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Comparative study of anatomical characteristics and chemical composition of essential oils of *Deverra tortuosa* (Desf.) DC. grown under different environmental conditions

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ABSTRACT

Environmental conditions are known to cause many physiological and biochemical changes in plant. This study aimed to investigate the variations in the anatomical features, phytohormones and volatile components, obtained by headspace gas of the aerial parts of *Deverra tortuosa* under the influence of different environmental conditions. The extracted phytohormones were analyzed by HPLC and the essential oils of plant samples were analyzed by using headspace gas chromatography–mass spectrometry (GC-MS). The concentration of growth promoting hormones, gibberellic, indole acetic acid and zeatin reached the maximum values of (6.75, 2.57 and 3.13 µg/g, respectively) in Sidi Barrani sample. The data of headspace GC-MS analysis indicated that the chemical composition of the essential oils of *Deverra tortuosa* grown in different geographic locations has shown a wide variation in the main constituents and the percentage of the major component, sabinene. Its percentages were 59.22, 52.73 and 80.44 % in Sidi Barrani, El-Alamein and Al-Arish samples, respectively. The volatile oils of Sidi Barrani ecotype contained remarkable compounds, these compounds were hexane, 2, 4-dimethyl-, perilla alcohol and camphenol. Moreover, monoterpenes such as l-limonene and cis-sabinene hydrate were detected only in Al-Arish ecotype sample. Whereas El-Alamein ecotype was characterized by the presence of other 13 monoterpene compounds in fairly good amount. The presence of high concentration of phytotoxic monoterpene, α-pinene (28.88%), β-myrcene (9.49%) and o-cymene (7.13%) in the oil sample of Sidi Barrani, Al-Arish and El-Alamein, respectively, might be an adaptive response to the low level of soil nutrients to decrease the competition by other plants for soil nutrients. The results of the principal component analysis (PCA) with environmental data indicated a strong relationship among the concentrations of photosynthetic pigments, phytohormones, as well as the main constituents of the essential oils of three ecotypes of *Deverra tortuosa* and their geographical origin. The results of this study provided preliminary evidence of the presence of three chemotypes of *Deverra tortuosa* and clarified how far the environmental conditions could affect the anatomical feature and chemical composition of *D. tortuosa* and the quality of its essential oils.

Keywords: Anatomy, *Deverra tortuosa*, essential oils, headspace, phytohormone

1. Introduction

Environmental conditions and Edaphic factors are capable of changing the anatomical characters (Özörgücü *et al*., 1991), the chemical composition of plant and its phytochemical activity (Jimoh *et al*., 2019). Nature of soil (pH, minerals concentration), growing site (altitude, latitude), light, and precipitation are generally related to the environmental factors, that might exert qualitative and quantitative changes in essential oils (Barra, 2009). It was reported that the type of soil and its mineral concentration affects the growth of plant and the production of secondary metabolites and have a direct relationship with vitamins and phenolic compounds in plants (Tsao *et al*., 2006).

The variations in the production of certain classes of secondary metabolites, such as essential

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oils, under the influence of different environmental and geographical conditions, can be used in distinguishing between different chemical patterns of the plants of the same species, and to differentiate between their ecotypes or chemotypes. Also, they may be regarded as chemical markers for plant sample of a specific geographical region, season and /or phenological phase (Telascrea *et al.,* 2007; Jones *et al.,* 2013; Vilela *et al.,* 2013). Essential oils are highly variable mixtures contain terpenes as a predominant constituent and phenyl propane, they may contain moderate amount of hydrocarbons or sulfur compounds (Barra, 2009). Terpenes possess acyclic or cyclic structures. They are classified into monoterpenes, sesquiterpenes, diterpenes, tetraterpenes and polyterpenes, based on the number of isoprene structure (Zwenger and Basu, 2008; Degenhardt and Kollner, 2009; Garcia *et al*., 2008). The production and the quality of essential oils depend on the external environmental conditions and are indicator to the environmental adaptability of the plants (Stević *et al*., 2011) as they can play an important role in the interaction between plants and environment and help the plant to adapt to the stress conditions (Abu Darwish and Abu-Dieyeh, 2009; Abu-Darwish, 2009).

The genus *Deverra* belongs to the family Apiaceae, which includes 24 genera and 49 species in the wild flora of Egypt (subfamilies Apioideae and Saniculoideae) sensu (Drude, 1898; Boulos, 2000). Plants of family Apiaceae are generally known to be rich in essential oils, that have antimicrobial activity against bacteria and fungi (Al-Gabya and Allam, 2000). The genus *Deverra* has two species, *D. tortuosa* and *D. triradiata*. *Deverra tortuosa* is known in Arabic as "Guezzah". It is a strong glabrous perennial shrub, xerophytic and salt tolerant plant. So, it is growing naturally in almost all the phytogeographical regions of Egypt especially desert wadis and sandy and stony plains, and widely distributed in Tunisia, Libya, Egypt, Palestine, and Saudi Arabia. Therefore, it can be used as a fodder in dry lands during summer due to its high palatability by livestock, especially camels (Boulos, 2000). The species of *Deverra* are used in traditional medicine to treat asthma, fevers, hepatitis, rheumatism (Vérité *et al*., 2004) and hypertension (El-Mokasabi, 2014).

Previous studies on *Deverra tortuosa* indicated that the methanolic extracts of *Deverra* was found to have high scavenging ability and antioxidant activity under stress conditions (El-Lamey, 2015), also, the extracted essential oils by hydrodistillation method, have a significant antimicrobial activity against the Gram-positive bacteria and very potent activity against *Streptococcus pyogenes* (Mighri *et al*., 2015).But there is no report about the analysis of volatile composition of *Deverra tortuosa* by headspace method. It was reported that the volatile composition of the medicinal plant can be determined exactly by headspace GC-MS analysis and suggested the use of this method of analysis in analytical control of herbal crude drugs (Al-Fatimi, 2018).

This study aimed to investigate the variation in the anatomical features, phytohormonal concentration and volatile components obtained by headspace gas of the aerial parts of *Deverra tortuosa* under the influence of different environmental conditions.

2. Materials and methods

2.1. Plant materials

The aerial parts of *Deverra tortuosa* plant were collected from three habitats in February 2020. These habitats are located at Sidi Barrani Desert, Marsa Matruh (between Latitudes 31°14'57.2"N and Longitudes 25°40'19.6"E), Wadi Al Arish at North Sinai (between30°39'56" N and 34°9'47" E), and Wadi El Natrun – El Alamein Road (between Latitudes 30°43'35.2"N and Longitudes 29°26'26.8"E). The plant was identified and authenticated in Desert Research Center Herbarium.

2.2. Ecological studies

2.2.1. The climate data

The climate data consist of rainfall rate and average temperature of the studied habitats, provided by Applied Agricultural Meteorological Laboratory.

2.2.2. Soil analysis

For soil analysis, soil samples were collected from the soil supporting the investigated plant at 3 random points at 0-30cm depth.

2.2.2.1. Soil physical properties

Soil texture (Granulometric analysis) was determined through mechanical analysis by the sieve method (Jackson, 1967).

2.2.2.2. Soil chemical analysis

The electrical conductivity (EC) and pH for each sample were determined as a 1:2.5 dilution in deionized water (Page, 1987). The concentrations of sodium and potassium in the soil solution were determined by using flame photometer (Jenway, PFP-7) and the concentration of chloride (Cl) was determined by titrating the soil solution against silver nitrate (0.5N) and using 1% potassium chromate as an indicator (Jackson ,1967). The concentrations of magnesium (Mg) and calcium (Ca) were determined by titration with ethylene diamine tetra-acetic acid (EDTA) according to the method of (Rowell, 1994). However, the concentrations of carbonate (CO_3) and bicarbonate ions (HCO_3) were determined by titration, using 0.1N HCl and methyl orange as an indicator (Rowell, 1994).

2.3. Anatomical examination

Fresh samples of *Deverra tortuosa*, collected from 3 different localities were kept in Ethyl Alcohol solution to fix and prepare them for anatomical studies. From each locality, five samples were sectioned by using microtome according to Paraffin Sectioning Method (Bani *et al*., 2011; Mavi *et al*., 2011). The staining slices were examined under Leica light microscope model DM-500, the images were obtained by using digital camera Leica ICC 50 HD with LAS E7 software version 2.1.0 2012.

2.4. Determination of photosynthetic pigments

The concentrations of chlorophyll-a (Chl. a), chlorophyll-b (Chl. b) and carotenoids were determined by spectrophotometric method (Sumanta *et al*., 2014). Half gram of fresh plant leaf sample was homogenized in tissue homogenizer with 10 ml of 80% Acetone. The homogenate was centrifuge at 10,000 rpm for 15 min at 4^0C and 0.5 ml of supernatant was mixed with 4.5ml of solvent. Then, the absorbance was read at 663, 644 and 452.5 nm (using UV/VIS spectrophotometer ChromTech CT-2400). The concentration of Chlorophyll-a, Chlorophyll-b and Carotenoids were calculated according to the following equations:

Chl. a=12.25A663.2 – 279A646.8 …………………………………………………………….(1)
Chl. b=21.5A646.8 – 5.1A663.2……………………………………………………………….(2)
C x+c= (1000A470 – 1.82Ca –85.02Cb)/198………………………………………………….(3)

Where: A = Absorbance, Chl.a = chlorophyll a, Chl. b = chlorophyll b, C x+c = carotenoids) and the results were expressed as (mg/100g FW).

2.5. Determination of phytohormones

The plant hormones: gibberellic acid (GA_3), indole acetic acid (IAA), abscisic acid (ABA) and Zeatin were determined by reversed phase high performance liquid chromatography (HPLC) (Kelen *et al*., 2004).

2.5.1. Extraction procedure

Ten grams of fresh plant sample was homogenized in 70% methanol, then stirred at 4°C overnight. The extract was filtered and evaporated under vacuum. The aqueous phase pH was adjusted to 8.5 using 0.1 M phosphate buffer and partitioned twice using ethyl acetate. The aqueous phase pH adjusted to 2.5 using 1 N hydrochloric acid (HCl) after removed the phase of ethyl acetate. The extract of phytohormones was partitioned three times with diethyl ether. Then, the phase of diethyl ether was dried under vacuum. The obtained residue was dissolved in 1 ml methanol and stored at 4°C for further analysis.

2.5.2. Analysis of phytohormones by reversed phase high performance liquid chromatography (HPLC)

The extracted phytohormones were analyzed by HPLC (Shimadzu) on reverse phase C18 column (250 x 4.60mm) at the temperature of 25ºC. Separation and quantitation were carried out with a mobile phase of acetonitrile: water (26: 74) and 30 mM phosphoric acid. Maintained the pH at 4 by using 1N sodium hydroxide. A constant flow rate of 0.4 ml/min was used for analyte separation and the elution of the phytohormones was observed at 208, 265, 270 and 280 nm (Kelen *et al*., 2004).

2.6. Analysis of volatile oils by Headspace Gas Chromatography–Mass Spectrometry (GC-MS).

The essential oils of plant samples were analysis by using Headspace method in combination with GC-MS. The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Headspace temperature program: oven temperature 80°C, needle temperature 85°C, transfer line temperature 90 °C and incubation time 20 min. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:30, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50-550 and solvent delay 3 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

2.7. Statistical analysis

The results were analysis with XLSTAT 2020.3.1.27 -Method: Partial Least Squares Regression (PLS). The application of PLSR allowed to investigate the correlations among environmental conditions (soil chemical and physical properties and some climatic factors, such as temperature and rainfall rate), and the production of photosynthetic pigments, phytohormones and the main constituents of *Deverra* volatile oils.

3. Results and Discussion

3.1. Plant description

Deverra tortuosa (Desf.) DC. Synonym: *Pituranthos tortuosus* (Desf.) Benth. ex Asch. & Schweinf. Strongly aromatic glabrous desert shrubs of leafless appearance. Densely branches of bushy appearance with numerous blue-green slender tortuos branches. Most of the capillary leaves being reduced to sheaths. Fruit rounded ovoid, hairy of strong aromatic scent when squeezed. Stems dichotomously branched, striate; leaves caducous; basal leaves 3-8 cm, 2-pinnatisect into linear-subulate, acute lobes; petiole sheathing, with broad scarious margin; lower cauline leaves with sheaths to 1.5 cm; blade 1-2.5 cm, ternatisect, the lobes linear-subulate; upper leaves reduced to sheaths with filiform apices; umbels mostly terminal; peduncle 1.5- 4 cm, stout; umbel-rays, few or numerous, 6-10, 1-2 cm, subequal; bracts 2-3 x 1-1.5 mm, triangular, the margin scarious, the apex mucronate; bracteoles minute; bracts and bracteoles persistent; pedicel 0-1.5 mm; flowers hardly opening; petals almost glabrous; styles longer than the depressed stylopodium; fruit 1-1.5 mm, globose, hirsute (Täckholm ,1974;Boulos, 1999;Boulos, 2002) (Fig.1).

3.2. Description of the study areas and their meteorological data

The climate data of Sidi Baranni indicated that the average winter temperature varies between 17.9° C and 19.6°C. The month with the lowest average high temperature is January. In summer season, the warmest month (with the highest average high temperature) is August (29°C). The highest rainfall rate is recorded in January (39 mm), while the months with the lowest rainfall rate are June, July and August.

The second habitat where the plants were collected is Wadi Al-Arish in North Sinai, Egypt. Wadi El-Arish is one of the most important geographical features of northern Sinai, located within 33° and 35° longitude 29° and 31.25° latitude, its length extended to about 250 Km, and its basin is about

20,000 Km^2. located within 33° and 35° longitude 29° and 31.25° latitude. The average high temperature in winter at Wadi AL-Arish varies between 18.8 °C and 20.5 °C and the lowest average high temperature is recorded in January. In summer season the month with the highest average high temperature is July (31.6 °C) and varies between 29.3 to 31.6 °C. The month with the highest rainfall is January (28 mm). The dry period covered 3 months from June to September.

Fig. 1. *Deverra tortuosa* at El-Alamein habitat, inflorescence with terminal umbel (A-C); Branches and leaves sheaths of *D. tortuosa* at Sidi Baranni habitat (D); Branches and leaves sheaths of *D. tortuosa* at Al-Arish habitat(E).

In El-Alamein Road habitat, the average winter temperature varies between 17.8 °C and 19.7 °C. The month with the lowest average high temperature is January. The average summer temperature varies between 28.7 °C and 30. 5 °C. The month with the highest average high temperature is August (30.5 °C). The highest rainfall rate is recorded in January (29 mm). The dry period extended to five months from May to September.

3.3. The physical and chemical properties of the soils at different geographic locations

The results of soil analysis Tables (1,2) showed some differences among the three locations, especially regarding to the percentage of silt and $CaCO_3$, as well as the level of macronutrients Ca^{++}, K^+, Cl^- and HCO_3^-. The obtained data revealed that *Deverra tortuosa* can grow in alkaline calcareous soil. Soil texture was loamy sand at all the studied habitats. The analysis of Sidi Barrani soil indicated that the soil particles contained the lowest values of Ca^{++} (3 meql^{-1}) and Na^{+} (1.4 meql^{-1}) and the highest percentage of clay (3.14 %) and $CaCO_3$ (27.25 %). Whereas the soil of Al-Arish contained the lowest values of Mg^{++}(1.5 meql$^{-1)}$ and Cl^{-} (1.9 meql$^{-1)}$. The soil of El- Alamein was characteristic by the highest content of available soil nutrient (EC) mainly, Ca^{++} (11 meql^{-1}) and Cl^{-} (3.85 meql^{-1}) and highest percentage of silt (18.12 %) in compare with the soil of the other habitats.

Table 1: Soil physical properties

Location	Soil depth	Soil Particles Distribution			Soil Texture Class
		Sand%	Silt%	Clay%	
Sidi Barrani (I)	0-30	84.26	12.60	3.14	Loamy Sand
Al-Arish (II)	0-30	84.42	14.92	0.67	Loamy Sand
El- Alamein (III)	0-30	79.23	18.12	2.65	Loamy Sand

Table 2: Soil chemical properties

Location	pH 1:2.5	EC dS/m	Cation (milliequivalent/Liter)				Anion (milliequivalent/Liter)		$CaCO_3$%
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	
I	8.72	0.70	3	2	1.4	1.14	2.25	4.95	27.25
II	8.37	0.84	4	1.5	2.3	0.93	1.9	7	7.5
III	8.45	1.53	11	2.05	2.03	0.49	3.85	4.2	13.13

3.4. Anatomical characteristics

3.4.1. The effect of different environmental conditions on the anatomy of *Deverra tortuosa* stem

In stem cross section, the outermost epidermal layer of the stem was covered by a thick cuticle layer, which was clearly distinguished in El-Alamein sections. As shown in Table (3) and Fig. (2), the stem of Sidi Barrani samples has the largest diameter with the average of 2.93±0.049 mm, while the stem diameter of El-Alamein and Al –Arish samples were calculated as 2.07±0.022 and 1.81±0.013 mm, respectively. The stem has three rows of epidermal cells in Sidi Barrani section and two rows in the other stem sections. The variation in stem diameters is related to cell expansion rates and strongly depends on the availability of water for vascular cambium tissues (Wimmer *et al*., 2002). Therefore, the increase in the average of stem diameter of Sidi Barrani samples is probably due to the increase in the rainfall rate (39 mm) in winter season at this habitat.

Deverra tortuosa belongs to the scleromorphic plants, which characterized by the presence of sclerenchymatic tissues that give their organs mechanical strength (Batanouny, 2011). Since the root of *Deverra* may reach a depth of 5 meters, to the wetted soil layers in a small wadi at the Cairo to Suez Road (Walter and Breckle, 2013). The stems of scleromorphic plants as *Deverra* are characterized by the presence of green tissues at their periphery as well as ribs and furrows along the green branches. They also characterized by the presence of sunken stomata in the furrows and sclerenchymatic strand at the center portion of the ribs. This strand is accompanied laterally by one or two rows of colourless parenchyma cells that act as storing cells for water, between these cells and the epidermis there are photosynthetic tissues (Fahn, 1989). Cortex consists of 3-4 layered parenchymatic tissues containing chloroplast and crystals. There are several numbers of secretory canals (Schizogenous duct) that are embedded in the cortex and enclosed by a sheath of thick-walled cells, which prevent compression or collapse through turgor of the surrounding tissue. The highest number of these secretory canals with the average of 36.66 was recorded in the stem section of Sidi Barrani sample, while the number of these canals in the samples of El-Alamein and Al -Arish with the average of 22.33. Also, the area of these ducts showed a variation between sections of stem, as the largest area of secretory canal was calculated in Sidi Barrani section with the average of 3518.5 µm, while the smallest area was calculated as 986.7 µm in Al -Arish section.

The vascular bundles arranged regularly and collaterally forming a circuit around the parenchymatic cells of the pith and attached to each other with sclerenchymatic cells. The average length of vascular bundle was 283.4 µm in Sidi Barrani section and decreased to 114.1 and 276.6 µm in Al-Arish and El-Alamein stem sections, respectively. Also, the average width of vascular bundle and the diameter of xylem vessels tend to reduce (decrease) in Al-Arish stem sections and El-Alamein in compare with Sidi Barrani section. The exposure of plant tissues to low water availability have generally shown reduction in cell size and increase in cell wall thickness (Pitman *et al*., 1983; Guerfel *et al*., 2009), also the vascular tissue area and the vessel diameters in stem of stressed plant were smaller than those in the unstressed one (Ristic and Cass, 1991; Kutlu *et al*., 2009). These variations may be related to loss of water and act as an adaptive strategy to stress.

On the other hand, in comparing the stem sections of El Alamein in winter with the stem sections in summer to examine the effect of season on the anatomical feature of *Deverra tortuosa*, it is clearly observed that the average diameter of stem, vessel diameter and area of pith region were reduced. However, the average secretory ducts area was enlarged and the number of sclerenchymatic cells between vascular bundles was increased. This increase in sclerenchyma tissue provides an advantage against the loss of water (Yentür, 2003). Also, the deposition of phenolic compounds in the epidermis and cortex was observed in the summer stem section of El-Alamein.

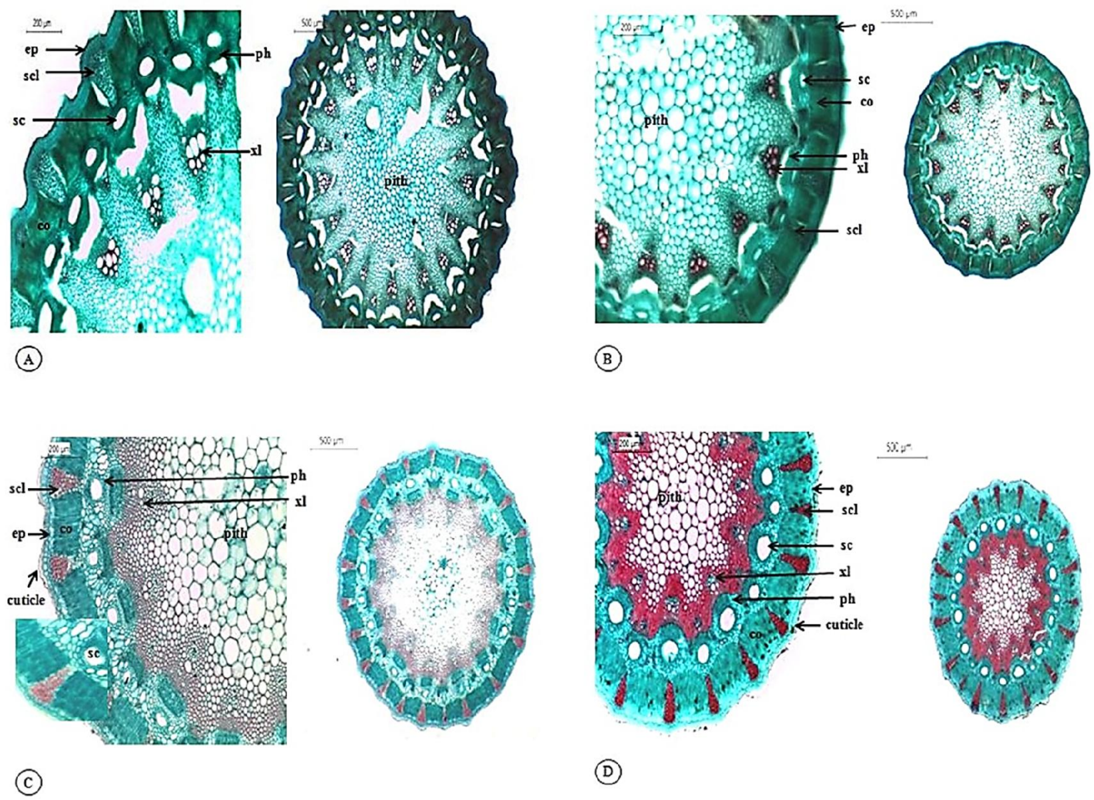

Fig. 2: Stem sections of *Deverra tortuosa* at different habitats. A) Sidi Barrani habitat; B) Al-Arish habitat; C) El-Alamein habitat in winter D) El-Alamein habitat in summer; c: cuticle, co: cortex, col: collenchymas, ep: epidermis, ph: phloem, sc: secretory canal, scl: sclerenchyma, xl: xylem. (Scale 200, 500 μm)

Table 3: Effect of different habitats on the anatomical characters of *Deverra* stem

Characters	Sidi Barrani Winter	Al-Arish Winter	El-Alamaine Winter	LSD	El-Alamaine Summer
Average diameter of stem (mm)	2.959[a]	1.812[b]	2.073[c]	0.0272	1.480
Width of cortex (mm)	0.195[a]	0.107[b]	0.135[b]	0.0297	0.164
Average secretory canal number	36.66[a]	22.33[b]	22.33[b]	1.153	22.33
Average area of secretory canal (μm)	3518.5[a]	986.79[c]	2522.18[b]	517.2	2828.5
Average length of vascular bundle μm)	283.40[a]	114.17[b]	276.63[a]	46.350	63.33
Average width of vascular bundle (μm)	161.33[a]	132.02[a]	178.73[a]	47.088	56.86

The values are the mean (n=3) In each row values followed by different letters are significantly different at $p < 0.05$ by LSD test.

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3.4.2. The effect of habitats on the content of photosynthetic pigments

As shown in Table (4), the concentration of photosynthetic pigments was significantly affected by different environmental conditions. The chlorophyll is the main source of energy and plays an important role in plant physiology. The leaf chlorophyll concentration can refract the physiological condition of a leaf or plant and is used as an indicator of chloroplast content, photosynthetic mechanism and the rate of utilization energy (Srichaikul *et al*., 2011). Also, the concentration of photosynthetic pigments is used as an indicator of the photosynthetic rate, which has a key role in the quality and the production of essential oil as secondary metabolites (Tawfeeq, 2017).

The highest concentrations of chlorophyll a (3.72mg/100gFW) and carotenoids (3.613mg/100gFW) pigments, as well as the highest ratio (7.746) of chlorophyll a to chlorophyll b (Chl. a/Chl. b) were detected in El-Arish sample. The high concentration of carotenoid may protect the plants from photo-oxidative damage and involved in quenching of $^1O_2$ and peroxyl radical (Demmig-Adams and Adams, 1996; Knox and Dodge, 1985). The decrease in the concentration of chlorophyll and carotenoids in El-Alamein sample may be due to the exposure of plant to drought stress, since the dry period extended to five months at this habitat. The decline in total chlorophyll concentration under stress conditions was also reported in other species due to the sensitivity of this pigments to the environmental stress (Ladjal *et al*., 2000; Younis *et al*., 2000; Terzi*et al*., 2010).

The main environmental factors related to the variation of photosynthetic pigments of *Deverra tortuosa* were the soil macronutrients HCO_3^-, Mg^{++}, Cl^-, the temperature as well as the percentage of clay and sand particles in soil sample. The high or low temperatures may reduce the efficiency of photosynthesis process of the leaves of St. John's wort plants and resulted in suppression of CO_2 assimilation (Dixon and Paiva, 1995).

Table 4: Effect of different habitats on the concentration of photosynthetic pigments

Location	Chlorophyll a (mg/100g FW)	Chlorophyll b (mg/100g FW)	Carotenoids (mg/100g FW)	Chl. a / Chl. b
Sidi Barrani	3.48^a	2.873^a	0.366^b	1.212^b
El-Arish	3.72^a	0.486^b	3.613^a	7.746^a
El- Alamein	2.35^b	2.673^a	0.413^b	0.881^a
LSD	0.264	0.232	0.386	1.493

The values are the mean (n=3) In each column values followed by different letters are significantly different at $p < 0.05$ by LSD test

3.4.3. The effect of different habitats on the content of phytohormones

Phytohormones play an important role in the regulation of germination, growth, reproduction and protective responses of plants against stress. The endogenous level of these compounds could assist in predicting the adaptive mechanisms of tolerant plants (Velitcukova and Fedina, 1998) as many proteins expressed and upregulated of their genes by plants under stress are induced by phytohormones (Hamayun *et al*., 2010).

The obtained results revealed that the concentration of growth promoting hormones, GA_3, IAA and zeatin reached to the maximum values of (6.75, 2.57 and 3.13 µg/g, respectively) in *Deverra* sample, collected from Sidi Barrani habitat, while the concentration of stress hormone ABA reached to the minimum value of (1.02 µg/g) in the same sample (Table 5). The increase in the average of stem diameter in Sidi Barrani sample may be related to this increase in the levels of zeatin, IAA and GA_3 due to the role of these endogenous hormones in stimulating the division of cells and/or the enlargement of cells and consequently growth (Taiz and Zeiger, 1998).

The values of GA_3, IAA were affected by the variation in environmental factors and slightly decreased to 4.52 and 1.51 µg/g, respectively at Al-Arish habitat. However, at El-Alamein habitat, the concentration of GA_3, IAA and zeatin decreased to 2.28, 1.4 and 1.91 µg/g, respectively, while the concentration of ABA increased to7.53 µg/g and reached the maximum value. The decrease in the level of IAA and the increase in the level of ABA in El-Alamein sample may be attributed to the exposure of plant to drought stress, since the dry period extended to five months at this habitat. The increase in ABA concentration is usually associated with leaf or soil water potential, as it acts as an endogenous messenger to limit transpiration and to regulate the plant's water status through guard cells as well as by induction of genes (Zhu, 2002; Zhang *et al*., 2006; Wilkinson and Davies, 2010).

Whereas the exposure of rice plants to stress such as salinity stress for 5 days, resulted in significantly reduced IAA levels (Nilsen and Orcutt, 1996)

Principal component analysis (PCA) was conducted to determine the relationship between environmental data (the rate of rainfall, the average the temperature, soil chemical and physical properties). The result of PCA analysis with environmental data and PLS plot (Figs. 3&4) indicated a clear relationship between the concentration of phytohormones of three ecotypes of *Deverra tortuosa* and their geographical origin. The variation of phytohormones in the aerial parts of *Deverra tortuosa* was correlated with the rate of rainfall, soil pH, the percentage of $CaCO_3$, as well as the soil macronutrients Na^+, K^+ and the percentage of silt in soil particles. There were strong direct correlations among the rate of rainfall and the concentration of phytohormones, GA_3 (r = 0.767), IAA (r = 0.969) and zeatin (r = 0.950), whereas the correlation of rainfall rate and the concentration of ABA was inverse (r =-828). Similarly, Dong *et al*., (2019) demonstrated that soybean hormones affected by drought stress, as the content of IAA, GA_3 and zeatin decreased with the increase in water stress, while the concentration of ABA increased. Also, it was reported that shade and drought stress have effects on the concentrations of auxin, cytokinin and abscisic acid (Davies, 2010).

Table 5: Effect of different habitats on the concentration of phytohormones

Location	GA_3 µg/g	IAA µg/g	ABA µg/g	Zeatin µg/g
Sidi Barrani	6.75	2.57	1.02	3.12
Al-Arish	4.52	1.51	4.84	0.84
El-Alamein	2.28	1.41	7.53	1.91

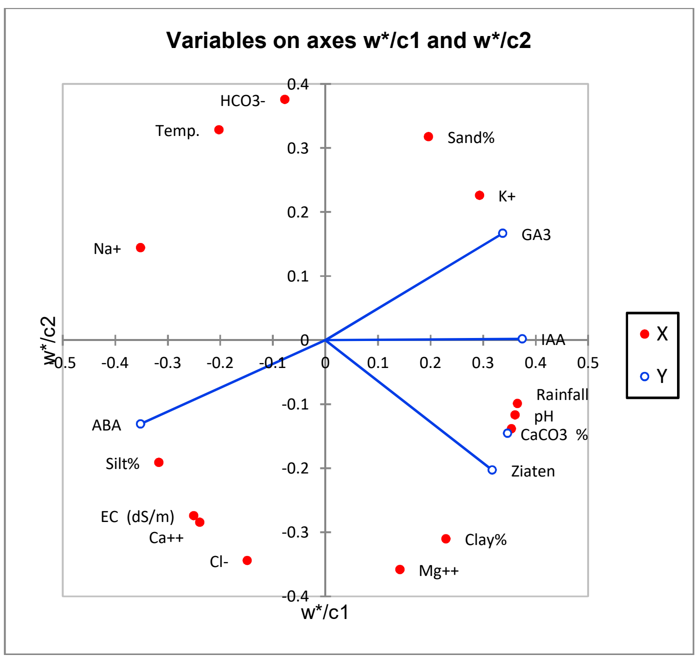

Fig. 3: PCA triplot for the hormones concentration of the aerial parts of *Deverra tortuosa* and the relationship with the environmental factors of Sidi Barrani, Al-Arish and El-Alamein habitats.

The high percentage of sand, high concentration of K^+ and low availability of other nutrient (EC) mainly, Ca^{++} in the soil of Sidi Barrani seem to be related to the high level of GA_3. As the correlations between the concentration of GA_3 and the concentration of K^+ (r = 0.980), as well as the percentage of sand (r = 0.853) were strong and direct, while the correlation between its concentration and Ca^{++} concentration (r = -0.918) and silt (r = -0.996) was inverse. Whereas, the concentration of IAA in Sidi Barrani plant sample was direct correlated with soil pH value (r = 0.956), as well as percentage of $CaCO_3$ (r = 0.937), and inversely correlated with the concentration of Na^+ (r = - 0.931).

The obtained results indicated that there were strong direct correlations among the soil pH value, the percentage of $CaCO_3$, as well as the percentage of clay and the content of zeatin (r = 0.964, 0.978 and 0.932 respectively).

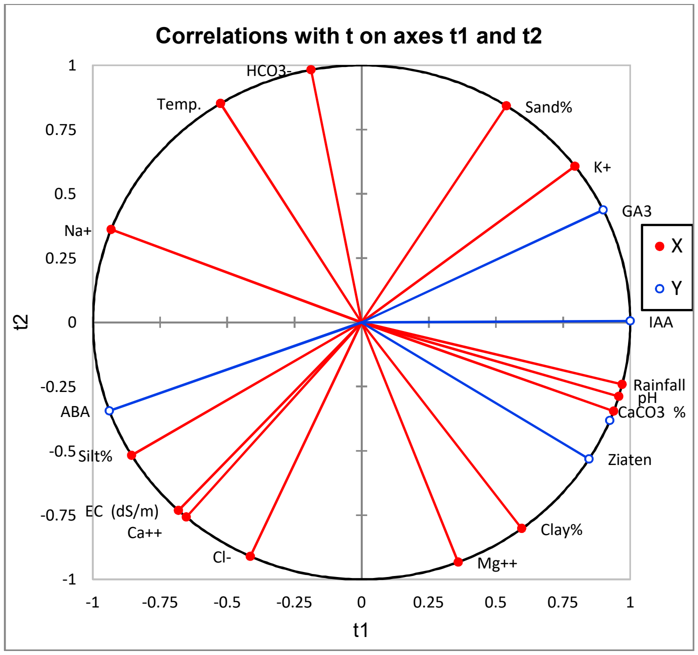

Fig. 4: PLS plot for the hormones concentration of the aerial parts of *Deverra tortuosa* and the relationship with the environmental factors of Sidi Barrani,Al-Arish and El-Alamein habitats.

3.4.5. The effect of different habitats on the main constituents of the essential oils

The main constituents of the essential oils of *Deverra tortuosa* and their percentage are listed in Table (6). The components were identified in the aerial parts of *Deverra tortuosa* at the different habitats according to the retention time and molecular weights of compounds. The number of identified essential compounds in the headspace gas of *Deverra tortuosa* were 8 compounds in Sidi Barrani sample, 6 compounds in Al-Arish and 19 compounds in El-Alamein sample. α-thujene, α-pinene, (-)-sabinene were the common constituents of the volatile oils in all samples and present at different percentages.

The data of headspace GC-MS analysis of volatile oils indicated that the main components of volatile oil of *Deverra tortuosa* were influenced by the habitat in which the plants grow. The volatile oil of *Deverra* collected from Sidi Barrani was characterized by high monoterpene hydrocarbon content (92.24%) and low oxygenated monoterpene content (5.51%), whereas the oil from the plant grown in Al-Arish was mainly composed of monoterpene hydrocarbon (99.12%). In El-Alamein sample, the chemical composition of volatile oil was quite different, as its content from monoterpene hydrocarbon was decreased to 82.09%, while the content of oxygenated monoterpene was (5.86%). Moreover, six non terpenoid volatile compounds were identified in *Deverra tortuosa* oil for the first time. The monoterpenes were the predominant components in all the analyzed samples (mainly hydrocarbon monoterpenes such as sabinene and α-pinene). Sabinene was found to be the major component of the oil in all samples, its percentages were 59.22 and 52.73% in Sidi Barrani and El-Alamein samples, respectively and reached maximum value of 80.44 % in Al-Arish sample. The percentages of α-pinene were 4.49 and 3.81% in Al-Arish and El-Alamein, respectively and reached maximum value of 28.88% in Sidi Barrani sample. Similarly, Krifa *et al*. (2011) demonstrated that the essential oil of *Deverra tortuosa* was contained sabinene, α-pinene, limonene and terpinen-4-ol as major constituents, also, it was reported that sabinene and 4-terpineol were the major components in *D. tortuosa*, as their percentages reached 32.09% and 20.31%, respectively (Saad and Abdelgaleil, 2014).

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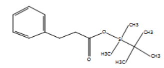
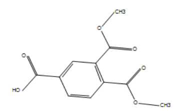
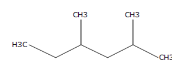
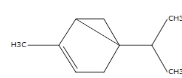
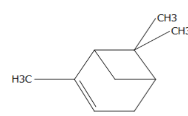
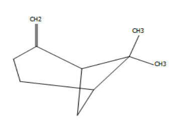
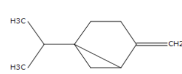
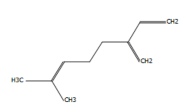
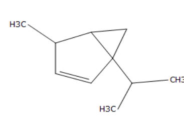
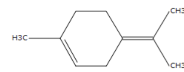
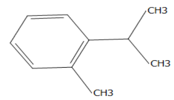
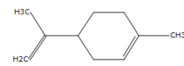
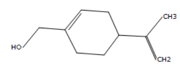
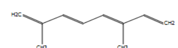
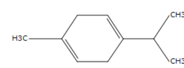
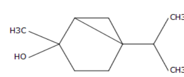
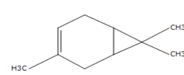
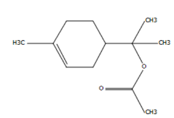
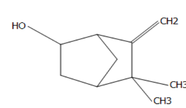
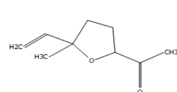
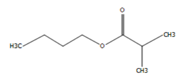
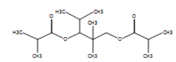
Table 6: Effect of different habitats on the main constituents of the essential oils

No.	Component	RT (min)	Molecular formula	Stereo structure	Sidi Barrani	Al Arish	El-Alamein Road
					Area sum%	Area sum%	Area sum%
1	Benzene propanoic acid, TBDMS derivative	7.50	$C_{15}H_{24}O_2Si$		-	-	1.12
2	1,2,4-Benzene tricarboxylic acid, 1,2-dimethyl ester	8.02	$C_{11}H_{10}O_6$		-	-	1.25
3	Hexane, 2,4-dimethyl-	10.43	C_8H_{18}		1.7	-	-
4	Nonanal	10.48	$C_9H_{18}O$		-	-	1.26
5	α-Thujene	11.40	$C_{10}H_{16}$		1.26	1.82	6.02
6	α-Pinene, (-)-	11.63	$C_{10}H_{16}$		28.88	4.49	3.81
7	2-β- Pinene	12.21	$C_{10}H_{16}$		-	-	0.5
8	Sabinene	13.02	$C_{10}H_{16}$		59.22	80.44	52.73
9	β- Myrcene	13.60	$C_{10}H_{16}$		-	9.49	0.58
10	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	14.20	$C_{10}H_{16}$		-	-	1.1

11	α-Terpinolene	14.63	$C_{10}H_{16}$		-	-	3.41
12	o-Cymene	14.86	$C_{10}H_{14}$		2.88	-	7.13
13	L-limonene	14.99	$C_{10}H_{16}$		-	2.88	-
14	Perilla alcohol	15.00	$C_{10}H_{16}O$		2.45	-	-
15	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	15.08	$C_{10}H_{14}$		-	-	3.06
16	γ-Terpinene	16.17	$C_{10}H_{16}$		-	-	5.46
17	*cis*-Sabinene hydrate	16.43	$C_{10}H_{18}O$		-	0.9	-
18	*trans*-Sabinene hydrate	16.43	$C_{10}H_{18}O$		1.4	-	2.96
19	δ -3-carene	17.26	$C_{10}H_{16}$		-	-	1.35
20	1- *p* -menthen-8-yl acetate	17.65	$C_{12}H_{20}O_2$		-	-	1.22
21	Camphenol, 6-	18.54	$C_{10}H_{16}O$		1.66	-	-
22	*trans*-Arbusculone	20.49	$C_9H_{14}O_2$		-	-	1.68

23	Propanoic acid, 2-methyl-, butyl ester	27.11	$C_8H_{16}O_2$		-	-	2.74
24	Pentan-1,3-dioldiisobutyrate, 2,2,4-trimethyl-	35.52	$C_{16}H_{30}O_4$		-	-	2.6
	Total (%)				99.45	100.02	103.44

RT, Retention time (as min)

The other volatile components which represented with high levels were β- myrcene (9.49%) in Al – Arish and o-cymene (7.13%) in El-Alamein. The volatile oil of Sidi Barrani ecotype was contained remarkable compounds, these compounds were hexane, 2,4-dimethyl- (1.7%), perilla alcohol (2.45%) and camphenol, 6- (1 .66%). Other monoterpenes such as l-limonene and cis-sabinene hydrate were detected only in Al –Arish ecotype sample. Meanwhile El-Alamein ecotype was characterized by the presence of other 13 compounds in fairly good amount, these compounds were benzene propanoic acid, TBDMS derivative (1.12%), 1,2,4-benzenetricarboxylic acid, 1,2-dimethyl ester (1.25%), nonanal (1.26%), 2-β-pinene (0.5%), bicyclo[3.1.0] hex-2-ene, 4 -methyl-1-(1methylethyl)-(1.1%), α-alpha.-terpinolene(3.41%), 2,6-dimethyl-1,3,5,7 octatetraene, E,E- (3.06%), γ-terpinene (5.46%), δ -3-carene(1.35%),1- p -menthen-8-yl acetate(1.22%), trans-Arbusculone (1.68%), propanoic acid, 2-methyl-, butyl ester (2.74%) and pentan-1,3-dioldiisobutyrate, 2,2,4-trimethyl- (2.6%).

The data of headspace GC-MS analysis of essential oils of *Deverra tortuosa* provided preliminary evidence of the presence of three chemotypes of *Deverra tortuosa* due to the significant difference in the main constituents of the essential oils of the three ecotypes. This agrees with the hypothesis that the exposure of a plant to different exogenous factors (biotic and abiotic) for a long period could cause it to undergo genetic modification. Which can alter the biosynthetic pathways and leads to quantitative and qualitative variations in the volatile products determining chemotypes (Barra, 2009). This result is supported by previous studies on *Deverra tortuosa*, which indicated that the chemical composition of the essential oil of *Deverra tortuosa* grown in different geographic location has shown a wide variation even in their main constituents. For example, the major component of essential oils of *Deverra tortuosa* grown in Saudi Arabian was found to be apiol with the percentage ranged between 65.73% and 74.41% (Guetat *et al*., 2019). Whereas the oil from *Deverra* grown in southern Sinai, Egypt contained 32 compounds and alcohols representing more than 42% of the oil, while camphene (31%) was a major one. These variations in the composition of volatile oils may related to different factors, such as plant age, season, pH of the soil, the geographic location, growth at different altitudes, the plant genotype (Saleh *et al*., 1987; Perry *et al*.,1999; Hussain *et al*., 2008) or essential oil isolation technique (Skwirzyńska *et al*., 2013; Khalajee *et al*., 2017).

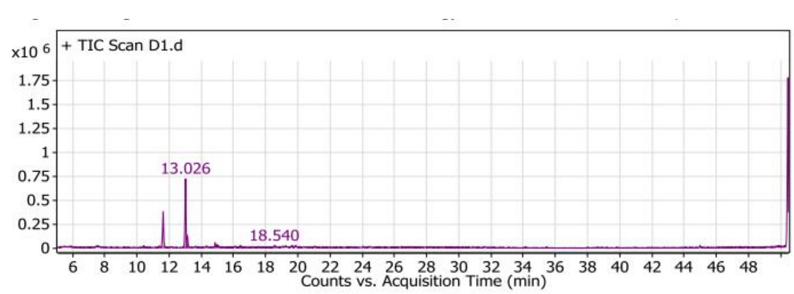


Fig. 5. Chromatogram of essential oil of *Deverra tortuosa* collected from Sidi Barrani Desert

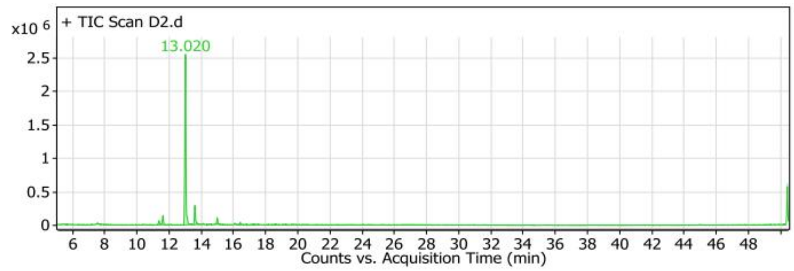


Fig. 6: Chromatogram of essential oil of *Deverra tortuosa* collected from Al-Arish

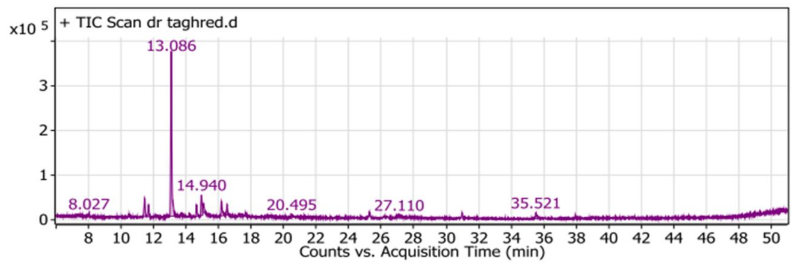


Fig. 7: Chromatogram of essential oil of *Deverra tortuosa* collected from El-Alamein Road

The result of the PCA analysis with environmental data and PLS plot (Figs. 8& 9) indicated a strong relationship between the main constituents of the essential oils of three chemotypes of *Deverra tortuosa* and their geographical origin. The main environmental factors related to the variation on the main constituents of the volatile oils of *Deverra tortuosa* were the percentage of sand and silt in soil particles, the soil macronutrients Cl^-, Ca^{++} and K^+, soil EC, the rate of rainfall, as well as the average temperature. The obtained results indicated that there was a direct relation among the rate of rainfall (r = 0.981), levels of soil pH (r = 0.970), as well as the percentage of $CaCO_3$ (r = 0.954) and the level of α-pinene (comp.6), hexane, 2,4-dimethyl-(comp.3), perilla alcohol (comp.14) and camphenol, 6-(comp.21). In a previous study of the influence of calcareous soil on the essential oil of *Thymus spinulosus*, it was reported that the plants grown in this soil were characterized by high monoterpene concentration (De Feo *et al*., 2003). Thus, the higher proportion of the constituent α-pinene (28.88 %) in oil sample of *Deverra* from Sidi Barrani can be related to the high percentage of $CaCO_3$ as well as the low level of Ca^{++} and Na^+ in the soil of this region in comparison with the soil of other regions.

The monoterpenes, β-and α-pinene are very common in plants volatile oils and presence in a wide range of mediterranean, tropical and coniferous species (Areco *et al*., 2014). They have allelopathic and phytotoxic effects as the inhibition of the seed germination and growth of other plants (Abrahim *et al*., 2003; Nishida *et al*., 2005; Amri *et al*., 2012). The monoterpenes, mainly as β-and α-pinene, limonene and myrcene found to be accumulated in resin ducts found in the needles, twings and trunks in Gymnosperms like Pine are toxic to numerous insects including bark beetles (Turlings *et al*., 1995). Also, o-cymene exhibited stronger fumigant and contact toxicity (Feng *et al*., 2021). Therefore, the high concentration of α-pinene, β- myrcene and o-cymene in the oil sample of Sidi Barrani, Al-Arish and El-Alamein, respectively, might be an adaptive response to the low level of soil nutrients to decrease the competition by other plants for soil nutrients. Similarly, it was reported that the variation in the production of terpenes in *Tithonia diversifolia*, seems to be a direct response to different abiotic environmental conditions (Sampaio and Da Costa, 2018).

The influence of the soil pH on the variation of in the chemical constituents of essential oils has been reported in *Rosmarinus officinalis* L. (Manunta, 1986) and *Erygium Campestre* L (Palà-Paul *et al*., 2008). It was found that the distribution of terpenes was strongly influenced by pH and the population of *Erygium Campestre* L growing in acid soil contained a high percentage of myrcene and a lower percentage of β-curumene. A recent publication demonstrated that the variation in the composition of the volatile oils of two chemotypes of *Tithonia diversifolia* growing in different regions may be related to soil nutrients, mainly Ca and P and climatic factors (Sampaio and Da Costa, 2018) as well as geographical distribution (Ajao and Moteetee, 2017).

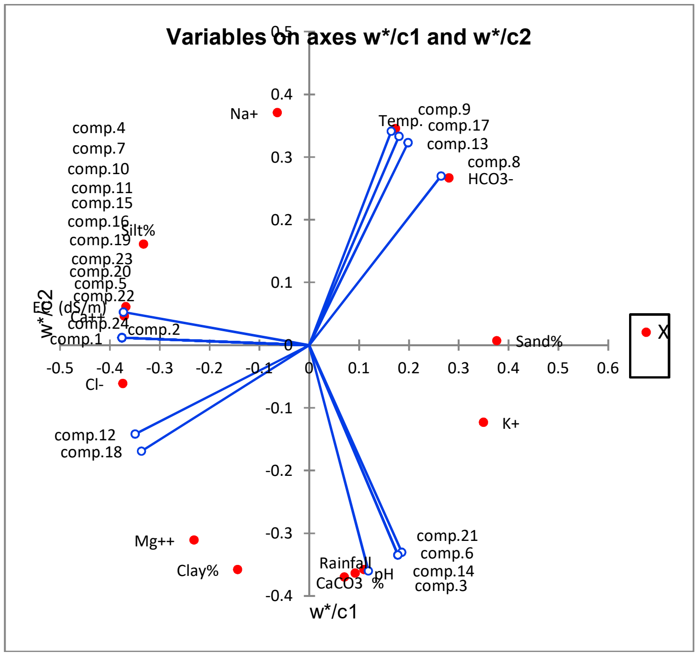


Fig. 8: PCA triplot for the GC–MS data obtained from the volatile oils of the aerial parts of *Deverra tortuosa* collected during the year of 2020 and the relationship with the environmental factors of Sidi Barrani, Al-Arish and El-A lamein habitats.

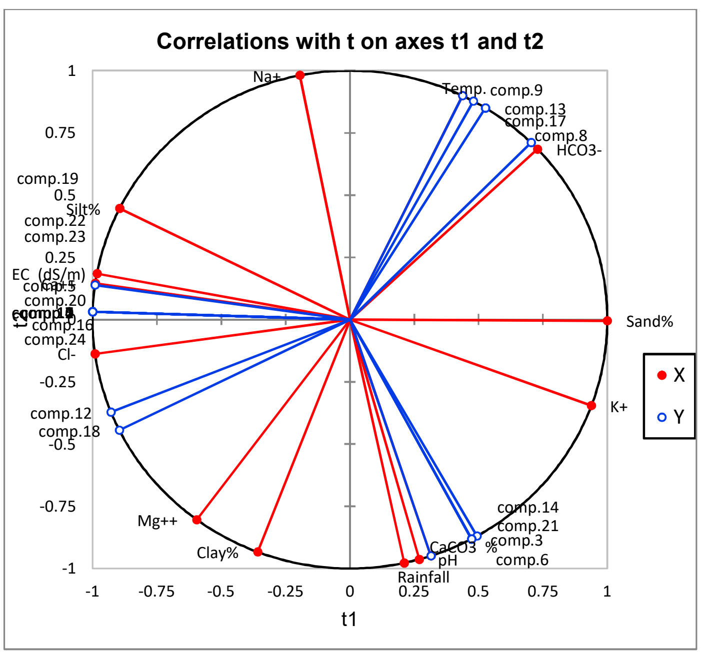


Fig. 9: PLS plot for the GC–MS data obtained from the volatile oils of the aerial parts of *Deverra tortuosa* and the relationship with the environmental factors of Sidi Barrani, Al-Arish and El-Alamein habitats

The level of sabinene (comp.8), the most abundant constituents in all samples of volatile oil of *Deverra* was significantly correlated with the concentration of HCO_3^- (r = 0.999) and high average temperature (r = 0.947) and inversely correlated with the concentration of Mg^{++} (r = - 0.990) and the percentage of clay (r = -0.916). Therefore, the oil sample of *Deverra* collected from Al-Arish contained the highest proportional of sabinene might be due to the high average temperature, high concentration of HCO_3^-(7 meql^{-1}), low concentration of Mg^{++} (1.5 meql^{-1}) and low percentage of clay (0.67%) in comparison with the environmental conditions of the other habitats. Similarly, the production of monoterpenes, β- myrcene, L-limonene and cis-sabinene hydrate was strongly influenced by HCO_3^-, the percentage of clay in soil and the average temperature. Most monoterpenes have significant ecological importance in the interactions between plant and its environment, since they assist in protecting against heat stress and can make the photosynthetic apparatus more resistant to high temperature (Delfine *et al*., 2000).

The high availability of nutrient in soil (EC), high concentration of soil micronutrients, mainly Ca^{++}, Cl^- and high percentage of silt in El-Alamein soil seem to be related to the production of nonanal , α-thujene, 2-β- pinene, bicyclo[3.1.0] hex-2-ene, 4-methyl-1-(1-methylethyl)-, α-alpha.-terpinolene, 2,6-dimethyl-1,3,5,7 octatetraene, E,E-, γ-terpinene, δ -3-carene,1- p -menthen-8-yl acetate, trans-arbusculone, propanoic acid, 2-methyl-, butyl ester and pentan-1,3-dioldiisobutyrate, 2,2,4-trimethyl-.

From the obtained results, it could be indicated the strong relationships among the concentration of photosynthetic pigments, phytohormones, as well as the main constituents of the essential oils of three ecotypes of *Deverra tortuosa* and their geographical origin. It was reported that the production and the chemicals composition of the essential oil can be changed by the factors such as photosynthetic rate, light quality, climatic and seasonal changes, temperature, humidity, salinity and growth regulators (Sangwan *et al*., 2001). Since the growth of plant and the biosynthesis of terpenoids are regulated by plant growth regulators or plant hormones in different aromatic plants, which affect the quality and the quantity of terpenoids (Farooqi and Shukla,1990).

The mechanism involved in the influence of edaphoclimatic factors on the main constituents of the essential oils of *Deverra tortuosa* appears to be related to the effect of temperature and the rate of rainfall on the concentration of photosynthetic pigments, which mainly affects the production and the quality of volatile oils as secondary metabolic products (Tawfeeq *et al*., 2017). Since the increase in temperature, the extend of dry period and the low rate of rainfall, can cause a reduction in chlorophyll a and b and in the photosynthetic rate. Resulting in an increase in the production of secondary metabolic products as a defense mechanism against reactive oxygen species by diminishing light absorbing capacity that reduces the flow of electrons through the photosystems (Elfeky *et al*., 2007). The increased photosynthetic rate of winter leaves results in the production of larger amounts of carbon from photosynthesis that are largely invested in the biosynthesis of secondary metabolites including essential oils (Padda and Picha, 2008). Therefore, the production of essential oils and the biosynthesis of terpenoids are based on primary metabolism such as photosynthesis, oxidative pathways for carbon and supply of energy (Singh *et al*., 1990) and regulated by plant hormones.

4. General conclusion

The anatomical examination of stem sections *Deverra tortuosa* by light microscope indicated the presence of anatomical variations among the different ecotypes of *Deverra*. These variations were related to the number of epidermal layers, average diameter of stem, number of secretory ducts and average length and width of vascular bundles. The collected samples from Sidi Barrani habitat possessed maximum number of vascular bundles and secretory ducts, as well maximum stem diameter and the highest concentration of growth promoting hormones, GA_3, IAA and zeatin in comparison with the other samples.

The results of the PCA analysis with environmental data indicated a strong relationship among the concentration of photosynthetic pigments, phytohormones, as well as the main constituents of the essential oils of three ecotypes of *Deverra tortuosa* and their geographical origin, taking into account the influence of genetic and biotic environmental factors on the composition of essential oils, the main edaphoclimatic factors which may be related to the variation on the main constituents of the essential oils of *Deverra tortuosa* were the percentage of sand and silt particles in soil samples, the soil macronutrients Cl^-, Ca^{++} and K^+, soil EC, the rate of rainfall, as well as the average temperature.

The data of headspace GC-MS analysis indicated that the major component of the oil in all samples was sabinen and the chemical composition of the essential oils of *Deverra tortuosa* grown in different habitats has shown a wide variation. This variation in the main constituents of the essential oils of the three-ecotype provided preliminary evidence of the presence of three chemotypes of *Deverra tortuosa*.

The presence of high concentration of phytotoxic monoterpene, α-pinene, β- myrcene and o-cymene in the oil sample of Sidi Barrani , Al-Arish and El-Alamein, respectively might be an adaptive response to the low level of soil nutrients to decrease the competition by other plants for soil nutrients and seems to be a direct response to different abiotic environmental conditions. The volatile oil of Sidi Barrani ecotype contained remarkable compounds, these compounds were hexane, 2,4-dimethyl-, perilla alcohol and camphenol, 6-. Other monoterpenes such as l-limonene and cis-sabinene hydrate were detected only in Al –Arish ecotype sample. Meanwhile El-Alamein ecotype was characterized by the presence of other 13 monoterpenes compounds in fairly good amount, which proved the ecological importance of monoterpenes in the interactions between plant and its environment. From the results of this study, it is clear that the main components of essential oils were influenced by the habitat in which the plants grow. Therefore, the impact of environmental conditions on the productivity and quality of oils with economic values and medicinal benefits must be taken into consideration when introducing such plants for cultivation.

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