2002).

Chlorophylls and carotenoids are essential pigments of higher plant assimilatory tissues. They are capable of channeling the radiant energy of sunlight into the chemical energy of organic carbon compounds through the process of photosynthesis in the cell (Sims & Gamon, 2002). Amino acids can play different roles in plants; they can act as stress-reducing agents, source of nitrogen and hormone precursors (Maeda & Dudareva, 2012). Proline is probably the most widely distributed osmolyte, and it occurs not only in plants but also in many other organisms (Delauney, 1993). Besides osmotic adjustment other roles have been proposed for proline in osmotically stressed plant tissues: protection of plasma membrane integrity (Mansour *et al.*, 1998), a sink of energy or reducing power (Verbruggen *et al.*, 1996), a source for carbon and nitrogen (Ahmad & Hellebust, 1988).

The objectives of this study were to identify the physiological behavior of *T. polium* in various environments and seasons through the chemical estimates of the plant and the accompanying soil and the work of anatomical sections of the leaves and related it to the environmental factors during spring and autumn seasons in Wadi El-Arbaeen at South Sinai and in Wadi El-Obayed at Marsa Matruh, Egypt.

Materials and methods

Study Area and Plant Material

This study was conducted in 23 March 2016 (spring) and 27 September 2016 (autumn) in the two locations Wadi El-Arbaeen at South Sinai and Wadi El-Obayed at Marsa Matruh in Egypt. The samples of *Teucrium polium* L. (*T. polium*) plants and soil were collected from these the two locations to assess the results seasonally. *T. polium* from *Teucrium* class is an herbaceous, grassy, branchy, 10–35cm height, white cotton plant, which is usually found in the Bayer, rocky and sandy beaches worldwide (Ricci *et al*., 2005).

Climatic Factors

The mean values of the meteorological data of the two regions under study during the period from January to December 2016-2017 are presented in Table 1. The Meteorological data of temperature, relative humidity and rainfall were provided by the Applied Agricultural Meteorological Laboratory of the Desert Research Center, Egypt. During the study period in the two regions Wadi El-Arbaeen and Wadi El-Obayed, the highest decadal temperature was recorded in July (30.21°C and 29.19°C), while the lowest temperature recorded were 15.13°C and 12.30°C in January, respectively. As for relative humidity, the highest values were recorded in June at Wadi El-Arbaeen (80.24%) and in August at Wadi El-Obayed (72.82%) whilst the lowest values were showed in September (53.44% and 43.12%) and February at Wadi El-Arbaeen and Wadi El-Obayed, respectively. The highest values of rainfall were 3.11mm and 24.31mm in December and January during Wadi El-Arbaeen and Wadi El-Obayed, respectively, while the lowest values recorded from July to September during the two studied locations.

Table 1. Meteorological data in the two studied regions during 2016 and 2017 seasons.

Region / Months	Wadi El-Arbaeen			Wadi El-Obayed		
	Temperature	Relative humidity	Rainfall	Temperature	Relative humidity	Rainfall
January	15.13	58.33	2.50	12.30	73.11	24.31
February	17.21	58.23	2.40	16.20	43.12	17.66
March	16.21	56.24	1.90	16.30	49.31	8.29
April	24.34	55.23	1.70	19.90	60.21	7.18
May	25.22	70.21	0.40	23.11	59.21	3.42
June	28.21	80.24	0.30	26.12	61.22	0.00
July	30.21	60.13	0.00	29.19	69.11	0.00
August	28.21	60.91	0.00	28.99	72.82	0.00
September	26.11	53.44	0.00	27.11	69.43	0.00
October	19.14	56.22	0.30	20.12	65.21	5.21
November	16.23	59.41	2.11	17.20	62.19	7.31
December	15.31	60.42	3.11	14.90	55.91	12.11

Soil Analysis

Soil samples were collected as a profile at the two depths 0 - 20cm and 20 - 40cm during the spring and autumn seasons in Wadi El-Arbaeen and Wadi El-Obayed. Three replicates were taken from each stands and carried to the laboratory in plastic bags. The physical and chemical properties of soil including pH of the soil extract, electrical conductivity (EC), and mineral content (Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-) were estimated using a saturation paste (Tuzuner, 1990).

Plant Analysis

1- Physiological Studies

The succulence ratio of the shoot systems of both plants was calculated as the initial fresh weight/dry weight ratio (fr.wt./dry wt.) according to Dehan & Tal (1978). Chlorophyll (a and b) carotenoids were determined quantitatively as described by Metzner *et al*. (1965). Total protein was calculated by multiplying the total nitrogen% (modified Kieldahle) by 6.25 (Tripath *et al*., 1971). Total carbohydrate content of shoot system was extracted and estimated according to Chaplin and Kennedy (1994). Crude fiber of shoot system was determined in ashed-plant material by acidic method according to Askar & Treptow (1993). The contents of different major soluble cations (Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-) in plant extracts were determined as previously mentioned for soil extract. Total amino acids content were estimated using Clait Amino Acid Analyzer SW (Pellet & Young, 1980) at

Central Laboratories, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

Anatomical Studies

A random sample of 10 leaves from the top of each of the investigated plant growing under Wadi El-Arbaeen and Wadi El-Obayed locations were collected. The leaves were thoroughly washed and immediately immersed in FAA fixative solution (37% Formalin, glacial acetic acid and 50% ethanol in ratio of 1:1:18, respectively) for 48h; then they were stored in 70% (v/v) ethanol until use. Samples were embedded in wax and cross sectioned with a manual rotary microtome. The sections are finally mounted on slides using Canada balsam. A hot plate at 40^0C was used to dry the slides. The slice slides were examined using image analyzer (inverted microscope Zeiss axiovert 25) and light microscope and photographed with an Olympus BX 51 digital camera.

Statistical Analysis

The data collected were evaluated using analysis of variance and the significant differences were identified using the Fisher's least significance difference (LSD) method when the ANOVA suggested a significant difference at $P \leq 0.05$ and $P \leq 0.01$ (Steel & Torrie, 1980). The PCA was performed for better understanding of the relationships of soil properties with chemical properties and amino acids in plants. Statistical analysis was performed using SPSS 20.0 program.

Results and discussions

Soil Analysis

Mechanical and chemical properties of soil at the first (0-20cm) and the second (20-40cm) depths from up, mid and down streams in Wadi El-Arbaeen and Wadi El-Obayed are presented in Table 2. The two locations (L), the three sites (S), the two depths (D) as well as the first order interactions (L x S, L x D and S x D) and the second order interaction (L x S x D) were showed significant ($P < 0.05$) or highly significant ($P < 0.01$) effects for all mechanical and chemical properties, except the locations and LxD interaction for pH, the SxD and LxSxD interactions for EC and the interaction of LxSxD for clay percentage, which were showed insignificant effects. Sharaf *et al.* (2013) reported that the means of soil parameters show that there is a highly significant variation in soil physical properties, EC, water content, sodium, potassium, calcium, magnesium and chloride and moderate significance for pH in *T. polium* and some desert plants in South Sinai, Egypt. The soil show significant seasonal changes in the pH, EC values, and mineral compositions (Furtana *et al.*, 2013).

The highest values of coarse sand percentage had recorded in the second depth at the mid- and up-streams during the Wadi El-Arbaeen and Wadi El-Obayed, respectively. While, the highest values of fine sand percentage were found in the first and second depths at down- and mid-streams in Wadi El-Arbaeen and Wadi El-Obayed, respectively. During the second and first depths at down- and up-streams in Wadi El-Arbaeen and Wadi El-Obayed, the silt percentage had recorded the highest values, respectively. As for clay percentage, the highest values were found in the first depth at the down-stream under the two studied habitats. Among the mechanical properties soil, the coarse and fine sand percentages were revealed the highest values in the three streams in the Wadi El-Arbaeen and Wadi El-Obayed, respectively.

The water content values of the two depths at three streams under the two studied locations were higher in the spring season than in the autumn season. The highest values of water content and EC at the two depths had recorded during the mid- and down-streams in Wadi El-Arbaeen and Wadi El-Obayed, respectively. The soil depth is an important factor restricting the type of vegetation in the Egyptian desert wadis. A thin soil will be moister during the rainy season, but will be dried by the approach of the dry season, here ephemeral vegetation appear. Deep soil allows the storage of some water in the subsoil (El-Khatib, 1993). Soil moisture content was lower in the surface compared to other depths of all samples during dry season (Abdel Kawy, 2015). This may be attributed to the fact that surface layer of the desert soil is subjected to evaporation (Zahran, 1989). While, the maximum values of pH were showed in the up-and mid-streams during Wadi El-Arbaeen and Wadi El-Obayed, respectively. The pH values were higher than 7 in the two depths at the three streams during the two wadis, thus the two locations tended to be slightly alkaline. On the other hand, the values of other chemical properties (Cl^-, Ca^{2+}, Mg^{2+}, Na^+ and K^+) at the two depths in up-stream were higher than in the other streams during the two studied wadis. The values of water content at the two seasons and Mg^{2+} were higher in the second depth than in the first depth, while the opposite by pH, EC, Cl^-, Na^+ and K^+ were found in the three streams in the two locations. As for Ca^{2+} in the three streams, the highest values were showed at the second and first depths in Wadi El-Arbaeen and Wadi El-Obayed, respectively. This agrees with the earlier findings of El-Absy (2011) and Kasim *et al.* (2014) in *Artemisia judaica* and *Achillea fragrantissima* during the wet and dry season in Wadi El-Arbaeen.

The relationship between soil features and vegetation were found by Liangpeng *et al.* (2007); Morsy (2007); Youssef & Al-Fredan (2008), where the soil characteristics are the main factors influencing plant growth and the distribution of plant communities. The decreases of Mg^{+2} and K^+ in some cases may be antagonistic with another elements, the uptake of an element by plant may not being directly related to the absolute content of this element in the soil but rather to the relative proportion of that element to other elements in the soil solution (Charan *et al.*, 2013). In addition, the abundance of Ca^{+2} is linked to the presence of carbonates that add soluble Ca^{+2} to the exchangeable pool. Salama *et al.* (2016) stated that, the estimated soluble salts in the soil were dominated by Ca^{+2} and Cl^-. The seasonal changes in the osmolyte concentrations of plants were found to be dependent on soil properties such as EC, anions, and cations (Furtana *et al.*, 2013).

Table 2. Mechanical and chemical properties of soil at 0-20 and 20-40cm depths from up, mid and down streams in Wadi El-Arbaeen and Wadi El-Obayed.

Locations (L)	Sites (S)	Depth (D)	Mechanical properties (%)						Chemical properties						
			Coarse sand (1-0.5)	Fine sand (0.25-0.1)	Silt (0.05-0.002)	Clay <(0.002)	Water content: Spring	Water content: Autumn	pH	EC (dS/m)	Cl^- (meq/L)	Ca^{2+} (meq/L)	Mg^{2+} (meq/L)	Na^+ (meq/L)	K^+ (meq/L)
Wadi El-Arbaeen	Up	0-20	14.31	4.12	1.11	2.12	3.12	2.03	7.81	0.99	7.21	4.31	111.02	8.44	0.89
		20-40	12.40	7.11	1.92	1.12	4.12	3.20	7.72	0.89	6.20	5.11	111.34	7.22	0.77
	Mid	0-20	19.21	8.10	2.31	3.12	6.13	4.41	7.31	7.22	4.32	2.11	80.12	4.21	0.71
		20-40	20.20	9.12	3.13	2.03	8.30	6.13	7.22	6.71	3.41	4.22	90.23	3.23	0.45
	Down	0-20	17.11	10.11	11.12	9.40	5.21	3.12	7.21	6.41	0.91	2.11	100.11	4.01	0.51
		20-40	14.1	10.01	14.12	8.14	6.11	4.12	7.11	5.82	1.11	3.12	110.11	1.94	0.31
Wadi El-Obayed	Up	0-20	1.40	64.80	4.80	2.20	6.80	5.30	7.30	70.00	15.80	16.61	18.91	20.21	30.22
		20-40	3.50	60.00	2.70	1.80	7.30	6.80	7.10	60.00	7.10	7.91	22.41	13.67	6.72
	Mid	0-20	0.90	70.10	2.90	1.10	7.10	6.10	7.80	50.00	13.20	15.21	16.24	17.11	20.34
		20-40	2.30	89.10	0.90	0.80	9.10	7.20	7.60	60.00	6.20	7.23	18.61	10.11	4.31
	Down	0-20	1.30	63.20	3.80	2.10	9.20	8.20	7.20	68.00	14.90	13.22	17.11	19.22	18.22
		20-40	2.90	58.20	2.10	1.70	9.90	8.90	7.10	77.00	6.90	6.21	20.22	12.22	5.23
L.S.D.		L	**	**	**	**	**	**	ns	**	**	**	**	**	**
		S	**	**	**	**	**	**	**	**	**	**	**	**	**
		LxS	**	**	**	**	**	**	**	**	**	**	**	**	**
		D	**	**	**	**	**	**	*	**	**	**	**	**	**
		LxD	**	**	**	**	**	**	ns	**	**	**	**	**	**
		SxD	**	**	**	**	**	**	**	ns	**	**	**	**	**
		LxSxD	**	**	**	ns	**	**	**	ns	**	**	**	**	**

Plant Analysis

Physiological responses

According to statistical analysis, the locations (L), seasons (S) and LxS interaction were showed highly significant effect ($P<0.01$) for all chemical compositions in *T. polium*, except LxS interaction had significant effects ($P<0.05$) for succulence ratio and Mg^{2+} (Table 3). The seasons source had the highest effect for all studied chemical compositions in *T. polium*, followed by the locations, while the role of interaction between locations and seasons was the lowest effect. Chlorophyll a, Chlorophyll b and carotenoids contents were significantly different between years, seasons and between plants within years and seasons (Uvalle Sauceda *et al.*, 2008). The seasons, wadis and their interaction showed significant differences for succulence ratio, crude fiber, total available carbohydrates and total nitrogen, mineral elements (El-Absy, 2011 and Kasim *et al.*, 2014), chlorophyll a, chlorophyll b and carotenoids

(El-Absy, 2011) in *Artemisia judaica* and *Achillea fragrantissima* during the wet and dry seasons in Wadi El-Sheikh and Wadi El- Arbaeen.

The concentrations of succulence ratio, total protein and Mg^{2+} in *T. polium* were higher in the spring season than in the autumn season under the two studied Wadis. On the other hand, the concentrations of chlorophyll a, chlorophyll b, carotenoids, K^+ and Ca^{2+} were higher in the autumn season than in the spring season during the two studied habitats. The highest concentrations of total carbohydrate and Na^+ were recorded in the spring season at Wadi El-Arbaeen and in the autumn season at Wadi El-Obayed, while opposite had found for crude fiber concentration in *T. polium*. As for the two studied locations, the data show that the concentrations of succulence ratio, chlorophyll b, carotenoids, total protein, total carbohydrate, crude fiber, Na^+ and K^+ in the Wadi El-Arbaeen were higher than in the Wadi El-Obayed. Whilst, the Wadi El-Obayed were higher than the Wadi El-Arbaeen for chlorophyll a and Ca^{2+} and Mg^{2+} in *T. polium*. Uvalle Sauceda *et al.* (2008) mentioned that during fall and winter chlorophyll a and carotenoids were higher than summer or spring, while, during spring, summer and fall chlorophyll b resulted higher than in winter. In this study, chlorophylls in all plants were higher than carotenoids in *T. polium*. These finding are in agreement with Sims & Gamon (2002). The plants from different times exhibit different abilities to accumulate inorganic ions, soluble carbohydrates, and quaternary ammonium compounds. In the autumn, a decrease is observed in the concentration of Ca^{2+}, Mg^{2+} and total amount of soluble carbohydrates, while an increase is shown in the concentration of K^+. These compounds might have been accumulated in the cytoplasm by the plants to contribute to the osmotic adjustment (Furtana *et al.*, 2013). Exclusion of excess Na^+ from the cytoplasm and the accumulation in the vacuole represents one of the adaptive mechanisms during salt stress (Bartels & Ramanjulu, 2005). The involvement of Ca^{2+} signaling in response to osmotic and ionic stress is well documented (Bartels & Ramanjulu, 2005). Some previous studies on halophytes have shown that Na^+, K^+, and Cl^- accumulate to achieve osmotic adjustment by lowering solute potential and water potential in plant tissues (Yasseen & Abu-Al-Basal, 2008). Yearly and seasonal variations in plant pigments (chlorophyll a, chlorophyll b and carotenoids) might have been related to seasonal water deficits, excessive irradiance levels during summer and extreme low temperature in winter that could have affected leaf development and senescence (Uvalle Sauceda *et al.*, 2008). The plants growing in semi-arid environments have various physiological and morphological characteristics to acclimate to stressful environments, such as drought and strong light conditions (Sharaf *et al.*, 2013).

Table 3. Chemical compositions concentrations of *T. polium* in spring and autumn seasons during Wadi El-Arbaeen and Wadi El-Obayed.

Locations (L)	Wadi El-Arbaeen			Wadi El-Obayed			LSD		
Seasons (S) / Parameters	Spring	Autumn	Mean	Spring	Autumn	Mean	L	S	L x S
Succulence ratio (fr.wt./dry wt.)	1.73	1.62	1.68	1.67	1.55	1.61	**	**	*
Chlorophyll a (mg/100g fr.wt.)	3.06	3.90	3.48	2.73	4.70	3.72	**	**	**
Chlorophyll b (mg/100g fr.wt.)	1.35	5.32	3.34	1.47	2.25	1.86	**	**	**
Carotenoids (mg/100g fr.wt.)	1.84	3.49	2.67	1.91	2.19	2.05	**	**	**
Total protein (mg/100g dry.wt.)	7.12	7.08	7.10	6.87	6.85	6.86	**	**	**
T. carbohydrate (mg/100g dry.wt.)	27.55	26.88	27.22	24.18	26.58	25.38	**	**	**
Crude fiber (mg/100g dry.wt.)	38.15	41.15	39.65	40.30	36.65	38.48	**	**	**
Na^+ (mg/100g dry.wt.)	1.68	1.66	1.67	1.44	1.72	1.58	**	**	**
K^+ (mg/100g dry.wt.)	1.35	1.56	1.46	1.13	1.42	1.28	**	**	**
Ca^{2+} (mg/100g dry.wt.)	148.00	155.00	151.50	152.00	165.00	158.50	**	**	**
Mg^{2+} (mg/100g dry.wt.)	128.00	113.00	120.50	132.00	120.00	126.00	**	**	*

The values of amino acids concentrations in *T. polium* at the spring and autumn seasons during El-Arbaeen and Wadi El-Obayed are presented in Table 4. Statistical analysis for data revealed that amino acids (A), locations (L), seasons (S), the first order interactions (L x S, A x L and A x S) and the second order interaction (A x L x S) had highly significant effect ($P < 0.01$). The amino acids and the second order interaction had the highest dominant effect, followed by A x S and then A x L as sub dominant. The concentrations of the amino acids i.e., histidine, leucine and proline in Wadi El-Arbaeen and the amino acids i.e., aspartic acid, glycine, alanine, methionine, phenylalanine, lysine and tryptophan in Wadi El-Obayed during the spring season were higher than the autumn season for *T. polium*. Based on the two studied Wadis, most amino acids concentrations of *T. polium* in the Wadi El-Arbaeen were higher than in the Wadi El-Obayed. The concentrations of eleven out of eighteen amino acids in the spring season were higher than the autumn season for *Zilla spinosa* and *Peganum harmala* in Wadi El-Arbaeen (Kamel & El-Absy, 2018). Proline ranks first in accumulation in *T. polium* at the spring and autumn seasons, which contributed about 25% and 26% of the total amino acids during Wadi El-Arbaeen and Wadi El-Obayed, respectively, followed by leucine, aspartic acid, glutamic acid, isoleucine and alanine with a different arrangement at the spring and autumn seasons under in two different habitats. Proline concentration in winter and summer plants of *T. polium* showed that values are significantly higher in winter plants (Lianopoulou *et al.*, 2014). While, the three amino acids i.e., methionine, cysteine and histidine had the lowest accumulation compared to the other amino acids in *T. polium* at the spring and autumn seasons in the two studied habitats. The results obtained in present study agree with previous studies by Movafeghia *et al.* (2009), Ahmed *et al.* (2013) and Kamel & El-Absy (2018). Generally, amino acids content in *T. polium* were differed from one locations to another at the spring and autumn seasons under the two locations. These results agrees with the earlier findings of El-Absy (2011) and Kasim *et al.* (2014) in *Artemisia judaica* and *Achillea fragrantissima* as well as Kamel & El-Absy (2018) in *Zilla spinosa* and *Peganum harmala* during the wet and dry seasons in Wadi El-Arbaeen.

The content of proline shows wide variations in the different locations (Youssef *et al.*, 2003). Such variations may be dependent on various factors, such as the environmental factors of habitats and energy constraints (Furtana *et al.*, 2013). In the autumn, a decrease is observed in the proline content in *Limonium anatolicum* and *Limonium lilacinum*, while an increase is seen in Limonium iconicum (Furtana *et al.*, 2013). Glycine betaine is thought to protect the plant by maintaining the water balance between the plant cell and the environment and by stabilizing macromolecules (Bartels & Ramanjulu, 2005). Among the characteristic responses to water stress is the accumulation of proline. Greater amounts of proline in wilted leaves, compared to non-wilted leaves, have been reported in *T. polium* and some desert plants in South Sinai, Egypt (Sharaf *et al.*, 2013) and other plants (Barnett & Naylor, 1966; Routley, 1966 and Stewart, 1972). Therefore suggests that the phenomenon is a general one. Proline have more intimate association with survival adaptability of plants at high altitudes. This might be interpreted on the bases of subjection of higher altitudes to more stressful conditions (Sharaf *et al.*, 2013) as well as it is plays an important role in the stimulation of root elongation at low water potentials (Yamada *et al.*, 2005).

Anatomical Studies

Leaves are sessile and simple, oblong-linear, spatulate, crenate margin and obtuse apex, opposite blade, shorter than flowers 10–15mm long , 3–5mm wide, crenulated at least in upper half, strongly revolute (Bukhari *et al.*, 2015). In *T. polium*, both surfaces are covered by the epidermis. The upper and the lower epidermises consist of a single layer rectangular to ovoidal cells, and is surrounded by a cuticle layer (Dinç *et al.*, 2011). The data of different anatomical characters in leaves of *T. polium* are presented in Table 5. The data analysis of the means for anatomical characters obtained from *T. polium* during Wadi El-Arbaeen and Wadi El-Obayed locations show significant differentiation for *T. polium* plants under the two locations (Fig. 1 and Fig. 2, respectively). Wadi El-Arbaeen location was largely higher than Wadi El-Obayed location for all leaf anatomical characters in *T. polium* except sector width at right and north of leaf.

Table 4. Amino acids values (mg/g) in the spring and autumn seasons during Wadi El-Arbaeen and Wadi El-Obayed for *T. polium*.

Locations (L)	Wadi El-Arbaeen			Wadi El-Obayed		
Seasons (S) / Amino acids (A)	Spring	Autumn	Mean	Spring	Autumn	Mean
Aspartic acid	8.20	9.10	8.65	11.40	7.33	9.37
Glutamic acid	6.80	7.33	7.07	8.60	9.10	8.85
Serine	1.72	2.70	2.21	2.30	2.60	2.45
Histidine	1.61	0.78	1.20	1.05	1.10	1.08
Glycine	1.76	4.16	2.96	3.08	1.80	2.44
Threonine	2.32	3.40	2.86	1.75	2.50	2.13
Arginine	6.11	6.20	6.16	6.10	3.99	5.05
Alanine	6.15	6.60	6.38	7.11	5.20	6.16
Tyrosine	2.40	4.10	3.25	2.90	3.10	3.00
Cysteine	0.82	0.91	0.87	0.77	1.10	0.94
Valine	3.10	6.30	4.70	3.30	5.10	4.20
Methionine	0.84	0.86	0.85	0.93	0.80	0.87
Phenylalanine	5.20	5.40	5.30	6.12	5.30	5.71
Isoleucine	6.18	7.10	6.64	5.20	8.10	6.65
Leucine	9.70	8.12	8.91	8.20	8.60	8.40
Lysine	4.20	5.10	4.65	3.82	3.73	3.78
Tryptophan	1.90	2.80	2.35	2.10	1.39	1.75
Proline	30.99	19.04	25.02	24.27	29.16	26.72
	L	**	L x S	**	L x S x A	**
L.S.D.	S	**	L x A	**	CV%	1.01
	A	**	S x A	**		

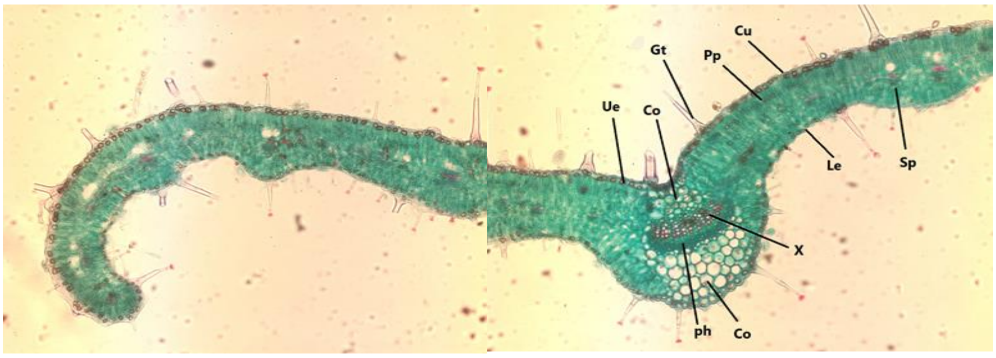

Fig. 1. Cross-sections of *T. polium* leaves from Wadi El-Arbaeen. Cu, cuticle; ue, upper epidermis; co, collenchyma; x, xylem; ph, phloem; pp, palisade parenchyma; sp, spongy parenchyma; le, lower epidermis; gt, glandular trichome.

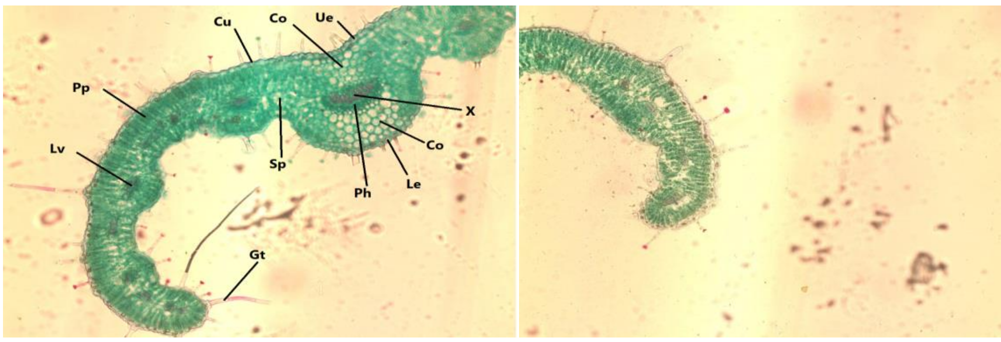

Fig. 2. Cross-sections of *T. polium* leaves from Wadi El-Obayed. Cu, cuticle; ue, upper epidermis; co, collenchyma; x, xylem; ph, phloem; pp, palisade parenchyma; sp, spongy parenchyma; le, lower epidermis; gt, glandular trichome;lv, laterale vascular bundle.

This result indicated that the environmental factors has a much greater influence on anatomical characters in *T. polium*. Dinç *et al.* (2011) mentioned that, the upper epidermis cells are larger than the lower ones. They added the upper epidermis cell walls are fairly thicker than the lower ones, both epidermises are covered with a cuticle and the upper cuticle layer is about as thick as the lower one.

Table 5. Anatomical characters of leaf in *T. polium* during Wadi El-Arbaeen and Wadi El-Obayed locations.

Characters \ Locations		Wadi El-Arbaeen			Wadi El-Obayed		
		Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE
Length of plant sector (µm)		4884.37	5087.92	4986.73±58.76	2921.34	3021.89	2971.71±29.03
Width of plant sector (µm)	Right	122.02	132.02	127.02±2.89	213.51	229.51	221.51±4.62
	Medium	351.28	371.28	361.28±5.77	329.22	347.22	338.22±5.20
	North	162.53	176.53	169.53±4.04	174.47	186.47	180.47±3.46
Interveinal distance of plant cell (µm)		296.62	349.09	316.62±16.38	72.19	138.32	112.84±20.54
Size of upper epidermal cell (µm)		253.58	264.92	260.19±3.41	78.87	85.31	82.13±186
Size of lower epidermal cell (µm)		360.31	396.42	382.81±11.33	46.11	55.19	51.99±2.95
Thickness of upper cuticle layer (µm)		13.19	23.14	16.99±3.10	11.83	12.83	12.32±0.29
Thickness of lower cuticle layer (µm)		18.30	22.11	20.37±1.11	10.48	13.64	11.80±0.95
Size of palisade tissue (µm)		332.31	391.55	365.91±17.56	224.42	282.31	254.78±16.77

SE: Statndard Error.

Correlation Coefficient Analysis

The principal component analysis (PCA) was performed to better understand the soil properties relationships with chemical compositions and amino acids in *T. polium* plants during the two studied locations. Relationships between soil properties and chemical compositions in plants under Wadi El-Arbaeen and Wadi El-Obayed are shown in Fig. 3. During Wadi El-Arbaeen location, Mg^{2+} and Ca^{2+} in soil had positive correlation with K^+, Na^+ and total protein in plants, while soil properties (Na^+, Cl^-, K^+ and pH) had positively correlated with total carbohydrate, Mg^{2+} and succulence ratio, however water content and EC was positive correlation with other plant chemical compositions. As for Wadi El-Obayed location, pH with Mg^{2+}, succulence ratio and crude fiber; soil properties (Na^+, Mg^{2+}, Ca^{2+}, Cl^- and K^+) with total protein; water content and EC with other plant chemical compositions were positive correlation.

According to biplot analysis in Fig. 4, the soil properties viz., pH, Cl^-, K^+, Na^+ and Ca^{2+} with the amino acids i.e., histidine, methionine, leucine and proline; Mg^{2+} with histidine, leucine and proline as well as water content and EC with all amino acids except previously amino acids were exhibited positive correlation under Wadi El-Arbaeen site. In respect to Wadi El-Obayed site (Fig. 2), the positive correlation among Mg^{2+} and amino acids (serine, tyrosine and leucine); among soil properties (pH, Cl^-, K^+, Na^+ and Ca^{2+}) and amino acids (aspartic, histidine, arginine, alanine, methionine, phenylalanine, lysine and tryptophan) as well as among soil properties (water content and EC) and amino acids (glycine, serine, threonine, cysteine, valine, isoleucine, leucine and proline). Kamel & El-Absy (2018) studied the correlation between soil analysis and amino acids in *Zilla spinosa* and *Peganum harmala* in South Sinai, Egypt, and they mentioned that the amino acids i.e. aspartic acid, cysteine, methionine, phenylalanine, tryptophan and proline were positively correlated with pH, K^+, Na^+, Ca^{2+}, Cl^-, EC and Mg^{2+} in Wadi El-Arbaeen. They added, the amino acids aspartic acid, methionine and isoleucine with pH, K^+, Na^+, water content (WC) and EC, as well as the amino acid tryptophan with Cl^- and Ca^{2+} showed positive correlation in Wadi Ghrandal. Soil pH had negative correlation with glucosamine (Prommer *et al.*, 2014) and with amino acids (Cao *et al.*, 2016). While, soil pH had positive correlation with muramic acid, L-alanine, D-alanine, and mDAP (Prommer *et al.*, 2014) and with D-alanine (Padan *et al.*, 2005).

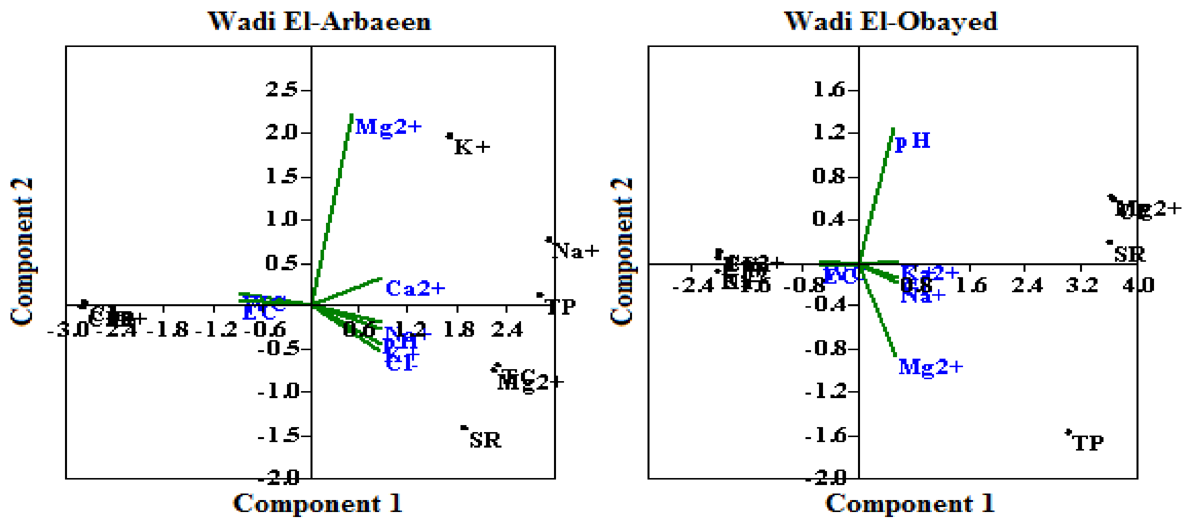

Fig. 3. Biplot diagram based on first two principal component axes of soil properties and plant chemical properties in *T. polium* during Wadi El-Arbaeen and Wadi El-Obayed. WC: water content; EC: electrical conductivity.

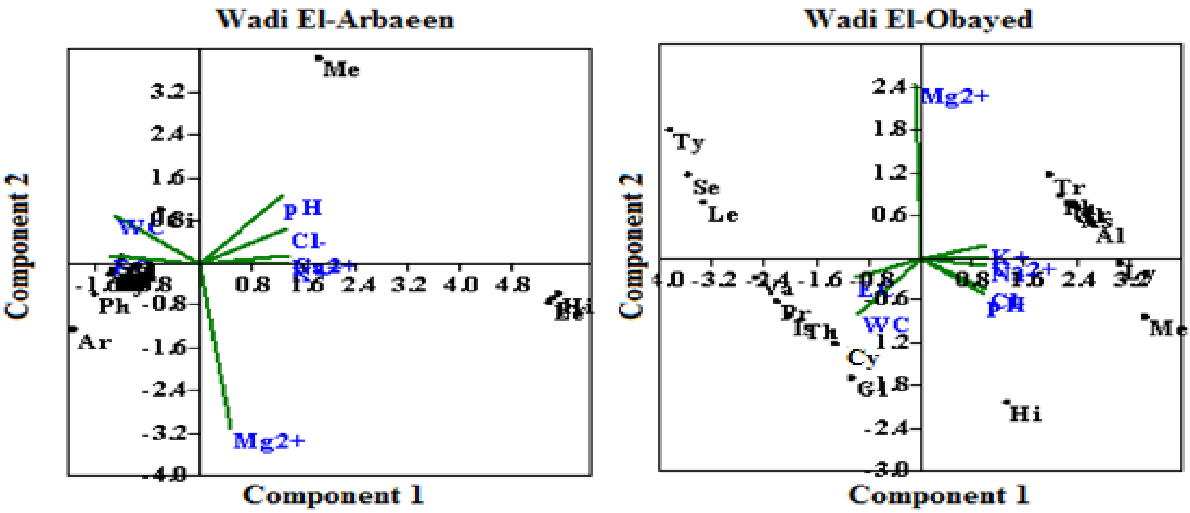

Fig. 4. Biplot diagram based on first two principal component axes of soil properties and amino acids in *T. polium* during Wadi El-Arbaeen and Wadi El-Obayed. As: aspartic; Gl: glutamic; Se: serine; Hi: histidine; Gl: glycine; Th: threonine ; Ar: arginine; Al: alanine; Ty: tyrosine; Cy: cysteine; Va: valine; Me: methionine; Ph: phenylalanine; Is: isoleucine; Le: leucine; Ly: lysine; Tr: tryptophan ; Pr: proline.

Conclusion

Most soil properties, plant chemical compositions, amino acids and leaf anatomical characters of *T. solium* in the Wadi El-Arbaeen were higher than in the Wadi El-Obayed. Most soil properties were showed positive correlated with most chemical compositions and amino acids in plants during the two locations. It seemed that the *T. polium* plants in Wadi El-Arbaeen and Wadi El-Obayed locations was adapted with drought conditions by mobilizing physiological, anatomical and biochemical defence mechanisms.

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