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Changes in some chemical compounds of *Retama raetam* (Forssk.) Webb & Berthel. in response to different environmental conditions

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Abstract

Climate changes, storage of water, rising in atmospheric temperature and other environmental stresses have harmful effects on the plant growth and productivity. Also they negatively influence the morpho-physiology of plants, as plant physiology is heavily affected by temperature fluctuations. Understanding the stress-resistance processes in plants has appeared as a very important issue in order to develop stress-resistant plants. So, the aim of this study was to evaluate the change in the physiological responses and chemical composition of *Retama raetam* in response to the environmental conditions. *Retama raetam* was collected from Wadi Sudr at South Sinai and Hammam Cleopatra region at Mersa Matruh in dry and wet seasons. The results of this study indicate that there was a significant difference between habitats in the contents of minerals, carbohydrates, soluble sugars and alkaloids. The deficiency of water and stress conditions in the dry season, induced a reduction in osmotic potential at both habitats, which was accompanied by the accumulation of osmolytes or osmoprotectants; such as inorganic ions (Ca^{2+}, mg^{2+}, K^{+}), carbohydrates, soluble sugars and organic acids (oxalic, citric, malic) considered as a protective metabolic adaptation that could exert beneficial effects upon drought tolerance of *Retama reteam*. Meanwhile, the content of total alkaloids in *Retama raetam* was significantly affected by habitats and/or seasons, as its values were significantly increased in dry season to 6.2mg/g at Wadi Sudr and 6.5mg/g at Cleopatra. These results revealed that the chemical composition of the plants was significantly affected by seasons and environmental conditions, which may have a negative effect due to the accumulation of some toxic compounds under stress condition, like alkaloids.

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Introduction

Climate change, shortage of water and global warming have devastating effects on the plant productivity and food security, which raises the world's concern. According to the report of FAO in 2007 all cultivated areas in the world are affected by climate changes and only 3.5% of areas are safe from environmental limitations. Morphological, biological, and biochemical mechanisms of plants have been severely affected by abiotic stresses. Zhao *et al.* (2017) reported that the climate change impact on yields of major crop caused a reduction in yield of 3.2%, 6%, 3.1%, and 7.4% in rice, wheat, soybean, and maize respectively.

Metabolomics is an interesting approach that has been used in the plant sciences, especially in ecological studies, to investigate the effects of environmental factors on plant metabolism. The output data have been utilized to compare between different species from the same family, or individuals from populations from the same species growing under different environmental conditions, or variations in the production of individuals metabolites within the same population at different seasons (Jones *et al.*, 2013; Kim *et al.*, 2010; Bundy *et al.*, 2009; Arbona *et al.*, 2013) Others reported that same species of plant that growing under different environmental conditions show significant differences in the production and accumulation of the primary and secondary metabolites (Pavarini *et al.*, 2012; Ramakrishna and Ravishankar, 2011; Wink, 1988). Therefore, the study of these variations is very useful in the chemical characterization of plants of the same species which are collected from different regions. Moreover, understanding the physiological mechanism of the stress- tolerant plant under different environmental conditions has appeared as a very important task for plant scientists in order to develop stress-resistant plants (Singh, 2018).

In this study, the investigated plant was *Retama raetam* (Forssk.) Webb & Berthel, Commonly known as 'raetam' or 'broom bush'. It contains flavones (Kassem *et al.*, 2000) quinolizidine alkaloids, dipiperidine alkaloids (El-Shazly *et al.*, 1960), lupin alkaloids (Abdel-Halim, 1992; Abdel-Halim, 1995). *Retama* has been used in folk remedies for diabetes, hypertension and some renal diseases (Caceres *et al.*, 1987; Burits and Bucar 2000) and its flowers extracts have antioxidant, and antiviral activities and high doses might have hepatotoxic, nephrotoxic, and mutagenic effects (Algandaby, 2015). On the other hand, Wink (1987) reported that quinolizidine alkaloids act as chemical defence compounds against herbivores, and microorganisms and competing plants.

The genus *Retama* (syn. Lygos) belongs to tribe Gentisae of the Fabaceae family and includes four species. It is a desert shrub that grows abundantly in North Africa and East Mediterranean regions (Mittler *et al.*, 2001; El Bahri *et al.*, 1999) and Sinai (Boulos, 1999), it inhabits desert wadis and sandy plains in the deserts of Egypt and can tolerate arid environments. So, it is considered a suitable model for studying drought responses due to its ability to survive under different environmental conditions and extreme drought conditions.

The aim of this study was to evaluate the change in the physiological responses and chemical composition of *Retama raetam* in response to different environmental conditions.

Material and methods

Plant description

Retama raetam (Forssk.) Webb & Berthel. (Family: Fabaceae), was recorded in North Africa and East of the Mediterranean Sea and inhabits desert wadis and sandy plains in the deserts of Egypt. It is a desert shrub of 0.5 to 2m in height, with multiple stems, either erect or spreading. Old branches are brown in colour while young ones are dark green and grooved. Flowers are white with purplish tip, aggregated in racemes (1-to-5-flowered), and fruits are indehiscent legumes containing hard coated brownish seeds (Boulos 1999, 2009)

The species of *Retama* can use water from deep and subsurface layers by developing an additional surface root system "T-root system" (Veste *et al.*, 2008) and extending the roots to reach the depth of 10m (Zohary and Fahn (1952) and its branches are slender to reduce the surface area exposed to the dry air of the desert and to help in reducing transpiration (Lambers and Poorter, 1992)

Ecological studies

Study area

The study was carried out in two areas; the first area was Wadi Sudr in Ras Sudr (29° 35´30″ N, 32° 42´20″ E), South Sinai, and the second area was at Hammam Cleopatra region at Mersa Matruh (31° 22´ 18.4N″, 27° 12´ 05.1″E).

Wadi Sudr is one of the most developed wadis of the northern group of south-west Sinai. The wadi is bounded by Gabel El-Raha (c-600m) from the North and Sinn Bishr (618m) from the South. The main trunk of the wadi extends roughly in NE-SW direction for a distance of about 55Km and pours at Ras Sudr. The climate is influenced by the orographic impact of the high mountains (Migahid *et al*., 1959; Issar and Gilad, 1982). During the period of study in 2017, the average annual temperature was 23.3˚C. The average annual maximum and minimum temperatures were 29.1 and 17.8˚C, respectively. While the total annual precipitation was 1.26mm and the dry period extended from April to December. The annual average wind speed was 10.1Km/h. The average annual humidity was 49.9%.

The Second locality was Hammam Cleopatra region at Mersa Matruh, situated within a region of dry climate. This area is classified as arid with mild winter and warm summer (UNESCO, 1977). The mean annual rainfall was 116.52mm (increasing from West to East and from South to North). In 2017, the average annual temperature was 20.4˚C, and the average annual maximum temperature was 24.8˚C, while the average annual minimum temperature was 16.1˚C. The average annual humidity was 67.5%. These Meteorological data indicated that the studied habitats have an arid type climate with high temperature especially during the dry period.

Osborne *et al.* (2000) reported that the average annual temperature has increased by 0.75˚C during the past century and precipitation has shown marked variation throughout the Mediterranean basin .This change affected the wild vegetation of South Sinai in general and resulted in rarity of trees and change in The composition of the vegetation (Moustafa *et al*., 2001)

Soil analysis

For soil analysis, soil samples were collected from the soil supporting the investigated plant at 3 random points from each location at two depths; The 1st depth from 0-20cm and the 2nd depth from 20-40cm at Wadi Sudr. While at Hammam Cleopatra the soil samples were taken at 0-30 and 30-50cm.

Soil physical properties

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

Soil chemical analysis

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

Physiological studies

Plant water content

Plant water content, the difference between fresh weight (FW) and dry weight (DW), was calculated on a dry basis using the following formula:

$$\text{Plant water content (ml /100gDW)} = \frac{FW-DW}{DW} \times 100$$

Determination of plants osmotic potential

The fresh plants were collected in liquid nitrogen, and then stored at-20˚C until the time of measurement then, the plant samples were thawed at room temperature and pressed to free the cell sap according to the cryoscopic method as described by Simmelsgaard (1976).The osmotic potential of the expressed sap was measured with Freezing Point Osmometer (Osmomat 030 - Gonotec - Berlin - Germany), which was calibrated with KCl.

Plant chemical analysis

The aerial parts of *Retama raetam* were collected in winter (January) and summer (August) seasons of 2017 from Wadi Sudr at South Sinai and Hammam Cleopatra region at Mersa Matruh, Egypt. The collected plant materials were dried in the oven at 70°C to a constant weight and ground to fine powder and subjected to various analyses.

Mineral analysis

Determination of total nitrogen and protein contents

Nitrogen (N) content of samples were estimated by the method described by Kjeldahl (1983) and crude protein was calculated as N×6.25 (James, 1995).

Determination of calcium, magnesium, Potassium and sodium

Half gm of dried samples were placed in a small Kjeldahl and digested with 10ml concentrated nitric acid and, the mixtures were heated gently on a hot plate until the production of brown fumes of (NO_2)had ceased, (2-4ml) of perchloric acid was added after cooling. The mixtures were reheated and allowed to evaporated to a small volume, then diluted to 100ml with distilled water and used for mineral analysis (Baker and Smith, 1974). The contents of calcium, magnesium, Potassium and sodium were determined according to the method of Rowell (1994).

Determination of sulphur, phosphorus and chloride

The plant materials were ashed in porcelain crucibles in a muffle furnace at 550 for 4-6 hours and used for the determination of sulphur and phosphorus by using turbidimetic and phosphomolybdat methods, respectively. The chloride was extracted from ashed material by dilute nitric acid (0.01N), then titrated with standard silver nitrate (Jackson and Thomas, 1960).

The concentrations of manganese, copper, zinc and iron were determined by using ICP Emission Spectroscopy (Jones, 1977).

Determination of total carbohydrates and total soluble sugars

The total carbohydrates were extracted by dissolving 0.3gm of plant powder in 10ml of 3%HCl. the tube was sealed and heated at 100˚C for a period of 2-5hours. The extracted sugars were estimated using the phenol–sulfuric acid assay (Buysse and Merck, 1993). For determination of total soluble sugars, the plant sample (2.5gm) was extracted twice in 40ml of boiling water and twice in 40ml of aqueous boiling ethanol (80%v/v) and clarified using saturated neutral lead acetate solution (AOAC method 2000). The clarified extracts were evaporated under reduced pressure until dryness, then dissolved in 100ml of distilled water and the total soluble sugars were estimated using the phenol–sulfuric acid assay.

Determination of total amino acids

The total amino acids of *Retama raetam* were determined by using Amino Acid Analyzer apparatus model (LC 3000 Eppendorf, Central Lab. of Desert Research Center) according to the method of Pellet and Young (1980).

Determination of organic acids

Organic acids in *Retama raetam* were determined by HPLC according to the method of Zbigniew *et al.* (1991). Oneml of each sample was diluted 10ml with water and 35µl was injected into HPLC Hewllet Packard (series 1050) with auto sampling injector, ultraviolet (UV) detector set at 210 nm and quaternary HP pump (series 1100). Packed column Hypesil BDS- C18, 4.0 × 250mm was used to separate organic acid. The column temperature was maintained at 55°C, at flow rate 1ml/min. Organic acids standard from Sigma Co. were dissolved in a mobile phase (phosphoric acid) and injected into HPLC. Retention time and peak area were used to calculation of organic acids concentration by data analysis of Hewllet Packard Software.

Quantitative estimation of total alkaloids

The total alkaloids were determined by using the gravimetric method of Harbone, (1973). 10g of plant samples were transferred to 500ml conical flask and 400ml of 10% acetic acid in ethanol was added and covered and allow to stand for 4h, then filtrated and concentrated on a water bath at 60°C to 100ml. drops of concentrated ammonium hydroxide were added to the extract to precipitate the alkaloid. The solution was centrifuged and the precipitate was collected and washed with dilute ammonium hydroxide (15%) and filtered. The precipitated alkaloid was dried in an oven and weighed. The percentage of the total alkaloid (w/w) was calculated based on dried plant material.

Statistical analysis

The data were statically analysed using the statistical program (CoStat Version 6.4). The significant differences between means were calculated by a split plot analysis of variance (ANOVA) using Student Newman–Keul's (SNK) test. Differences with $P < 0.05$ were considered as significant (Glantz, 1992).

Results and discussion

Soil physical and chemical properties

Physicochemical characters of Wadi Sudr at South Sinai and Hammam Cleopatra soils at Matrouh are represented in Tables (1) and (2). Soil samples collected from the different habitats are sandy in texture. The analysis of soil particles indicated that the soil of Wadi Sudr was characterized by a high percentage of coarse sand in the surface layer. Meanwhile, the soil of Cleopatra was characterized by a high percentage of medium sand at first and second depth. The pH values fluctuated in the basic range. Generally, no significant differences in soil pH due to location changes were noticed. The Soils of both habitats are rich in calcium carbonate, its percentages were 25.50 and 30.10% in Wadi Sudr and 36.46 and 40.52% in Cleopatra in the first and second depth, respectively. The presence of $CaCO_3$ directly or indirectly affects the chemistry and availability of nitrogen, phosphorus, iron, zinc, magnesium, calcium, potassium and copper to plants due to reduced solubility at alkaline pH values (Marschner, 1995).

Table 1. Physical proprieties of the soil supporting *Retama raetam* at Hammam Cleopatra and Ras Sudr habitats.

Locality	Soil Depth (cm)	$CaCO_3$%	Soil Particles Distribution (%)						Soil Texture class
			Very Coarse Sand	Coarse Sand	Medium Sand	Fine Sand	Very Fine Sand	Clay & Slit	
1)Wadi Sudr	0 - 20	25.50	32.25	30.21	16.32	13.00	7.11	1.11	Sandy
	20 - 40	30.10	15.10	5.12	25.32	45.05	8.57	0.84	Sandy
2)Hammam Cleopatra	0-30	36.46	0.34	13.86	54.32	29.33	0.85	1.30	Sandy
	30-50	40.52	0.25	20.25	51.91	25.89	0.91	0.79	Sandy

Table 2. Chemical properties of the soil supporting *Retama raetam* at Wadi Sudr and Hammam Cleopatra habitats.

Locality	Soil Depth (cm)	pH	EC dS/m	Cation (milliequivalent/Liter)				Anion (milliequivalent/Liter)			P (ppm)
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	SO_4^{--}	
1	0 - 20	7.87	4.52	2.10	2.82	13.68	1.31	12.53	6.01	1.37	0.3
	20 - 40	7.79	4.10	2.69	2.50	13.32	2.14	10.52	6.52	3.61	0.8
2	0 - 30	8.15	1.21	3.48	2.52	8.50	1.18	6.41	8.72	0.55	0.1
	30 –50	8.31	1.60	3.80	3.78	10.85	1.41	8.55	9.65	1.64	0.5

El-Nennah *et al.* (1981) and Moustafa and Zayed (1996) reported that South Sinai soils are gravelly in wadis and plains, sand to loamy sand in texture, alkaline, nonsaline to slightly saline and this agrees with our results.

The obtained results of soil moisture content (Fig. 1) revealed that there was a significant variations in the soil moisture content between habitats, as its values at 1st and 2nd depths in winter were 1.16±0.44 and 2.63±0.50% at Wadi Sudr and 6.83±0.56 and

8.21±0.55% at Cleopatra, respectively. Meanwhile, its values were significantly decreased in dry season to 0.25±0.10 and 0.45±0.12 g% at Wadi Sudr and 2.26±0.45 and 3.13±0.30g% at Cleopatra at 1st and 2nd depth, respectively. It could be concluded from these results that *Retama raetam* is one of the xerophytic plants and it can grow in various types of soils. Moreover, it tolerates water deficiency and is adapted to survive extreme drought conditions, like that in Wadi Sudr.

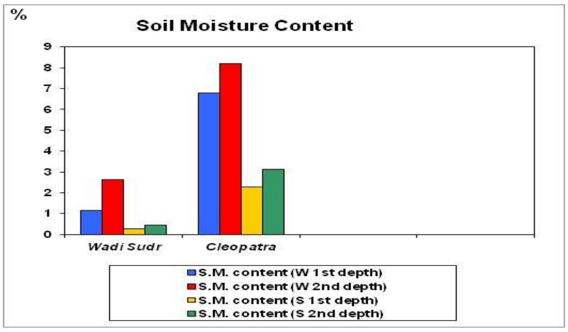

Fig. 1. Soil moisture content.

The results of soils chemical analysis indicated that the highest values of Ca^{++} (3.80meq/L) and HCO_3^- (9.65meq/L) were recorded in the soil of Hammam Cleopatra, while the lowest values (2.10meq/L) and (6.01%) were detected in the soil of Wadi Sudr, respectively. There was no considerable variation in the content of mg^{++} and K^+ between the soil samples in winter.

The soil of Wadi Sudr is characterized by high content of Na^+ and Cl^- at 1st and 2nd depth. Its values were 12.53 and 10.52meq/L at Wadi Sudr and 6.41 and 8.55meq/L at Hammam Cleopatra, respectively. The increase in the concentration of NaCl has been reported to cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with nutrients such as $Ca^{2+}$and K^+. Once sodium gets into the cytoplasm, it inhibits the activities of many enzymes (Jouyban, 2012). High concentrations of Na^+ in the soil solution may depress nutrient-ion activities and produce extreme ratios of Na^+/Ca^{2+} or Na^+/K^+ (Grattana and Grieveb, 1999). Increases in cations and their salts, particularly NaCl generates external osmotic potential in the soil that can prevent or reduce the influx of water into the root.

Osmotic potential and plant water content

The measured osmotic potential (Ψs) and plant water content of *Retama* were illustrated in Table (3) and Figs. (2 and 3), as the level of osmotic potential was significantly decreased by stress at both habitats, as its values decreased from - 0.987 and - 1.186MPa in winter to - 1.544 and - 1.791MPa in summer at Wadi Sudr and Cleopatra, respectively. Although the plant is growing in dry environments and suffers from a deficit of water, it still maintains a moderate content of water, as the percentages of plant water content were 56 and 58.8% in winter and decreased to 36.9 and 52.2 in dry season at Wadi Sudr and Cleopatra, respectively. These results indicate that the plant has the ability to uptake of water and maintain moderate content of water in its cells in dry period, that may be attributed to the accumulation of compatible solutes, which assist the cell to readjust its internal osmotic potential under stress conditions. Since the reduction in osmotic potential and accumulation of solutes in response to drought stress is assisting the plants to cope with stress conditions (Patakas and Noitsakis, 1999; Morgan, 1984), to maintain the cell turgor and to reduce the effect of water stress (Subbarao *et al.*, 2000). Osmolyte accumulation is one of the drought tolerance mechanisms which allows cells to manage their dehydration and membrane structural integrity to give tolerance against drought and cellular dehydration.

Table 3. Osmotic potential (Ψs) of cell sap and plant water content of *Retama reatam*.

Studied Factor	Locality				LSD
	Wadi Sudr		Hammam Cleopatra		
	Winter Mean±SD	Summer Mean±SD	Winter Mean±SD	Summer Mean±SD	
Osmotic Potential (MPa)	- 0.987 ± 0.02^d	- 1.544 ± 0.11^b	- 1.186 ± 0.06^c	- 1.791 ± 0.02^a	0.076
	Plant Water Content				
Plant Water Content ml/100gDW	56 ± 2.82^b	36.9 ± 0.79^d	58.8 ± 2.86^a	52.2 ± 3.05^c	1.105

The values are the mean ±SD (n=3).

Means in a single row followed by the same letters are not significantly different ($P < 0.05$)

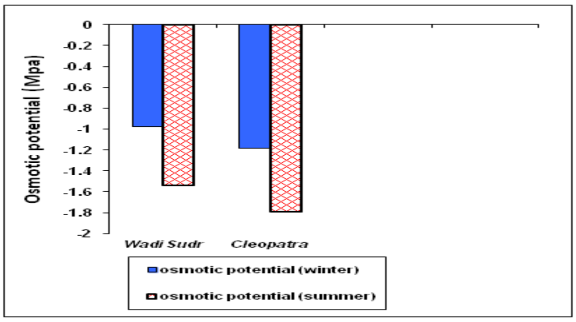

Fig. 2. Plant osmotic potential.

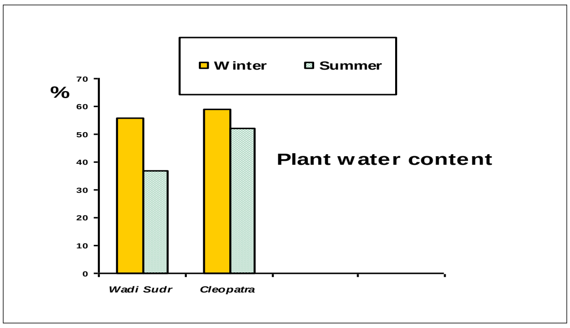

Fig. 3. Plant water content.

Physiological studies

Minerals

The results of mineral analyses (Table 4) indicated that the content of nitrogen in *Retama raetam* was significantly affected by habitats and tended to decrease significantly in dry season, as its values were 3.71 and 4.25g% in winter and 1.88 and 2.15g% in summer at Wadi Sudr and Cleopatra, respectively. Meanwhile, the content of potassium was significantly increased in summer season and significantly different between habitats, since its values were 0.78 and 1.37g% in winter and increased to 1.06 and 1.54g% in summer season at Wadi Sudr and Cleopatra, Respectively. The maintenance of high concentration of K^+ in aerial parts of plants, can be considered necessary for the natural function of cellular and organic physiology (Wyn Jones *et al.*, 1979; Jeschke, 1980, 1984).

Table 4. Mineral composition of *Retama reatm* at different habitats.

Minerals	Locality				LSD
	Wadi Sudr		Hammam Cleopatra		
	Winter Mean±SD	Summer Mean±SD	Winter Mean±SD	Summer Mean±SD	
Nitrogen g%	3.71± 0.50 b	1.88± 0.06 c	4.25 ± 0.38 a	2.15 ± 0.27 c	0.378
Sodium g%	0.50 ± 0.04 a	0.71 ± 0.05 a	0.87 ± 0.06 a	0.90 ± 0.03 a	6.544
Potassium g%	0.78 ± 0.07 d	1.06 ± 0.19 c	1.37 ± 0.07 b	1.54 ± 0.21 a	0.128
Calcium g%	1.44 ± 0.05 c	1.80 ± 0.04a	1.64 ± 0.07 b	1.60 ± 0.04 b	0.080
Magnesium g%	1.03 ± 0.12 c	1.14 ± 0.04 b	0.50 ± 0.04 d	1.43 ± 0.05 a	0.082
Phosphorus g%	0.29 ± 0.04 a	0.40 ± 0.05 a	0.03 ± 0.00 a	0.05 ± 0.00 a	4.952
Sulphur g%	0.20 ± 0.01 a	0.18 ± 0.04 a	0.19 ± 0.05 a	0.23 ± 0.06 a	6.925
Chloride g%	0.04 ± 0.00 a	0.0 3± 0.00 a	0.04 ± 0.00 a	0.03 ± 0.00 a	2.998
ironmg/100g	30.6 ± 0.47 a	29.2 ± 0.57 b	26.1 ± 0.59 c	31.1 ± 0.26 a	0.737
manganesemg/ 100g	8.67 ± 0.06 c	9.47 ± 0.07 a	6.84 ± 0.21 d	9.02 ± 0.11 b	0.173
Zincmg/100g	0.70 ±1.04 c	1.90 ± 0.04 b	3.75 ± 0.12 a	3.45 ± 0.03a	0.612
Coppermg/100g	11.2 ± 0.28 c	13.8 ± 0.03 b	11.2 ± 0.32 c	18.2 ± 0.62 a	0.252

The values are the mean ±SD (n=3).

Means in a single row followed by the same letters are not significantly different (P <0.05)

The contents of calcium and magnesium in *Retama raetam* were significantly increased in summer season at Wadi Sudr, as their values were 1.44 and 1.03g% in winter and increased to 1.80 and 1.14g% in summer. Meanwhile, at Cleopatra, there was no significant change between seasons in calcium content, while the content of magnesium was significantly increased in dry season. The accumulation of high concentrations of Ca^{2+} andmg^{2+} can counteract the harmful effects of Na^+ and Cl^- and considered as a protective adaptive response (Bannister, 1976).

The content of manganese was significantly different between habitats and significantly increased under stress condition, as its values were significantly increased from 8.67 and 6.84mg/100g in wet season to 9.47 and 9.02mg/100g, in dry season, at Wadi Sudr and Cleopatra respectively. Similarity, the content of zinc was significantly affected by habitats, as its values reached the maximum values of 3.75 and 3.45mg/100g at Cleopatra in wet and dry seasons, respectively. It has been reported that Zn reduces Cu toxicity and improves Zn uptake in plants (Hafeez *et al.*, 2013) it also plays a major role in abiotic resistance in various plants.

The results of statistical analysis revealed that there was no significant difference in the content of sodium, phosphorus, sulphur and chloride in *Retama raetma* between seasons and/or habitats. However, the contents of iron, manganese and zinc were significantly affected by seasons and/or habitats.

According to Peuke and Rennenberg (2011), the concentrations of Ca, mg and K in *Retama* are considered in the proper range of the plants' requirement, as their values were greater than 0.1% of dry mass and each of these is considered essential plant nutrient and plays important roles in photosynthesis, enzyme activation, plant structure and growth. Regardless of special functions in physiology and structure, the increase in the concentration of minerals in the dry season may be attributed to their contribution to osmotic adjustment, since the accumulation of ions in vacuole provides less energy-consuming solutes for osmotic adjustment (Flowers *et al.*, 1977).

Total amino acids

As shown in Table 5, the concentrations of total amino acids (protein amino acids and free amino acids) in *Retama raetam* were affected by seasons and/or habitats, as their concentrations were decreased from 115.19 and 114.9mg/g in winter to 108.3 and 88.4mg/g in summer season at Wadi Sudr and Cleopatra, respectively. The dominants amino acids at both habitats in dry and wet seasons were glutamic acid and leucine, in addition to aspartic acid at Wadi Sudr. The concentration of all detected amino acids tended to decrease with stress conditions, except for aspartic acid, as its values were increased in dry season, the aspartate family pathway appears to be essential for generating energy, mainly via lysine catabolism, as stress causes energy deprivation (Planch *et al.*, 2015). It is well known that free amino acids have several functions in plant growth and maybe a part of adaptive responses contributing to osmotic adjustment and has been taken as an index for drought tolerant potential of many plants (Duby, 1994; Gadallah, 1995). However, it would be difficult to recognize their role in this research, as the results obtained from the samples analyzes contain the amino acids involved in protein formation and free amino acids. However, the percentage of crude protein in *Retama raetam* was affected by seasons, as its values were 23.2 and 26.5 g% in winter and decreased to 11.7 and 13.4g% in summer at Wadi Sudr and Cleopatra, respectively (Table 6).

Table 5. Total amino acids in *Retama reteam* at different habitats.

Locality	Seasons	Total Amino Acids mg/g													
		Aspartic acid	Threonine	Serine	Glutamic acid	Glycine	Alanine	Valine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine
Wadi	Winter	11.19	4.7	6.1	16.3	3.1	9	7.2	5.2	12.1	8.1	8.4	6.5	8.5	8.8
Sudr	Summer	12.6	4.5	6.2	15.3	2.7	8.5	6.6	5	10.6	7.2	8	5.3	8.6	7.2
H.	Winter	6.5	4.3	5.9	15.4	2.6	8.8	6.7	10.5	11.1	7	9.9	7.1	8.7	10.4
Cleopatra	Summer	9.1	3.9	5.1	11.8	2.3	7.6	5.9	4	9.2	6.5	6.4	3.4	6.1	7.1

The alternations of proteins under water stress conditions have been observed in many plant species; *Pisum sativum* (Al-Jebory, 2012), *Leucaena leucocephala*, *Prosopis chilensis* (El-Lamey, 2015a) and *Deverra tortuosa* (El-Lamey, 2015b) and moth bean leaves (Vyas *et al.*, 1996). Since the reduction in the rate of protein synthesis and changes in the type of producing protein under stress conditions maybe due to the reduced number of polysomal complexes in tissues(Kabiri *et al.*, 2014) and the oxidation of amino acids by the generated ROS under drought stress(Al-Jebory, 2012)

Table 6. The percentage of crude protein in *Retama reteam* at different habitats.

Locality	Seasons	Crude Protein% Mean±SD
Wadi Sudr	Winter	23.2 ± 3.149 [b]
	Summer	11.7 ± 0.397 [c]
H. Cleopatre	Winter	26.5 ± 2.430 [a]
	Summer	13.4 ± 1.710 [c]
LSD		2.359

The values are the mean ±SD (n=3).

Means in a single row followed by the same letters are not significantly different (P <0.05)

Organic acids

As shown in Table 7, ascorbic acid was the dominant acid followed by citric acid and oxalic acid at both habitats, as its values were 1460 and 2300mg/100g in winter and decreased to 1270 and 2300mg/100g in summer at Wadi Sudr and Hammam Cleopatra, respectively. Meanwhile, the concentrations of citric acid were 360 and 770mg/100g in winter and 350 and 580mg/100g in summer season at the same habitats, respectively. However, oxalic acid tended to increase in dry season and its values were affected by season and/or habitats. The obtained results indicate that the plant response to stress conditions by accumulation of malic acid, as its value increased from 1 to 10mg /100g at Cleopatra. These results suggest that the malic and oxalic acids have important roles in response to drought in *Retama* and their levels can be related to drought tolerance. Similarly, drought induced notable malic and oxalic acid productions in four *Triticum aestivum* L genotypes (Marček *et al.*, 2019). From the previous results it can be concluded that drought stress induced accumulation of the oxalic acids and citric acid in *Retama raetma* at both habitats, in addition to malic acid at Cleopatra. Since environmental or agronomic practices may affect the expression of genes in the seeds, which may affect the organic acid contents (Kader, 2008).

Organic acids are important metabolites of the tricarboxylic acid cycle in plants (Kader, 2008). Not only they act as the intermediates in carbon metabolism, but also involved in various roles in the plant adaptation to nutrient deficiency and metal stress (López-Bucio *et al.,* 2000), and involved in osmotic adjustment as compatible solutes (Morgan, 1984).

Table 7. Organic acids in *Retama reteam* at different habitats.

Locality	Seasons	Organic Acids mg/100g						
		Oxalic Acids	Citric Acid	Lactic Acid	Ascorbic Acid	Formic Acid	Succinic Acid	Malic Acid
Wadi Sudr	Winter	160	360	-	1460	5	-	-
	Summer	240	350	-	1270		-	-
H. Cleopatra	Winter	400	770	130	2300	-	-	1
	Summer	500	580	-	1170	-	-	10

Total soluble sugars and total carbohydrates and alkaloids

It was obvious from Table 8, that there was no significant difference between seasons in the concentration of total soluble sugars at Wadi Sudr, while at Cleopatra, as its values were significantly increased under stress condition. Meanwhile, the concentrations of total carbohydrates in *Ratama* tended to significantly increase in dry seasons and affected by seasons and/or habitats, as its values significantly increased by stress from 8.26 and 8.01g% in the wet season to 12.26 and 11.5 g% in the dry season at Wadi Sudr and Cleopatra, respectively. The same behaviour has been recorded in other drought-tolerant wheat genotypes under stress conditions (Goggin and Setter, 2004; Wardlaw and Willenbrink, 2000). The organic solutes are a well -known compatible osmolytes, act as osmoprotectants, antioxidants and exert beneficial effects upon drought tolerance trees (Larher *et al.*, 2009), as carbohydrates, which may contribute as ROS scavengers and membrane stabilizer (Bohnert and Jensen, 1996).

Table 8. Total soluble sugars and total carbohydrates and alkaloids in *Retama reteam* at different habitats.

Locality	Season	Total Soluble Sugars g% Mean±SD	Total Carbohydrates g% Mean±SD	Total Alkaloidsmg/g Mean±SD
Wadi Sudr	Winter	2.99 ± 0.02 [a]	8.30 ± 0.30 [c]	5.70 ± 0.40 [c]
	Summer	3.37 ± 0.50 [a]	12.3 ± 0.50 [a]	6.20 ± 0.40 [b]
H.Cleopatra	Winter	2.57 ± 0.05 [b]	8.01 ± 0.15 [d]	5.43 ± 0.42 [d]
	Summer	3.05 ± 0.30 [a]	11.5 ± 0.12 [b]	6.50 ± 0.53 [a]
LSD		0.312	0.210	0.065

The values are the mean ±SD (n=3).

Means in a single row followed by the same letters are not significantly different (P <0.05)

Similarly, high concentration of alkaloid was detected in dry seasons, as its values were significantly increased by stress from 5.7 and 5.4mg/g% in winter to 6.2 and 6.5mg/g% in summer at Wadi Sudr and Cleopatra, respectively. The elevation of the level of alkaloid under water stress conditions has been observed in many plant species; *Nicotiana tabacum* (Van Bavel, 1953; Waller and Nowacki, 1978), *Ephedra alata* and *Capparis sinaica* and *Capparis spinosa* (El-Lamey, 2005) *Catharanthus roseus* L (Yu *et al.*, 2018). The mechanism by which water deficits increase the alkaloids levels has been the subject of some speculations. One possible explanation is that stress raises the levels of amino acids and amides, which can serve as a biosynthetic precursor to alkaloids, while other explanation attributed the accumulation of alkaloids to a decline in protein synthesis.

Conclusion

The data of climate and the reduction in water content/potential in leaves of *Retama reteam* indicate that the plant faces a wide range of environmental stresses. The results indicate that *Retama reteam* tended to accumulate osmolytes or osmoprotectants in response to stress, such as inorganic ions (Ca^{2+}, mg^{2+}, K^{+}), carbohydrates, soluble sugars and organic acids (oxalic, citric, malic), which maybe considered as a protective metabolic adaptation that could exert beneficial effects upon drought tolerance of *Retama reteam*. These results revealed that the chemical composition of the plant was significantly affected by seasons and environmental conditions, which may have a negative effect due to the accumulation of some toxic compounds under stress conditions, like alkaloids. So it is an important issue to understand the physiological mechanism of the stress-tolerant plant under different environmental conditions, to realize how variations in plant metabolism can be a response to changes in the surrounding environmental conditions, to develop stress-resistant plants and to explore the genetic basis underlying these mechanisms.

References

Abdel-Halim OB, Sekine T, Saito K, Halim AF, Abdel-Fattah H, Murakoshi. 1992. I. (þ)-12a-hydroxylupanine, a lupin alkaloid from *Lygos raetam*. Phytochemistry **31,** 3251e3.

Abdel-Halim OB. 1995. ()-6a-Hydroxylupanine, a lupin alkaloid from *Lygos raetam* var. sarcocarpa. Phytochemistry **40,** 1323e5.

Algandabymm. 2015. Assessment of acute and subacute toxic effects of the Saudi folk herb *Retama raetam* in rats. Journal of the Chinese Medical Association **78(12),** 691-701.

Al-jebory EI. 2012. Effect of water stress on carbohydrate metabolism during *Pisum sativum* seedlings growth. Euphrates Journal of Agriculture Science **4,** 1-12.

AOAC. 2000. Official Methods of Analysis, 17th Ed. Association of Official Analytical Chemists. Washington D.C., U.S.A.

Arbona V, Manzi M, Ollas C De, Gómez-Cadenas A. 2013. Metabolomics as a tool to investigate abiotic stress tolerance in plants. International Journal of Molecular Sciences **14,** 4885-4911.

Baker AS, Smith RL. 1974. Preparation of solutions for atomic absorption analysis of Fe, Mn, Zn and Cu in plant tissue. Journal of Agriculture and Food Chemistry **22,** 103.

Bannister P. 1976. Introduction to physiological plant ecology. Blackwell Scientific Publications. Oxford, London.

Bohnert HJ, Jensen RG. 1996. Strategies for engineering water-stress tolerance in plants. Trends Biotechnol **14,** 89-97.

Boulos L. 1999. Flora of Egypt. Vol. One (Azollaceae - Oxalidaceae). Al-Hadara Publishing, Cairo, Egypt.

Boulos L. 2009. Flora of Egypt Check list. Revised Annotated Edition. Al-Hadara Publishing, Cairo, Egypt.

Bundy JG, Davey MP, Viant, MR. 2009. Environmental metabolomics: a critical review and future perspectives. Metabolomics **5,** 3 - 21.

Burits M, Bucar F. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy **14,** 323-328.

Buysse J, Merck XR. 1993. An improved colorimetric method to quantify sugar content of plant tissue. Journal of Experimental Botany **44(267),** 1627-1629.

Caceres A, Giron LM, Martinez AM. 1987. Diuretic activity of plants used for the treatments of urinary ailments in Guatemaela. J Ethnopharmacol **43,** 197-201.

Duby RS. 1994. Protein synthesis by plants under stressful conditions. In handbook of plant and crop stress. Ed. Pessarki M. pp. 277-299. New York. Marcel Decker Inc.

El Bahri L, Djegham M, Bellil H. 1999. *Retama raetam* W: a poisonous plant of North Africa. Veterinary Human Toxicology **41,** 33 - 35.

El-Nennah M, El-Kadi MA, Kamh RN, El-Sherif S. 1981. “Forms of copper and zinc in soils of Sinai". Desert Institute Bulletin, ARE **31(B),** 129-139.

El-Lamey TM. 2005. The effect of some ecological factors on the chemical compounds in some Plant xerophytes for enhancing their use. Ph.D. Thesis, Institute of Environmental Studies and Research, Ain Shams University, Cairo, Egypt.

El-Lamey TM. 2015a. Morphological and anatomical responses of *Leucaena leucocephala* (Lam.) de Wit. and *Prosopis chillensis* (Molina) Stuntz. to Ras Sudr conditions. Journal of Applied Environmental and Biological Sciences **5(7),** 43-51.

El-Lamey TM. 2015b. Contribution of solutes to the osmotic adjustment of *Deverra tortuosa* (Desf.) DC. Journal of Biodiversity and Environmental Sciences **7(1),** 380-395.

El-Shazly A, Ateyaa AM, Witte L, Wink M. 1996. Quinolizidine alkaloid profiles of *Retama raetam*, *R. sphaerocarpa* and *R. monosperma*. Z Naturforsch **51,** 301e8.

FAO. 2007. (http://www.fao.org/home/en/).

Flowers TJ, Troke PF, Yeo AR. 1977. The mechanisms of salt tolerance in halophytes. Annual Review of Plant Physiology **28,** 89 - 121.

Gadallah MAA. 1995. Effect of water stress, abscisic acid and proline on cotton plants. Journal of Arid Environments **30,** 315-325.

Glantz SA. 1992. “Primer of Biostatistics” 3rd ed, New York: McGraw Hill.

Goggin DE, Setter TL. 2004. Fructosyltransferase activity and fructan accumulation during development in wheat exposed to terminal drought. Functional Plant Biology **31,** 11-21.

Grattana SR, Grievebcm. 1999. Salinity-mineral nutrient relations in horticultural crops. Scientia Horticulturae **78,** 127-157.

Hafeez B, Khanif YM, Saleem M. 2013. Role of zinc in plant nutrition - A review. American Journal of Experimental Agriculture **3,** 374-391.

Harborne JB. 1973. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd., London.

Issar A, Gilad D. 1982. Ground water flow systems in the arid crystalline province of Southern Sinai. Journal of Hydrogeology Science **27(3),** 309-325.

Jackson WA, Thomas GW. 1960. Effect of KCl and dolometric limestone on growth and ion uptakeof sweet potato. Soil Science **89,** 347-352.

Jacksonml. 1967. Soil chemical analysis. Hall of India Private, New Delhi, India. Printice. Hall Inc NJ 248 p.

James CS. 1995. Analytical chemistry of foods. Blackie Academic and Professional Publisher. An imprint of Champman and Hall 178 p.

Jeschke WD. 1980. Involvement of proton fluxes in K^+-Na^+ selectivity at the plasmalemma; K^+-dependent net extrusion of sodium in barley roots and the effect of anions and pH on sodium fluxes. Z. Pflanzenphysiol **98,** 155-175.

Jeschke WD. 1984. Effects of transpiration on potassium and sodium fluxes in root cells and the regulation of ion distribution between roots and shoots of barley seedlings. Journal of Plant Physiology **117,** 267-285.

Jones JB. 1977. Elemental analysis of soil extracts and plant tissue ash by plasma emission spectroscopy. Communication in Soil Science and Plant Analysis **8,** 349-365.

Jones OAH *et al.* 2013. Metabolomics and its use in ecology. Austral Ecology **38,** 713 -720.

Jouban Z. 2012. The effects of salt stress on plant growth. Engineering, Technology & Applied Science **2(1),** 7-10.

Kabiri R, Nasibi F, Farahbakhsh H. 2014. Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in *Nigella sativa* plant under hydroponic culture. Plant Protection Science **50,** 43 - 51.

Kader AA. 2008. Flavor quality of fruits and vegetables. Journal of the Science of Food and Agriculture **88,** 1863.

Kassem M, Mosharrafa SA, Saleh NAM, Abdel-Wahab SM. 2000. Two flavonoids from *Retama raetam*. Fitoterapia **71,** 649e54.

KimH K, Choi YH, Verpoorte R. 2010. NMR-based metabolomic analysis of plants. Nature Protocols **5,** 536 - 549.

Kjeldahl J. 1983. The Kjeldahl determine of nitrogen: retrospect and prospect. Trends in analytical Chemistry **13(4),** 138 p.

Lambers H, Poorter H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Advances in Ecological Research **23,** 187-261.

Larher FR, Lugan R, Gagneul D, Guyot S, Monnier C, Lespinasse Y, Bouchereau A. 2009. A reassessment of the prevalent organic solutes constitutively accumulated and potentially involved in osmotic adjustment in pear leaves. Environmental and Experimental Botany **66,** 230-241.

López-Bucio J, Nieto-Jacobo MF, Ramírez-Rodríguez V, Herrera-Estrella L. 2000. Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. Plant Science **160(1),** 1-13.

Marček T, Hamow KA´, Ve´gh B, Janda T, Darko E. 2019. Metabolic response to drought in six winter wheat genotypes. PLoS ONE **14(2),** e0212411.

Marschner H. 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Migahid AM, El Shafi MA, Abo El Rahman AA, Hammouda MA. 1959. Ecological observations in western and southern Sinai. Bulletin de la Societe de Geographie d'Egypte **32,** 165 -205.

Mittler R, Merquiol E, Hallak-Herr E *et al.* 2001. Living under a "dormant" canopy: a molecular acclimation mechanism of the desert plant *Retama raetam*. Plant Journal **25,** 407 - 416.

Morgan JM. 1984. Osmoregulation and water stress in higher plants. Annual Review of Plant Physiology **35,** 299-319

Moustafa AA, Zaghloul MS, Abdel-Wahab RH, Shaker M. 2001. Evaluation of plant diversity and endemism in Saint Catherine Protectorate, South Sinai, Egypt. Egyptian Journal of Botany **41,** 123-141**.**

Moustafa AM, Zayed AM. 1996. "Effect of environmental factors on the flora of alluvial fans in southern Sinai". Journal of Arid Environments **32,** 431-443.

Osborne CP, Mitchell PL, Sheehy JE, Woodward FI. 2000. Modeling the recent historical impacts of atmospheric CO_2 and climate change on Mediterranean vegetation. Global Change Biology **6,** 445-458.

Page AL. 1987. Methods of soil analysis, part 2.nchemical and microbiological properties-agronomy monograph. No. 9. American Society of Agronomy Inc., Madison 167-179.

Patakas A, Noitsakis B. 1999. Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions. Journal of Plant Physiology **154,** 767-774.

Pavarini DP, Pavarini SP, Niehues M, Lopes NP. 2012. Exogenous influences on plant secondary metabolite levels. Animal Feed Science and Technology **176,** 5-16.

Pellet PL, Young V. 1980. Nutritional Evaluation of Protein Foods. The United Nations University, Tokyo pp. 154.

Peuke AD, Rennenberg H. 2011. Impacts of drought on mineral macro-and microelements in provenances of beech (*Fagus sylvatica* L.) seedlings. Tree Physiology **31,** 196 - 207.

Planchet E, Anis M, Limami JP, d'Mello. 2015. Amino acid synthesis under abiotic stress. Amino acids in higher plants, CAB International, pp. 262-276, 978-1-78064-263-5.

Ramakrishna A, Ravishankar GA. 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling and Behavior **6,** 1720-1731.

Rowell DL. 1994. Soil science: methods and application. Longman Publishers, Singapore 350 p.

Simmelsgaard SE. 1976. Adaptation to water stress in wheat. Physiologia Plantarum **37,** 167-174.

Singh P, Basu S, Kumar G. 2018. Polyamines Metabolism: A Way Ahead for Abiotic Stress Tolerance in Crop Plants. In Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants; Elsevier: Amsterdam, The Netherlands pp. 39-55.

Subbarao GV, Nam NH, Chauhan YS, Johansen C. 2000. Osmotic adjustment, water relations and carbohydrate remobilization in *pigeonpea* under water deficits. Journal of Plant Physiology **157,** 651-659.

UNESCO. 1977. Map of the World Distribution of Arid Regions, MAB, Paris.

Van Bavel CHM. 1953. Chemical composition to tobacco leaves as affected by soil moisture conditions. Agronomy Journal **45,** 611-614.

Veste M, Staudinger M, Küppers M. 2008. Spatial and temporal variability of soil water in drylands: plant water potential as a diagnostic tool. Forestry Studies in China10 74-80.

Vyas SP, Kathju S, Garg BK, Lahiri AN. 1996. Activities of nitrate reductase and ammonia assimilating enzymes of moth bean under water stress. Science and culture Journal **62,** 213-214.

Waller GR, Nowacki EK. 1978. Alkaloid biology and metabolism in plants. Plenum Press, New York.

Wardlaw IF, Willenbrink J. 2000. Mobilization of fructan reserves and changes in enzyme activities in wheat stems correlate with water stress during kernel filling. New Phytologist **148,** 413 - 422.

Wink M. 1987. Chemical Ecology of quinolizidine Alkaloids. In Allelochemicals. Role in agriculture and forestry. G.R. Waler, ed., Amer. Chem. Soc. Symp Ser. **330,** 525-533, Washington, DC.

Wink M. 1988. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theoretical and Applied Genetics **75,** 225-233.

Wyn Jones RG, Brady CJ, Speirs J. 1979. Ionic and osmotic relation in plant cell. In recent advances in the biochemistry of cereal: Iaidman, D. L. and Wyn Jones, R. G., Eds pp. 63-103. Academic Press. London.

Yu B, Liu Y, Pan Y, Liu J, Wang H, *et al.* 2018. Light enhanced the biosynthesis of terpenoid indole alkaloids to meet the opening of cotyledons in process of photomorphogenesis of *Catharanthus roseus*. Plant Growth Regulation **84(3),** 617-626.

Zbigniew JW, Bogumit T, Marck S. 1991. Chromatographic determination of citric acid monitoring the mould process. Journal of Chromatography A **558(1),** 302- 305.

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P. 2017. Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of The National Academy of Sciences. USA **114,** 9326-9331.

Zohary M, Fahn A. 1952. Ecological studies on East Mediterranean dune plants. Bull Res Counc Israel **1,** 38-53.