

# **Original Research Article**

## **Effect of different habitats conditions on *Citrullus colocynthis* (L.) Schrad. growing naturally in Egypt and Kingdom of Saudi Arabia**

### **Abstract**

Harsh environmental conditions are major factors limiting plants production and development, including *Citrullus colocynthis*. The present investigation provides an analysis of soil properties and plant compounds of *C. colocynthis* in Wadi Hebran, Saint Katherine, South Sinai, Egypt and Wadi Al-Akhder, Tabuk Province, KSA at dry and wet seasons, and focuses on the relationship between the environmental factors and phytochemical compounds using principal component analysis (PCA). According to ANOVA, the experimental factors (locations, depths, seasons and their interactions significantly influenced the physico-chemical soil properties of soil and chemicals present in compounds of *C. colocynthis* ( $P < 0.05$  or  $0.01$ ). Wadi Hebran at both seasons significantly promoted most mechanical and chemical properties in soil and most chemical compositions in *C. colocynthis* compared with Wadi Al-Akhder. While the significantly positive highest impact on photosynthetic pigments contents was found in Wadi Al-Akhder at both seasons. The dry season in the two locations significantly increased the total carotenoids, Chl.a/b, total pigment, P %, total carbohydrate, proline, catalase enzyme, peroxidase enzyme, ascorbic acid and malondiadehyde compared to the wet season. Based on PCA analysis, the first two PCs explain more than 84% of the total variance in the original variables and mainly distinguished the soil and plant variables in different groups during each location or between the two locations at both seasons. The first PC showed the highest positive correlation with some soil variables (EC,  $K^+$ , and  $Cl^-$ ) and plant variables (P, total carbohydrate, proline, catalase enzyme, peroxidase enzyme and malondiadehyde). While the second PC exhibited a highly positively correlated with  $Ca^{2+}$ ,  $S^-$  and water content in soil and with Chl.b,  $K^+$ ,  $Ca^{2+}$ , N, water content, crude protein, crude fiber, total ash and superoxide enzyme in *C. colocynthis* plants. Positive correlations among most soil and plant variables were found, but they differed in their degree and consistency in quantity. PCA application indicated that the above soil variables are related closely to the above plant variables of *C. colocynthis* in the two locations. The results of PCA could be useful and used as a suitable method for studying the stresses tolerance mechanisms of plants under hard environmental conditions.

**Key words:** *C. colocynthis*, soil properties, phytochemical compounds, PCA.

### **1. Introduction**

Climate changes could play a major role in modifying species distribution in desert areas controlling environmental heterogeneity and disturbances effects on plant species diversity (Khafagi *et al.*, 2013). Ackerly *et al.* (2000) stated that ecophysiological studies have been powerful in elucidating plant function and identifying traits that are adaptive in specific environmental conditions. Drought stress is a major factor limiting plant growth, production and development, thus, water is essential for plant growth. High drought stresses led to decreased photosynthesis, disrupted physiological processes and reduced plant biomass, at last, expected plant death (Do *et al.*, 2013; Saberi *et al.*, 2017). Drought stress tolerance is a complex trait,

due to interaction among several factors (Blum, 1988). To adapt and thrive to drought stress, plant species have developed various strategies to deal with extreme changes in the environment in all kinds of climates and terrains (Wang *et al.*, 2014). Aldamegh *et al.*, (2013) mentioned that dry weather conditions may activate the production of secondary phytochemical compounds with high concentrations. Also, Pradhan *et al.*, (2020) and Salama *et al.*, (2021) added that stress conditions induce the accumulation of numerous reactive oxygen species and osmolytes such as proline, soluble proteins, soluble sugars, and betaine, which will play a critical role during stress **acclimatization** in plants. Maybe, these compounds made these plants more capable of resistance to salinity and drought stresses (Al-Ghamdi, 2015). In desert plants, drought tolerance is due to the plant's ability to preserve turgidity and water absorption (Sayed *et al.*, 2013). Also, it is achieved by morphological, physiological and molecular changes in plants (Levitt, 1980; Bartels & Sunkars, 2005).

The Sinai Peninsula is an arid to the extremely arid region and has a varied heritage of plant diversity, which is about 1285 species (Ayyad *et al.*, 2000). Because of the high rainfall, South Sinai is one of the three richest places in Egypt in terms of biodiversity, in addition to, the Mediterranean coast and Jebel Elba in the far southwest of the country (Gilbert & Zalat, 2021). In South Sinai, the flora consists of about 472 species; of which 19 species are Egyptian endemic species and 115 are of medicinal interest and about 170 are used in folk medicine (Fayed & Shaltout, 2004). Saint Katherine Protectorate is one of the most floristically varied spots in the Middle East (Boulos, 2009), with 410 species have been reported by Shaltout *et al.*, (2004).

The Kingdom of Saudi Arabia (KSA) is an arid region and it has a varied heritage of plant diversity (Al-Mutairi, 2017). KSA contains 2290 plant species belonging to 855 genera in 131 families, of which 9 species are Gymnosperms and 27 are Pteridophytes, with about 200 regional endemics (Thomas *et al.*, 2015). Some of these plants are showing important genetic resources of medicinal (Collentette, 1999). According to the theory of biogeography, KSA flora has strong ties with those of North Africa, East Africa and Mediterranean countries (Alfarhan, 1999). The climate of Tabuk is considered an arid region (Al-Nafie, 2008) and it had remarkable floristic diversity (Al-Mutairi *et al.*, 2016). Moawad & Ansari (2015) released a checklist on Tabuk area flora that includes 82 wild plant species belonging to 66 genera and 30 families. Alghanem *et al.*, (2020) identified plant species of Al-Wadi Al-akhder from Tabuk region, which registered 30 species belonging to 23 genera and 15 families.

The Cucurbitaceae family is considered as one of the families that possess economically important species that are used as food or fodder (Hussain *et al.*, 2014). This family comprises about 110 genera and 560–850 species of plants (Mariod *et al.*, 2017). *Citrullus colocynthis* (L.) Schrad (***C. colocynthis***) belongs to the Cucurbitaceae family, it is an uncultivated and annual useful plant that commonly grows in the desert regions of the world (as Egypt and Saudi Arabia), possess great tolerance to salinity and drought stresses as well as desert extreme conditions (Abdulridha *et al.*, 2020; Darwish *et al.*, 2021). Wang *et al.*, (2014) reported that *C. colocynthis* can survive in arid environments by maintaining its water content at sharp stress conditions. Previous studies on *C. colocynthis* reported many medicinal benefits against different ailments including decrease blood sugar and against breast cancer (Tannin-Spitz *et al.*, 2007), diabetes type II (Huseini *et al.*, 2009), anti-inflammatory and anti-bacterial activities (Shahid & Rao, 2014) and against colorectal cancer cell lines (Abdulridha *et al.*, 2020). In addition to its important medicinal applications a source of valuable oil (Dane *et al.*, 2007), seeds oil can be used as low-cost biodiesel (Giwa *et al.*, 2010), natural insecticide (Torkey *et al.*, 2009), and a viable source of

genes for promoting pest and disease resistance for the commonly cultivated watermelon cultivars (Levi *et al.*, 2017).

Soil pH also affects the physical, chemical and biological properties of the soil as well as plant growth (Al-Mujahidy *et al.*, 2013). Since soil and plant are interrelated, the soil organic matter content and nature and type of vegetation influence the physical, chemical, and biological properties of soil (Kim *et al.*, 1995; Singwane & Malinga, 2012). The influence of soil variables on the plant species had been studied out by several workers/studies, such as Al-Ghamdi (2015), Al-Mutairi (2017), Salama *et al.*, (2017) in *C. colocynthis* plants and El-Ghani & Amer (2003) in other plant species.

Several authors indicated a positive correlation between proline accumulation and drought stress in plants. Proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule (Hayat *et al.*, 2012). The plants produce a huge number of metabolites to adapt to the stress conditions as chlorophylls and carotenoids (Mibei *et al.*, 2016; El-Absy, 2021). Some studies have investigated metabolites in *C. colocynthis*, the results suggest that osmotic adjustment was the main water relationship adaptation to cope with drought stress (Sayed *et al.*, 2013; Wang *et al.*, 2014; Salama *et al.*, 2017). El-Absy (2021) explained that most of the chemical compounds in the plant were significantly affected by seasonal changes. This may reflect seasonal changes in physiological needs and effort, rather than availability in plant content (Estevez *et al.*, 2010).

The principal component analysis (PCA) is one of the Multivariate statistical techniques tools that can be used to study the relationship between the environmental parameters and plant variables. Many researchers have used the PCA to assess the relationship and diversity between measured soil properties and vegetation (Casas & Ninot, 2003; El-Ghani & Amer, 2003; Kooch *et al.*, 2008; Iwara, 2011; Ferraz *et al.*, 2019; Metwally *et al.*, 2019; Abdel-Fattah *et al.*, 2021; El-Absy, 2021).

The present study aims to identify the responses of the *C. colocynthis* growing in the arid desert regions in different environments (Egypt and KSA) and to show its adaptive mechanism to drought tolerance through: a) calculating soil physico-chemical properties associated with plants, b) measuring morphological, anatomical and physiological parameters in the plants and (c) estimating the relation between the environmental factors and phytochemical compounds using PCA, to understand its adaptive behavior and the possibility of osmotic adjustment adopted by these plants to tolerate of harsh environmental conditions during the wet and dry seasons.

## 2. Materials and methods

### 2.1. Study area

The present study was carried out along two sites: Wadi Hebran, Saint Katherine, South Sinai, Egypt and Wadi Al-Akhder, Tabuk Province, Saudi Arabia. The two sites were visited from August 2019 (dry) to January 2020 (wet). Wadi Al-Akhder is the most important regional wadis in Tabuk region, and is situated about 120km from the Tabuk and does not directly cross Tabuk city. It lies located southeast of Tabuk at the intersection of 28° 1' 4" N and 36° 39' 19" E and extends between latitude: 28° 1' 4" N and longitude: 36° 39' 19" E. This site is created from Ghawanim Mountains in the east and is 68 km long and connected to Wadi Mishash Bani Atiyah and Tuus Al Arqanah in the upland. This site is characterized by a hot deserts climate, also has unique plant species communities due to its location in North part of KSA (Abushandi & Alatawi, 2015; Alghanem *et al.*, 2020). Wadi Hebran is part of the route from El Tur on the Gulf of Suez to Mount Sinai, and runs parallel to but north of

Wadi Isla, and which opens onto the Plain of El Qa'a. This site lies at the intersection of 28°25'35.9" N and 33°35'20.9" E. between latitude: 28°25'35.9" N and longitude: 33°35'20.9" E (Zalat *et al.*, 2008; Haggag, 2015).

The Meteorological data of temperature, relative humidity and rainfall were provided by the Applied Agricultural Meteorological Laboratory of the Desert Research Center, Egypt (Table 1). The grand mean of average temperature, relative humidity and rainfall in Wadi Hebran were higher than in Wadi Al-Akhder. The highest temperatures occur in August at both locations. The highest percentage of relative humidity were registered in May and January across Wadi Hebran and Wadi Al-Akhder, respectively. During/In the two locations, the rainfall rates were increased in December and January compared with other months.

Table 1. Monthly average temperature (°C), relative humidity (%) and rainfall (mm) at each location in both seasons.

Months	Wadi Hebran			Wadi Al-Akhder		
	Average temperature (°C)	Relative humidity (%)	Rainfall (mm)	Average temperature (°C)	Relative humidity (%)	Rainfall (mm)
Jan	14.7	59	3.4	12	48	3.7
Feb	15.2	58	2.3	13	37	2.8
Mar	17.8	63	2.2	16	28	0.6
Apr	25.7	69	0.2	23	21	0.8
May	29.1	70	0.2	28	18	1.8
Jun	26.7	69	0.0	31	17	0.0
Jul	28.8	64	0.0	31	19	0.0
Agu	30.3	63	0.0	33	21	0.0
Sep	29.4	65	0.0	30	23	2.2
Oct	24.5	60	1.7	26	28	0.0
Nov	21.1	59	1.9	14	41	0.0
Dec	17.4	55	3.6	15	46	2.9
Grand mean	23.39	62.83	1.29	22.67	28.92	1.23

## 2.2. Description of *C. colocynthis* (L.) Schrad.:

Handal (*C. colocynthis*) is a very scabrid herb with long trailing branches growing in the arid region with perennial nature (Fig. 1 and 2). It has long, fleshy and perennial rootstock. The stems are spread in all directions for some things over which to climb and rough in texture with prominent rough hairs at 3 meters in length. Leaves are deeply lobed arranged alternatively on the petioles length 5-10 cm and width 1.5-2 cm. The flowers are small yellow. Each plant produces 15 to 30 fruits very bitter, round in shape, smooth textured, fleshy mottled with dark-green, turning dry and yellow when ripe, each one contains seeds of about 200-300. Seeds small, smooth textured, brown. Reproduction by seeds and vegetative buds in rootstocks. The plants flourish in sandy loam, sub-desert soils, along sandy seacoasts, as well as when the annual temperature and annual rainfall averages range from 23 to 27°C and from 25 to 37cm, respectively (Duke, 1978; Al-Ghamdi *et al.*, 2009; El-Keblawy *et al.*, 2017 and Bhasin *et al.*, 2020).





**Fig. 1.** *C. colocynthis* in Wadi Al-Akhder, Tabuk Province, Saudi Arabia.



**Fig. 2.** *C. colocynthis* in Wadi Hebran, Saint Katherine, South Sinai, Egypt.

### **2.3. Soil physical and chemical properties**

Soil samples were collected from the soil associated with *C. colocynthis* carefully made from three random points at the two depths 0-20 cm and 20-40 cm from the two Wadis studied. The soil samples were carried to the laboratory in closed tins to be used for soil physical and chemical analyses. Soil samples were air-dried, sieved and used for mechanical analysis of soil particles as suggested by Jackson (1967) and Rowell (1994) for soil texture, and they are expressed as a percentage of the original weight. The soil moisture content was calculated according to the method described by Rowell (1994). The electrical conductivity (EC) and pH value for each sample were carried out using soil-water paste, according to Jackson (1962), EC was expressed as ds/m. The mineral contents of soil including sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), sulfur ( $\text{S}^-$ ) and chlorides ( $\text{Cl}^-$ ; meq/L) were determined using a saturation paste described by Tuzuner (1990).

### **2.4. Plant analysis**

The samples of *C. colocynthis* were collected manually in triplicates at random from the two sites studied during the dry and wet seasons of 2019 and 2020, respectively. The samples were placed in plastic bags at the sites, then transferred immediately to the laboratory for preparation. Drying of collected plant materials was done in oven at  $70^\circ\text{C}$  to a constant weight after which dried samples were milled to a

fine powder and stored in brown bags at room temperature pending minerals and metabolites determinations.

The concentrations of Sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ) and calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), and phosphorus (P) were determined by atomic absorption spectrophotometry (GBC Avanta E, Victoria, Australia) [Chapman, 1965]. Total nitrogen (N) content was determined using the micro-Kjeldahl method (Bremner, 1965). The photosynthetic pigments parameters were quantified spectrophotometrically, and using the wavelengths of 663, 645 and 470 nm, the chlorophyll a (Chl.a), chlorophyll b (Chl.b) and total carotenoids were calculated by equations of Lichtenthaler (1987), respectively. The crude protein % was determined by multiplying the total nitrogen by 6.25 according to Allen (1989). The plant water content was obtained following the equation described by Jin *et al.*, (2017). The proline and the total available carbohydrate contents were determined according to the methods of Bates *et al.*, (1972) and Chaplin & Kennedy (1994), respectively. Total ash and crude fiber were determined by AOAC (2000) methods. Soluble proteins were extracted according to Beauchamp and Fridovich (1971) and used in the assay of catalase (CAT; EC 1.11.1.6), peroxidase (POX; EC 1.11.1.7) and superoxide dismutase (SOD; 1.15.1.1) according to Kato & Shimizu (1987). Enzyme activities were expressed in  $\mu\text{M}$  of the substrate converted  $\text{min}^{-1} \text{g}^{-1}$  fresh weight. Ascorbic acid concentration was determined according to Rai (2001) and expressed in  $\text{mg } 100\text{ml}^{-1}$ . Malondialdehyde (MDA); a peroxidation product of the unsaturated fatty acid linolenic acid, 18:3) was estimated as  $\mu\text{M } \text{g}^{-1}$  fresh weight using the method of Heath & Packer (1968).

## 2.5. Statistical analysis:

The normality of data distribution was verified using the Komolgorov-Smirnov test. Then, the measured data were subjected to a two and three-way ANOVA test and the coefficient of variation (CV%) to determine the significant differences ( $p \leq 0.05$  and  $p \leq 0.01$ ) of the effect of experimental factors and their interactions according to the method of Steel & Torrie (1997). The obtained data were expressed as mean  $\pm$  standard error (SE) and multiple comparisons were determined using the least significant difference test (L.S.D) at 0.05 level of probability (Steel & Torrie, 1997). Principal component analysis (PCA) was applied for a better understanding of the relationship among traits studied across experimental factors. The ANOVA and PCA were performed using the computer software programs SPSS version 20, PAST version 4.03 and OriginPro 2018 b9.5.0.193.

## 3. Results

### 3.1. Soil analysis

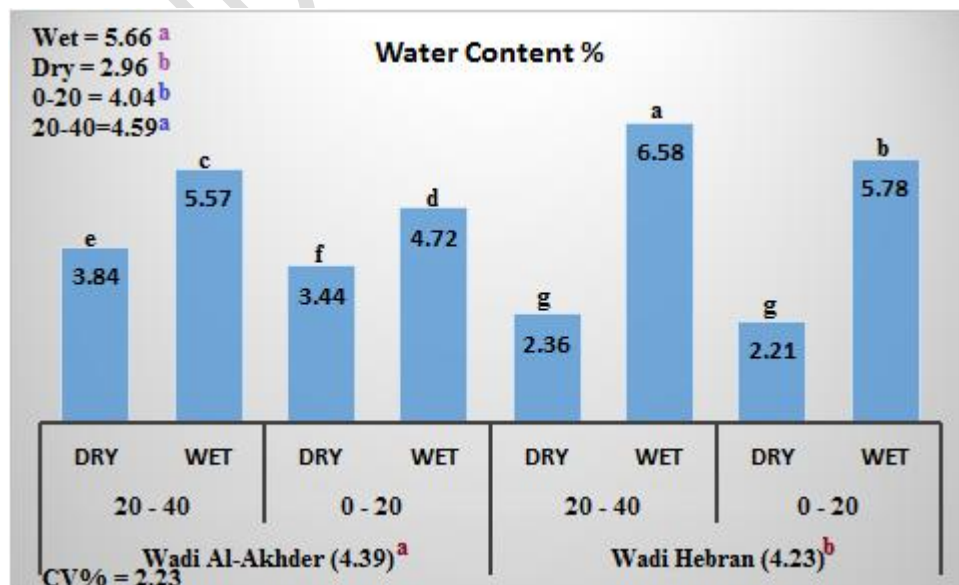
Table 2 outlines the detailed results of two-way ANOVA for the effects of locations (L), depths (D) and their interaction (LD) on mechanical properties % of the adjoining soil samples of *C. colocynthis*. All mechanical properties % in *C. colocynthis* soil were significantly affected ( $P < 0.05$  or  $0.01$ ) by L, D and interaction LD, except D on very fine sand. Significant differences between the two depths in the two wadis were found for mechanical properties % at *C. colocynthis* soil. The highest percentage of coarse sand was found in the soil associated soil of *C. colocynthis* in 0 - 20 depth at KSA location, followed by fine and medium sands, compared with 20 - 40 depth at Egypt location. Compared with other interactions LD, the maximum fine sand % and coarse sand % were registered by interactions Wadi Hebran x 20 - 40 depth and Wadi Al-Akhder x 0 - 20 depth, respectively.

Table 2. Effect of the locations, depths and their interaction on mechanical properties % of the adjoining soil samples of *C. colocynthis*.

Factors	Very Coarse Sand	Coarse Sand	Medium Sand	Fine Sand	Very Fine Sand	Clay and Silt	Soil Texture Class
Locations							
WH	9.02±0.40b	6.41±0.52b	23.54±0.71a	29.16±0.52a	22.51±0.37a	9.36±0.38a	Sandy
WAA	51.82±0.62a	25.11±0.35a	16.36±0.84b	2.37±0.40b	3.08±0.31b	1.26±0.21b	Sandy
P-Values	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	
Depths							
0 – 20 (D1)	31.29±3.69a	16.39±4.11a	18.40±1.73b	15.06±5.95b	12.78±4.29a	6.08±1.88a	Sandy
20 – 40 (D2)	29.54±3.45b	15.13±4.28b	21.50±1.55a	16.47±6.05a	12.81±4.42a	4.55±1.59b	Sandy
P-Values	0.00**	0.00**	0.00**	0.00**	0.81 <sup>ns</sup>	0.00**	
Locations x Depths							
WH x D1	9.64±0.48c	7.24±0.62c	22.19±0.65b	28.34±0.38b	22.34±0.52a	10.25±0.39a	Sandy
WH x D2	8.39±0.31d	5.58±0.46d	24.89±0.47a	29.98±0.46a	22.68±0.67a	8.48±0.42b	Sandy
WAA x D1	52.94±0.54a	25.54±0.39a	14.61±0.35d	1.78±0.64d	3.22±0.44b	1.91±0.31c	Sandy
WAA x D2	49.69±0.67b	24.68±0.33b	18.11±0.40c	2.95±0.29c	2.95±0.37b	1.62±0.34d	Sandy
P-Values	0.04*	0.05*	0.01*	0.09*	0.06*	0.00**	
C.V.%	1.09	1.83	0.98	1.92	1.80	0.75	

WH: Wadi Hebran; WAA: Wadi Al-Akhder; Statistically significant differences at  $*p \leq 0.05$  and  $**p \leq 0.01$ ; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at  $p \leq 0.05$  according to the LSD test

The results of three-way ANOVA revealed a significant effect ( $P < 0.05$  or  $0.01$ ) of the locations, depths, seasons as well as their first and second-order interactions on the water content % in *C. colocynthis* soil (Fig. 3). Significantly, the water content increased with Wadi Al-Akhder soil and decreased with Wadi Hebran soil. Also, the water content in soil is significantly increased in 20 - 40 depth compared to 0 - 20 depth. Naturally, it is significantly increased with the wet season than with the dry season. The interaction of 20-40 depth in Wadi Hebran during the wet season recorded a significantly higher water content % compared with the other interaction among factors studied. In contrast, when comparing all interactions, minimum water content % were found in 0-20 depth at the Wadi Hebran during the dry season.





**Fig.3.** Water content % at the soil associated with *C. colocynthis* in the two depths and the two seasons during Wadi Hebran; WAA: Wadi Al-Akhder. Statistically significant differences at  $*p \leq 0.05$  and  $**p \leq 0.01$ ; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at  $p \leq 0.05$  according to the LSD test

The ANOVA generally showed a significant effect ( $P < 0.05$  or  $0.01$ ) of L, D and interaction LD on all chemical properties in *C. colocynthis* soil (Table 3). The two wadis and depths differently influenced the chemical properties in soil of *C. colocynthis*. In the Wadi Hebran, a significantly higher  $\text{Ec}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{S}^-$  and  $\text{Cl}^-$  were noticed with respect to the Wadi Al-Akhder. As for the two depths,  $\text{Ec}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Cl}^-$  contents were higher in 20 - 40 than in 0 - 20. The highest  $\text{Ec}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in the Wadi Hebran x 20-40 interaction, the highest  $\text{Ca}^{2+}$  and  $\text{S}^-$  in the Wadi Hebran x 0-20 interaction and the highest pH and  $\text{Mg}^{2+}$  in the Wadi Al-Akhder x 20-40 interaction were observed.

Table 3. Effect of the locations, depths and their interaction on soil chemical properties of the adjoining soil samples of *C. colocynthis*.

Factors	Ec (ds/m)	pH	$\text{Na}^+$ (meq/L)	$\text{K}^+$ (meq/L)	$\text{Ca}^{2+}$ (meq/L)	$\text{Mg}^{2+}$ (meq/L)	$\text{S}^-$ (meq/L)	$\text{Cl}^-$ (meq/L)
Locations								
WH	2.91±0.11a	6.88±0.04b	7.75±1.26a	2.10±0.07a	8.43±0.85a	87.72±1.86b	2.36±0.65a	14.61±1.00a
WAA	1.53±0.22b	7.45±0.01a	6.27±1.23b	0.74±0.14b	5.20±0.20b	89.73±2.18a	1.46±0.22b	8.71±0.12b
P-Values	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Depths								
0 - 20 (D1)	1.87±0.38b	7.21±0.10a	4.19±0.33b	1.19±0.34b	7.54±1.24a	88.36±1.57b	2.38±0.64a	10.67±0.76b
20 - 40 (D2)	2.56±0.24a	7.12±0.15b	9.83±0.34a	1.64±0.27a	6.09±0.20b	89.09±2.47a	1.43±0.24b	12.65±1.88a
P-Values	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Locations x Depths								
WH x D1	2.71±0.14b	6.98±0.04b	4.94±0.09c	1.96±0.02b	10.32±0.17a	91.88±0.52b	3.81±0.09a	12.36±0.15b
WH x D2	3.11±0.12a	6.78±0.03c	10.57±0.18a	2.24±0.04a	6.53±0.19b	83.57±0.67d	0.91±0.10c	16.85±0.19a
WAA x D1	1.03±0.10d	7.45±0.02a	3.45±0.04d	0.43±0.02d	4.76±0.23d	84.85±0.49c	0.96±0.08c	8.97±0.09c
WAA x D2	2.02±0.11c	7.46±0.01a	9.09±0.02b	1.04±0.03c	5.64±0.10c	94.60±0.62a	1.96±0.11b	8.45±0.11d
P-Values	0.01*	0.00**	0.09*	0.00**	0.00**	0.00**	0.00**	0.00**
C.V.%	5.52	0.26	2.21	3.54	0.57	2.64	2.36	0.62

WH: Wadi Hebran; WAA: Wadi Al-Akhder; Statistically significant differences at  $*p \leq 0.05$  and  $**p \leq 0.01$ ; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at  $p \leq 0.05$  according to the LSD test.

### 3.2. Plant analysis

The effects of locations (L), seasons (S) and their interaction (LS) on all photosynthetic pigments contents in *C. colocynthis* were significant ( $P < 0.05$  or  $0.01$ ) (Table 4). The photosynthetic pigments contents of *C. colocynthis* growing in Wadi Al-Akhder were significantly greater than that of Wadi Hebran. A significant increase of Chl.a, Chl.b and Chl.a+b contents were observed in the wet season compared to the dry season. While, in the dry season a significant increase of the total carotenoids, Chl.a/b (more than 1) and total pigment contents were noticed than in the wet season. Relative to the effect of interaction LS, Wadi Al-Akhder in both seasons displayed significantly higher photosynthetic pigments contents compared with Wadi Hebran in both seasons.

Table 4. Effect of the locations, seasons and their interaction on photosynthetic pigments contents (g/100g fr. wt.) of *C. colocynthis*.

Factors	Chlorophyll a (Chl.a)	Chlorophyll b (Chl.b)	Total Carotenoids	Chl.a+b	Chl.a/b	Total Pigment
---------	--------------------------	--------------------------	----------------------	---------	---------	------------------



Locations						
WH	5.57±0.28b	4.73±0.81b	389.81±2.93b	10.30±1.07b	1.32±0.17b	400.10±3.90b
WAA	8.47±0.46a	5.31±0.82a	472.96±9.31a	13.78±1.28a	1.74±0.19a	486.74±8.05a
P-Values	0.00**	0.02*	0.00**	0.00**	0.00**	0.00**
Seasons						
Wet	7.84±0.74a	6.82±0.19a	423.87±12.67b	14.66±0.89a	1.14±0.09b	438.52±13.55b
Dry	6.21±0.56b	3.22±0.15b	438.90±24.57a	9.43±0.69b	1.92±0.12a	448.32±25.26a
P-Values	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Locations x Seasons						
WH x Wet	6.19±0.10c	6.50±0.29a	395.57±0.90c	12.69±0.19b	0.96±0.06d	408.26±0.72c
WH x Dry	4.96±0.09d	2.95±0.12b	384.04±2.99d	7.90±0.12d	1.69±0.10b	391.94±3.02d
WAA x Wet	9.49±0.08a	7.13±0.07a	452.16±0.94b	16.62±0.15a	1.33±0.03c	468.78±1.08b
WAA x Dry	7.46±0.11b	3.49±0.16b	493.76±0.41a	10.95±0.27c	2.15±0.07a	504.70±0.18a
P-Values	0.01*	0.03*	0.00**	0.08*	0.07*	0.00**
C.V.%	2.30	6.41	1.65	3.01	8.15	0.63

WH: Wadi Hebran; WAA: Wadi Al-Akhder; Statistically significant differences at \* $p \leq 0.05$  and \*\* $p \leq 0.01$ ; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at  $p \leq 0.05$  according to the LSD test.

In Table 5, the statistical evaluation exhibited a significant effect ( $P < 0.05$  or  $0.01$ ) of L, S and interaction LS on all mineral compositions of *C. colocynthis*, except  $\text{Na}^+$  was not significantly affected by the seasons. The contents of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , N and P showed a significant increase in Wadi Hebran than in Wadi Al-Akhder. Moreover,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and N contents displayed a significant increase in the wet season compared to the other season. The highest values were registered for  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and N contents in the interaction Wadi Hebran x Wet, for  $\text{Na}^+$  and P in the interaction Wadi Hebran x Dry and for  $\text{Mg}^{2+}$  in the interaction Wadi Al-Akhder x Wet.

Table 5. Effect of the locations, seasons and their interaction on mineral compositions of *C. colocynthis*.

Factors	$\text{Na}^+$ %	$\text{K}^+$ %	$\text{Ca}^{2+}$ %	$\text{Mg}^{2+}$ %	N %	P %
Locations						
WH	1.10±0.06a	2.03±0.07a	1.96±0.25a	1.56±0.15b	1.21±0.11a	0.70±0.08a
WAA	0.78±0.07b	1.47±0.25b	1.89±0.17b	2.05±0.07a	0.75±0.09b	0.33±0.05b
P-Values	0.00**	0.00**	0.04*	0.00**	0.00**	0.00**
Seasons						
Wet	0.95±0.01a	2.10±0.05a	2.39±0.05a	2.03±0.08a	1.20±0.12a	0.37±0.07b
Dry	0.93±0.13a	1.40±0.22b	1.46±0.03b	1.58±0.16b	0.77±0.10b	0.65±0.10a
P-Values	0.39 <sup>ns</sup>	0.00**	0.00**	0.00**	0.00**	0.00**
Locations x Seasons						
WH x Wet	0.97±0.03b	2.18±0.08a	2.50±0.04a	1.89±0.05b	1.45±0.06a	0.52±0.08b
WH x Dry	1.23±0.02a	1.89±0.12b	1.41±0.03d	1.23±0.03c	0.98±0.04b	0.87±0.10a
WAA x Wet	0.92±0.05c	2.02±0.10b	2.27±0.05b	2.17±0.06a	0.94±0.03b	0.22±0.04d
WAA x Dry	0.63±0.04d	0.91±0.09c	1.50±0.02c	1.93±0.01b	0.56±0.05c	0.44±0.06c
P-Values	0.00**	0.00**	0.00**	0.00**	0.09*	0.00**
C.V.%	2.61	4.33	2.27	4.23	3.87	0.95

WH: Wadi Hebran; WAA: Wadi Al-Akhder; Statistically significant differences at \* $p \leq 0.05$  and \*\* $p \leq 0.01$ ; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at  $p \leq 0.05$  according to the LSD test.

The statistical evaluation showed highly significant effects ( $P < 0.05$  or  $0.01$ ) of L, S and interaction LS on water content, crude protein, crude fiber, total ash, total carbohydrate and proline contents of *C. colocynthis* (Table 6). Significant higher these

chemical compositions of *C. colocynthis* were observed in Wadi Hebran than in Wadi Al-Akhder. Moreover, water content, crude protein, crude fiber and total ash contents were significantly higher during the wet season compared to the dry season. While, total carbohydrate and proline contents were significantly higher in the dry season than in the wet season. Regarding the effect of interaction LS and according to statistical significance, the highest water content, crude protein, crude fiber and total ash contents, as well as total carbohydrate and proline contents, were identified in the interactions of Wadi Hebran with the wet and dry seasons, respectively.

Table 6. Effect of the locations, seasons and their interaction on some chemical compositions of *C. colocynthis*.

Factors	Water Content %	Crude Protein	Crude Fiber	Total Ash	Total Carbohydrate	Proline
Locations						
WH	59.70±3.25a	7.67±0.63a	32.46±2.90a	23.17±0.88a	41.52±0.40a	13.22±0.99a
WAA	51.12±2.68b	4.70±0.52b	23.41±0.39b	19.83±0.48b	35.90±1.33b	6.90±1.06b
P-Values	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Seasons						
Wet	63.15±2.71a	7.46±0.72a	30.96±3.56a	22.91±0.99a	37.07±1.83b	7.81±1.48b
Dry	47.68±1.15b	4.90±0.62b	24.91±0.65b	20.08±0.59b	40.35±0.81a	12.31±1.37a
P-Values	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Locations x Seasons						
WH x Wet	69.19±0.23a	9.07±0.07a	38.83±1.17a	25.07±0.39a	41.10±0.49b	11.08±0.50b
WH x Dry	50.21±0.39c	6.27±0.19b	26.10±0.54b	21.26±0.32b	41.95±0.61a	15.37±0.31a
WAA x Wet	57.10±0.28b	5.86±0.09c	23.09±0.52c	20.76±0.29b	33.04±0.53d	4.54±0.09d
WAA x Dry	45.15±0.33d	3.53±0.03d	23.72±0.64bc	18.90±0.47c	38.76±0.62c	9.26±0.16c
P-Values	0.00**	0.04*	0.00**	0.01*	0.00**	0.06*
C.V.%	2.31	2.58	4.78	2.12	2.47	5.24

WH: Wadi Hebran; WAA: Wadi Al-Akhder; Statistically significant differences at \* $p \leq 0.05$  and \*\* $p \leq 0.01$ ; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at  $p \leq 0.05$  according to the LSD test.

As can be observed in Table 7, L, S and interaction LS exhibited statistically significant effects ( $P < 0.05$  or  $0.01$ ) on some antioxidant enzymes, ascorbic acid and malondiadehyde contents of *C. colocynthis*. The contents of catalase enzyme, peroxidase enzyme, superoxide enzyme, ascorbic acid and malondiadehyde have increased significantly during Wadi Hebran compared with their values in Wadi Al-Akhder. As for the seasons, catalase enzyme, peroxidase enzyme, ascorbic acid and malondiadehyde contents were significantly higher in the dry season than in the wet season. As for interaction LS, these contents in *C. colocynthis* were increased significantly in Wadi Hebran in both seasons.

Table 7. Effect of the locations, seasons and their interaction on some antioxidant enzymes ( $\mu\text{M/g fr. wt. mint.}^{-1}$ ), ascorbic acid ( $\text{mg/100 ml.}$ ) and malondiadehyde (MDA) contents ( $\mu\text{M/g fr. wt. min}^{-1}$ ) of *C. colocynthis*.

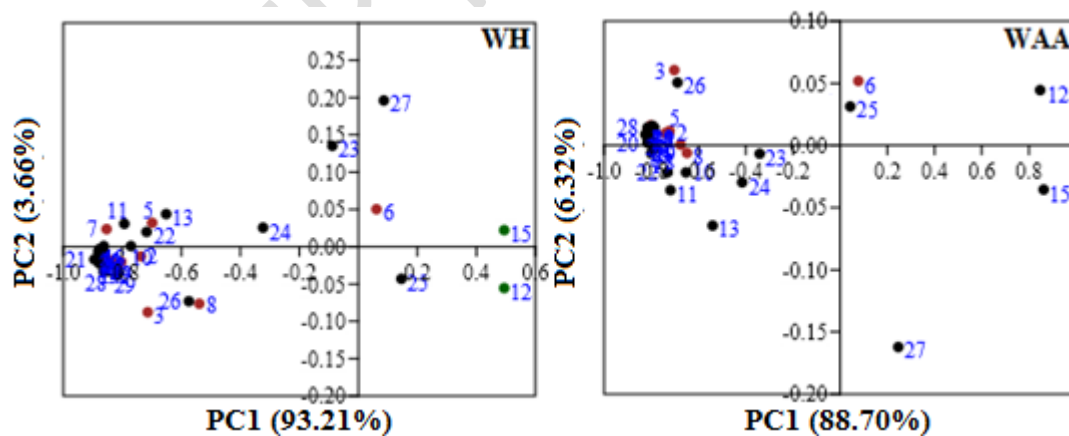
Factors	Catalase enzyme	Peroxidase enzyme	Superoxide enzyme	Ascorbic Acid	MDA
Locations					
WH	1.97±0.36a	4.02±0.37a	1.37±0.17a	1.63±0.16a	2.55±0.16a
WAA	0.76±0.18b	1.75±0.22b	0.93±0.09b	1.57±0.20b	2.04±0.20b
P-Values	0.00**	0.00**	0.00**	0.06*	0.00**
Seasons					
Wet	0.77±0.19b	2.22±0.43b	1.31±0.20a	1.55±0.20b	1.89±0.13b
Dry	1.96±0.37a	3.54±0.58a	1.00±0.06b	1.65±0.17a	2.71±0.10a

P-Values	0.00**	0.00**	0.00**	0.02*	0.00**
Locations x Seasons					
WH x Wet	1.17±0.04b	3.18±0.06b	1.75±0.03a	1.99±0.01a	2.19±0.06c
WH x Dry	2.77±0.05a	4.85±0.08a	1.00±0.05b	1.28±0.02b	2.92±0.02a
WAA x Wet	0.37±0.03c	1.26±0.04d	0.86±0.03c	1.11±0.06c	1.59±0.04d
WAA x Dry	1.15±0.04b	2.24±0.05c	1.00±0.06b	2.02±0.03a	2.49±0.03b
P-Values	0.00**	0.00**	0.00**	0.00**	0.00**
C.V.%	3.91	1.08	2.70	3.19	0.58

WH: Wadi Hebran; WAA: Wadi Al-Akhder; Statistically significant differences at \* $p \leq 0.05$  and \*\* $p \leq 0.01$ ; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at  $p \leq 0.05$  according to the LSD test.

### 3.3. Principal component analysis (PC):

The principal component analysis (PC) was used for an evident understanding of relationships between soil and plant variables in each site and between sites under this study. Out of all PCs, the two first main PCs (PC1 and PC2) were kept for the final analysis, in which, it has eigenvalues greater than one. The similarities and dissimilarities relationships between the soil and plant variables in each site are graphically displayed in a biplot of PC1 and PC2 (Fig. 4). The PC1 and PC2 contributed 96.87% and 95.01% of the total variation existing among soil and plant variables regarding Wadi Hebran and Wadi Al-Akhder sites, respectively. The PC1 only describes 93.21% and 88.70% of the measured data total variability under each Wadi Hebran and Wadi Al-Akhder sites in both seasons, respectively.  $Mg^{2+}$  soil and plant variables i.e., carotien, total pigment, total carbohydrate and water content of *C. colocynthis* contributed to the PC1 under each Wadi Hebran and Wadi Al-Akhder in both seasons. Thus,  $Mg^{2+}$  in soil had positively correlated with carotien, total pigment, total carbohydrate and water content in *C. colocynthis*. While the PC2 has lower positive correlations with  $Ca^{2+}$ ,  $Mg^{2+}$  and  $S^{-}$  in Wadi Hebran and all soil variables except  $Cl^{-}$  in Wadi Al-Akhder site as well as most plant variables of *C. colocynthis* in the two sites. The soil and plant variables inside each site were positively or negatively associated with each other.



**Fig.4.** Biplot diagram between PC1 and PC2 shows similarities and dissimilarities relationships between soil and plant variables during Wadi Hebran (WH) and Wadi Al-Akhder (WAA) in both seasons. 1: Ec; 2: pH; 3:  $Na^{+}$ ; 4:  $K^{+}$ ; 5:  $Ca^{2+}$ ; 6:  $Mg^{2+}$ ; 7:  $S^{-}$ ; 8:  $Cl^{-}$ ; 9: water content; 10: Chl.a; 11: Chl.b; 12: total arotenoids; 13: Chl.a+b; 14: Chl.a/b; 15: total pigment; 16:  $Na^{+}$ ; 17:  $K^{+}$ ; 18:  $Ca^{2+}$ ; 19:  $Mg^{2+}$ ; 20: N; 21: P; 22: water content; 23: crude protein; 24: crude fiber; 25: total ash; 26: total carbohydrate; 27: proline; 28: catalase enzyme; 29: peroxidase enzyme; 30: superoxide enzyme; 31: ascorbic acid; 32: malondiadehyde.

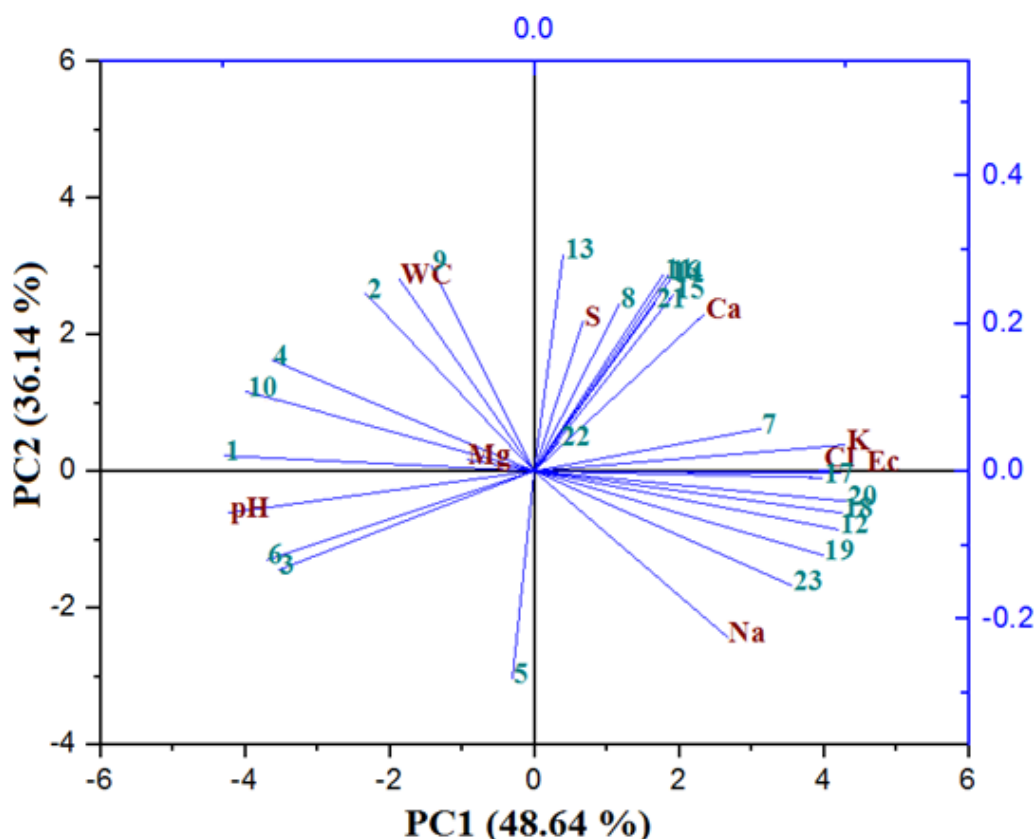
The PCA biplot in Fig. 5 identifies the relationships among soil and plant variables under the two sites in both seasons. The two first PCs explains 84.78% of

the total variance in the original variables of *C. colocynthis* in Wadi Hebran and Wadi Al-Akhder at both seasons. The PC1 and PC2 accounted for more than 48.64% and 36.14% of the total variance, respectively, and discriminated well the soil and plant variables of *C. colocynthis* on the two sites in both seasons. The PC1 and PC2 have positive correlations with most soil and plant variables in Wadi Hebran and Wadi Al-Akhder sites at both seasons, where PC1 is located in the first and fourth quarters and PC2 in the second and third quadrants. The highest positive correlations of PC1 were found with soil variables i.e., EC,  $K^+$ , and  $Cl^-$  as well as plant variables i.e., P, total carbohydrate, proline, catalase enzyme, peroxidase enzyme and malondiadehyde. While the PC2 is highly positively correlated with  $Ca^{2+}$ ,  $S^-$  and water content in soil and with Chl.b,  $K^+$ ,  $Ca^{2+}$ , N, water content, crude protein, crude fiber, total ash and Superoxide enzyme in *C. colocynthis* plants (Fig. 5).

Regarding relationships among soil properties, a positive correlation was found among EC,  $Na^+$ ,  $K^+$  and  $Cl^-$  and among  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $S^-$  and water content.  $Ca^{2+}$  showed positive correlations with EC,  $K^+$  and  $Cl^-$ . pH is positively correlated with Mg and water content.  $S^-$  has a positive correlation with EC and  $K^+$ . As for relationships among plant analysis, most plant variables in *C. colocynthis* had positive correlations across the two sites in the two seasons. Highest positive correlation had observed among Chl.a, Chl.b, Chl.a+b,  $Ca^{2+}$  and  $Mg^{2+}$ , between carotenoids and total pigment, between N and  $K^+$ , between  $Na^+$  and P, between  $Ca^{2+}$  and water content, among N, water content, crude protein, crude fiber, total ash and superoxide enzyme, among total carbohydrate, proline, catalase enzyme, peroxidase enzyme and malondiadehyde, as well as between superoxide enzyme and ascorbic acid.

The data of soil and plant variables studied displayed a positive correlation among most studied variables, but they differed in their degree and consistency in quantity. Strong positive correlations were observed for EC,  $Na^+$ ,  $K^+$  and  $Cl^-$  in soil with  $Na^+$ , P, total carbohydrate, proline, catalase enzyme, peroxidase enzyme and malondiadehyde in plant, for soil pH with Chl.a, carotenoids, total pigment and Mg in plant, for  $Ca^{2+}$  and  $S^-$  in soil with N, crude protein, cruse fiber, total ash, superoxide enzyme and ascorbic acid, for soil Mg with plant ascorbic acid, as well as for soil water content with Chl.b,  $Ca^{2+}$  and water content in *C. colocynthis* under Wadi Hebran and Wadi Al-Akhder locations.





**Fig.5.** Biplot diagram between PC1 and PC2 shows similarities and dissimilarities relationships between soil and plant variables during Wadi Hebran and Wadi Al-Akhder in both seasons. Ec; pH;  $\text{Na}^+$ ;  $\text{K}^+$ ;  $\text{Ca}^{2+}$ ;  $\text{Mg}^{2+}$ ; S; Cl; WC: water content; 1: Chl.a; 2: Chl.b; 3: total arotenoids; 4: Chl.a+b; 5: Chl.a/b; 6: total pigment; 7:  $\text{Na}^+$ ; 8:  $\text{K}^+$ ; 9:  $\text{Ca}^{2+}$ ; 10:  $\text{Mg}^{2+}$ ; 11: N; 12: P; 13: water content; 14: crude protein; 15: crude fiber; 16: total ash; 17: total carbohydrate; 18: proline; 19: catalase enzyme; 20: peroxidase enzyme; 21: superoxide enzyme; 22: ascorbic acid; 23: malondialdehyde.

#### 4. Discussion

The current study evaluated the soil proprieties and plant analysis and their relationships of *C. colocythis* under the two locations (Wadi Hebran and Wadi Al-Akhder) in the two seasons. Statistically, most mechanical and chemical properties of the adjoining soil samples of *C. colocythis* were significantly affected ( $p < 0.05$  or  $0.01$ ) by the locations, depths, and their interaction. Salama *et al.*, (2017) and Midhat *et al.*, (2021) confirm that significant differences in chemical properties of the soil supporting *C. colocythis*. Also Al-Mutairi (2017) stated that there is variation in measured soil properties of *C. colocythis* by locations, especially for pH and calcium. The present/Our study revealed that most mechanical and chemical properties of the adjoining soil samples of *C. colocythis* were significantly higher in Wadi Hebran location than in Wadi Al-Akhder location, regardless of the two depths. During the LD interactions, the highest values of most mechanical and chemical properties soil were found in 20-40 depths at Wadi Hebran location. In agreement with Al-Ghamdi (2015) and Bhasin *et al.*, (2020), the pH of soil associated with *C. colocythis* tended to be somewhat alkaline. Alkalinity may be due to the increase in total soluble salts in plant soil (Salama *et al.*, 2021). Generally, the adjoining soil of *C. colocythis* and collected from the two depths in Wadi Hebran and Wadi Al-Akhder are sandy in texture, as already reported by Al-Zahrani & Al-Amer (2006) and Bhasin *et al.*, (2020). Significant differences in environmental variables reflect the variance in the soil characteristics and texture (Al-Mutairi, 2017). Shaltout *et al.*,

(2020) reported that the diversity in plant species had been brought about by local differentiation in soil characteristics around individual plants. The plants can adapt and thrive in locations with different soil properties (Comole *et al.*, 2021). Also, the soil chemical and physical characteristics have a marked influence on the vegetation patterns of *C. colocynthis* (Al-Zahrani and Al-Amer 2006), through their effect on the water resources (Batanouny & Baeshin, 1983).

Significantly, the highest values of water content % were found by the interaction Wadi Hebran in 20-40 depth under the wet season compared to the other location in both seasons. These results may be due to the high seasonal rainfall rates during the study/studied period, and the extent of increase was 2.31% in Wadi Hebran than in Wadi Al-Akhder, thus enabling the plants to adapt to drought conditions. Also, due to increase silt and clay content in Wadi Hebran than in Wadi Al-Akhder, thus increase available water storage capacity, then increases the vegetation. These results similar to those described by Al-Ghamdi (2015) and Salama *et al.*, (2017) in soil *C. colocynthis*. Generally, the appearance and disappearance of plants species as *C. colocynthis* depend on affect microclimatic conditions like soil texture, soil water content and temperature (Bolstad *et al.*, 1998; Al-Ghamdi *et al.*, 2009 and Bhasin *et al.*, 2020).

The locations, seasons and their interaction had highly significant effects on most chemical compositions measured in *C. colocynthis*. In the study by Al-Zahrani and Al-Amer (2006), the locations significantly affected mineral compositions (N, P, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> and Cl<sup>-</sup>) in *C. colocynthis*. Also, Salama *et al.*, (2017) mentioned that the seasons showed significant effects on photosynthetic pigments, water content, ionic composition and metabolic components of *C. colocynthis*. The differences between seasons and locations were oftentimes weather-related. Thus, can be assumed that weather conditions were the cause of the significant differences for studied contents of *C. colocynthis*. In other species, similar results were recorded in the study of Kamel & El-Absy (2020), Al-Onazi *et al.*, (2021) and Salama *et al.*, (2021).

Results of plant analysis exhibited that there was a significant effect of increasing photosynthetic pigments contents of *C. colocynthis* in Wadi Al-Akhder during the two seasons. While, Wadi Hebran in the wet season showed significantly increased most chemical compositions studied of *C. colocynthis* compared to the Wadi Al-Akhder location in the two seasons. The contents of total carotenoids, Chl.a/b, total pigment, P%, total carbohydrate, proline, catalase enzyme, peroxidase enzyme, ascorbic acid and malondialdehyde have been significantly increased in the dry season than in the wet season in both locations. While the highest values of other components contents studied were registered in the wet season compared with their values in the dry season. Seasonal changes are the main factor affecting the chemical composition of plants (Sgarbossa *et al.*, 2019), as these reflect seasonal changes in physiological needs and efforts, rather than availability in plant content (Estevez *et al.*, 2010).

Salama *et al.*, (2017), the highest concentration of photosynthetic pigments and Chl.a/b ratio (more than 1) in the summer season were observed at *C. colocynthis*. Huang *et al.*, (2021) cleared that increased ratio of Chl.a/b may be due to the Chl.a content was higher than Chl.b content. During dry conditions and due to adaptive mechanisms, the desert plants attained higher concentrations of chlorophyll and carotenoids (Morsy *et al.*, 2008), enabling the plants to adapt to light conditions changes and stresses. While, Ait Said *et al.*, (2013) has hypothesized that a decrease in Chl.a can be considered as a protective adaptive mechanism that prevents increased photon absorption.

The plant species differ in the content of the minerals as well as the reactions under adverse conditions in the same region (Osugwu and Edeoga, *et al.*, 2012). The highest values of mineral compositions were of  $K^+$  and  $Ca^{2+}$  in Wadi Hebran, and  $Mg^{2+}$  in Wadi Al-Akhder. Some previous studies reported similar conclusions with the present/our results, for example, Salama *et al.*, (2017) in *C. colocynthis* and Kamel & El-Absy (2020) in *Lycium showii*. An increased concentration of  $Ca^{2+}$  and  $Mg^{2+}$  without reaching toxic levels counteracts the inhibitory effect of  $Na^+$  and may contribute to its physiological salt tolerance mechanisms, have also a role in large responsibility in physiological reactions within plant cells (Gul & Khan, 2008; Grigore *et al.*, 2012; Gil *et al.*, 2014). The  $Na^+$  and  $Cl^-$  are often stored in the vacuoles resulting in increased osmotic pressure (Wyn Jones, 1981) and avoid  $Na^+$  toxicity (Salama *et al.*, 2021),  $K^+$  ions are essential for reducing the uptake of  $Na^+$ . Concentrations of  $Na^+$  and  $K^+$ , as well as ion balance play important roles in plant salt tolerance (Zheng *et al.*, 2014). Salama *et al.*, (2015) stated that the absorption and removal of inorganic osmoregulatory ions like  $K^+$ ,  $Na^+$ ,  $Ca^{+2}$  and  $Mg^{+2}$  are useful means of osmotic gradient re-adjustment in stressed plants.

The present/Our results are as well in agreement with Salama *et al.*, (2017) for water content, with Gonzalez-Hernandez *et al.*, (2000) for crude protein, with Abebe *et al.*, (2012) for crude fiber and total ash, with Al-Qahtani *et al.*, (2020) for total carbohydrates and with Pouris *et al.*, (2020) for proline. The water content of *C. colocynthis* decreased due to the high temperature, where the transpiration rate increases in summer than in the winter (Salama *et al.*, 2017), thus the plants tend to reduce their internal water potential under abiotic stresses (Erdei *et al.*, 1990). Kasim *et al.*, (2008) reported that the protein types synthesis rich in certain amino acids may be the key to survival for species plants, where, the osmotic modification under stress conditions may be accompanied by protein accumulation to improve the plant species to drought stress tolerance (Salama *et al.*, 2021). Similarly, in studies by several researchers such as Dhaka and Meena (2018), Al-Qahtani *et al.*, (2020) and El-Absy (2021), the highest accumulation of total carbohydrates and proline observed in the dry season, which can be used as an indicator of disturbed physiological conditions as drought and salinity stresses in most plant species. The total carbohydrates and proline have a more intimate association with the survival adaptability of plants, which play an important role during drought by acting as compatible osmolytes to maintain cell turgor and favorable plant water status, thereby sustaining biological processes and soil water uptake (Verslues & Sharma, 2010 and Blum, 2017).

In line with this study, Kasim *et al.*, (2008) have already stated, *C. colocynthis* plants showed significantly higher levels of contents of catalase enzyme, peroxidase enzyme, ascorbic acid and malondialdehyde in the dry season than in wet season, while the opposite was found in case of superoxide enzyme. These results indicated increased activities of these compositions can be considered as a defense mechanism in *C. colocynthis* during the two locations at the dry seasons in this study. Such a decrease in uperoxide enzyme activity can be due to the formation of  $H_2O_2$  as a by-product which should be a potentially damaging agent at higher salt concentrations in the dry season (Kasim *et al.*, 2008). Sarker & Oba (2018) reported that the catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes play a vital role in the tolerance of *Amaranthus tricolor* and major ROS detoxification in the tolerant genotype.

Principal component analysis (PC) has been used to decrease the number of comparisons between variables and to estimate the similarities and dissimilarities relationships between soil and plant variables in each site and between sites under this

study. The first two PCs explain more than 95% and 84% of the total variance of all variables investigated in each location and between locations, respectively. The PC1 explained above 88% and 48% of the measured data total variability in the original variables under each location and between locations, respectively, in the present/our research and other studies like Kooch *et al.*, (2008), Ferraz *et al.*, (2019), Metwally *et al.*, (2019) and El-Absy (2021). Thus, it explains variance more than an individual attribute and it expresses more variability and support to select the variable with a positive loading factor. It is evident that the PC1 and PC2 can be interpreted as a response related to the soil and plant variables and which possess positive and negative contributions to locations and seasons in this study. Therefore, the PC1 is considered very important to increase the soil and plant variables studied under drought stress conditions. In agreement with Gil *et al.*, (2014) and El-Absy (2021), the first PC is correlated with soil variables i.e.,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Cl}^-$ , as well as with contents of water, crude protein, proline, total pigment, Chl.a+b and carotenoids in plants, and which related to water stress and to salt stress. Likewise, PC1 and PC2 characterized some soil variables and the PCA displayed noticeable variations of soil properties under the study region (Kooch *et al.*, 2008). Similar conclusions with this study were published by Khafagi *et al.*, (2013) and Al-Mutairi (2017), where the soil variables EC,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Cl}^-$  as well the soil variables EC,  $\text{Na}^+$ ,  $\text{S}^-$  and  $\text{Cl}^-$  were the most important factors controlling the community structure of plants in the region under study, respectively. The PC1 and PC2 had strongly correlated with some soil and plant variables investigated (Gil *et al.*, 2014).

The highest and strong positive correlations among EC,  $\text{K}^+$  and  $\text{Cl}^-$  as well as between  $\text{Ca}^{2+}$  and  $\text{S}^-$  in soil were observed in this study. The present/Our results are as well in agreement with Ferraz *et al.*, (2019), Neina (2019), Abdel-Fattah *et al.*, (2021) and El-Absy (2021), who reported that positive or negative significant correlations among soil properties. The results of PCA showed high positive correlations among plant variables measured in this study and other studies by Uvalle Saucedo *et al.*, (2008), Kaspary *et al.*, (2014), Sadeghi & Robati, (2015), Nejat & Sadeghi (2016) and Yinping *et al.*, (2021). Highest and strong positive correlations between Chl.b and  $\text{Ca}^{2+}$  as well as among P, proline, catalase enzyme and peroxidase enzyme were found in this study. The positive correlation among most plant variables such as between total chlorophyll and carotenoid concentrations plays an important role in protecting plants from stresses through photo-oxidation (Hendry & Price, 1993) and evaluating the ability of plants to capture light during shade (Kaspary *et al.*, (2020).

The biplot showed the degree of correlation amongst most soil and plant variables measured regarding each Wadi Hebran and Wadi Al-Akhder as well as between them in the two seasons. Most soil variables have strong positive correlations with most plant variables, for example, total carbohydrate, proline, catalase enzyme, peroxidase enzyme and malondialdehyde in *C. colocynthis* during Wadi Hebran and Wadi Al-Akhder at both seasons. These findings were consistent with Gil *et al.*, (2014) and El-Absy (2021). Using PCA, Gil *et al.*, (2011), Gil *et al.*, (2014) and El-Absy (2021) reported that positive correlations among most soil variables and the plant variables ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , proline and total carbohydrate), indicating the functional role of proline in the stress tolerance mechanisms of the plant species under study. Salama *et al.*, 2013 cleared that significant negative relationships were noticed for the plant species richness with the soil variables (EC,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Cl}^-$ ). High correlation results between soil and plant variables indicate an effect of soil chemical variables on plant variables, where, increasing these soil variables will increase plant variables. These results indicate that *C. colocynthis* and through their adaptive



behavior and the possibility of osmotic adjustment adopted can survive and endure a wide range of environmental conditions during the dry season.

## 5. Conclusions

According to ANOVA, significant effects ( $P < 0.05$  or  $0.01$ ) by the locations, depths and their interaction on most soil mechanical and chemical properties, and by the locations, depths and their interaction on most plant compositions were found. Most mechanical and chemical properties in soil and most chemical compositions in *C. colocynthis* were significantly increased in Wadi Hebran at both seasons than that of Wadi Al-Akhder. Wadi Al-Akhder in both seasons had the positive highest impact on photosynthetic pigments contents. The dry season significantly increased proline and other chemical compositions compared to the wet season. According to PCA, the first two PCs had highly and positively associated with the most soil and plant variables, and showed a strong positive correlation amongst most soil and plant variables investigated under the two locations in both seasons. Using PCA, estimating the relation between the environmental factors and phytochemical compounds might help to understand adaptive behavior and mechanisms in *C. colocynthis* to a tolerance of harsh environmental conditions.

## References

- Abdel-Fattah, M.K., Mohamed, E.S.;Wagdi, E.M., Shahin, S.A., Aldosari, A.A., Lasaponara, R. and Alnaimy, M.A. (2021). Quantitative Evaluation of Soil Quality Using Principal Component Analysis: The Case Study of El-Fayoum Depression Egypt. Sustainability, 13:1824. <https://doi.org/10.3390/su13041824>.
- Abdulridha, M.K., Al-Marzoqi A.H. and Ghasemian, A. (2020).The Anticancer Efficiency of *Citrullus colocynthis* Toward the Colorectal Cancer Therapy. Journal of Gastrointestinal Cancer, 51(2):439-444 <https://doi.org/10.1007/s12029-019-00299-6>.
- Abebe, A.T., Tolera, A., Holand, Ø., Ådnøy, T., and Eik, L.O. (2012). Seasonal variation in nutritive value of some browse and grass species in borana rangeland, southern Ethiopia. Tropical and Subtropical Agroecosystems, 15:261-271.
- Abushandi, E.H. and Alatawi, S. (2015). Dam site selection using remote sensing techniques and geographical information system to control flood events in Tabuk city. Journal of Waste Water Treatment and Analysis, 6(1):1-13. <http://dx.doi.org/10.4172/2157-7587.1000189>.
- Ackerly, D.D., Dudley, S.A., Sulton, S.E., Schmitt, J., Coleman, J.S. and Linder, C.R. (2000). The Evolution of Plant Ecophysiological Traits: Recent Advances and Future Directions: New research addresses natural selection, genetic constraints, and the adaptive evolution of plant ecophysiological traits, BioScience, 50(11):979-995, [https://doi.org/10.1641/0006-3568\(2000\)050\[0979:TEOPET\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2000)050[0979:TEOPET]2.0.CO;2).
- Ait Said, S. Torre, F., Derridi, A., Gauquelin, T. and Mevy, J.P. (2013). Gender, Mediterranean drought, and seasonality: photosystem II photochemistry in *Pistacia lentiscus*. Photosynthetica, 51:552-564. DOI: 10.1007/s11099-013-0055-9.
- Aldamegh, M.A., Abdallah, E.M. and Hsouna, A.B. (2013). Evaluation of antimicrobial and antioxidant properties of leaves of *Emex spinosa* and fruits

- of *Citrullus colocynthis* from Saudi Arabia. African Journal of Biotechnology, 2(34):5308-5313. DOI: 10.5897/AJB2013.12987.
- Alfarhan, A. (1999). A phytogeographical analysis of the floristic elements in Saudi Arabia. Pakistan Journal of Biological Sciences (Pakistan), 2(3):702-711. DOI : 10.3923/pjbs.1999.702.711.
- Al-Ghamdi, A.A.M. (2015). Ecological studies on the colocynth, *Citrullus colocynthis* (L.) (*Cucurbitaceae*) from Shada, Saudi Arabia and its insect repellent properties. Life Science Journal, 12(1):125-133.
- Al-Ghamdi, F.A., Al-Zahrani, H.S. and Al-Amer, K.H. (2009). Phytosociological studies of *Citrullus colocynthis* L., Growing in Different Altitudinal Sites in Saudi Arabia. Pakistan Journal of Biological Sciences, 12:779-785. DOI: 10.3923/pjbs.2009.779.785.
- Alghanem, S.M., Al-Atwi, H.Q., Al-Saiari, M.O., Al-Balawi, A.M., Al-Zahrani, S.A. and Al-Sayed A.M. (2020). Floristic Diversity and Perennial Vegetation Analysis of Al-Wadi Al-akhder, Tabuk Region, Saudi Arabia. International Journal of Plant Science and Ecology, 6(2):31-38.
- Allen, S.E. (1989): Chemical analysis of ecological materials. Blackwell Scientific Publications. Oxford, London Edinburgh. Pp. 368.
- Al-Mujahidy, S.M.J., Hassan, M.M., Rahman, M.M. and Mamun-or-Rashid, A. (2013): Study on measurement and statistical analysis of adherent soil chemical compositions of leguminous plants and their impact on nitrogen fixation. International Journal of Biosciences, 3:112-119. <http://dx.doi.org/10.12692/ijb/3.6.112-119>.
- Al-Mutairi, K.A. (2017). Influence of soil physical and chemical variables on species composition and richness of plants in the arid region of Tabuk, Saudi Arabia. Ekológia (Bratislava), 36(2):112-120. <https://doi.org/10.1515/eko-2017-0010>.
- Al-Mutairi, K.A., Al-Shami, S.A., Khorshid, Z.B., and Moawed, M.M. (2016). Floristic diversity of Tabuk province, North Saudi Arabia. The Journal of Animal & Plant Sciences, 26(4):1019-1025. Corpus ID: 146805936.
- Al-Nafie A. H. (2008). Phytogeography of Saudi Arabia. Saudi Journal Biology Science, 15(1):159-176.
- Al-Onazi et al., (2021)::::
- Al-Qahtani, H., Alfarhan, A.H., and Al-Othman, Z.M. (2020). Changes in chemical composition of *Zilla spinosa* Forssk. medicinal plants grown in Saudi Arabia in response to spatial and seasonal variations. Saudi Journal of Biological Sciences 27(10):2756-2769. <https://doi.org/10.1016/j.sjbs.2020.06.035>.
- Al-Zahrani, H.S. and Al-Amer, K.H. (2006). A comparative study on *Citrullus colocynthis* plant grown in different altitudinal locations in Saudi Arabia. American-Eurasian Journal of Scientific research, 1(1):1-7.
- AOAC, (2000). Official Methods of Analysis, 17<sup>th</sup> Ed. Association of Official Analytical Chemists (Washington D.C., U.S.A, 2000).
- Ayyad, M.A., Fakhry, A.M. and Moustafa, A.R.A. (2000). Plant biodiversity in the Saint Catherine area of the Sinai Peninsula, Egypt. Biodiversity and Conservation, 9:265-281. <https://doi.org/10.1023/A:1008973906522>.
- Bartels, D. and Sunkars, R. (2005). Drought and salt tolerance in plants. Cr Rev Plant Science, 24:23-58. <https://doi.org/10.1080/07352680590910410>.
- Batanouny, K.H. and Baeshin, N.A. (1983). Plant communities along the Medina-Badr road across the Hejaz Mountains, Saudi Arabia. Vegetatio, 53:3343. <https://doi.org/10.1007/BF00039769>.

- Bates, L.S., Waldren, R.P. and Teare I.D. (1972). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39:205-207.
- Beauchamp, C. and Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44:276-287.
- Bhasin, A., Singh, D.S. and Garg, R.K. (2020). Nutritional and medical importance of *Citrullus colocynthis*-A review. *Plant Archives*, 20(2):3400-3406. Corpus ID: 227298313.
- Blum, A. (1988) <sup>0</sup>. Plant breeding for stress environments. CRC Press, Boca Raton, FL. 38-78.
- Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant Cell Environ.* 40, 4–10. doi: 10.1111/pce.12800.
- Bolstad, P., Swank, W. and Vose, J. (1998). Predicting southern Appalachian **overstory** vegetation with digital terrain data. *Landscape Ecol.*, 13: 271-283.
- Boulos, L. (2009). Flora of Egypt Checklist, revised annotated edition. Al Hadara Publishing Egypt, Cairo.
- Bremner, J.M. (1965). Total nitrogen and inorganic forms of nitrogen. In: *Methods of Soil Analyses*. (Ed.): C.A. Black. American Society of Agronomy, Madison, Wisconsin, 1149-1237.
- Casas, C. and Ninot, J.M. (2003). Correlation **between** Species Composition **and** Soil **properties in the** Pastures **of** Plana De Vic (Catalonia, Spain). *Acta Botánica Barcinonensia*, 49:291-310.
- Chaplin, M.F. and Kennedy, J.F. (1994). *Carbohydrate Analysis*. A practical approach. 2nd Ed. Oxford Univ., Press Oxford, New York, Tokyo, 324 pp. 1994.
- Chapman, H. (1965). Cation-exchange capacity. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*; John Wiley & Sons: Hoboken, NJ, USA, 9:891-901.
- Collentette, S. (1999). Wild flowers of Saudi Arabia. Riyadh: National Commission for Wildlife Conservation and Development.
- Comole, A.A., Malan, P.W. and Tiawoun, M.A.P. (2021): Effects of *Prosopis velutina* invasion on soil characteristics along the riverine system of the **Molopo** river in north-west province, **South Africa**". *International Journal of Ecology*, vol. 2021, Article ID 6681577, 11. <https://doi.org/10.1155/2021/6681577>.
- Dane, F., Liu, J. and Zhang, C. (2007). **Phylogeography/Phytogeography** of the Bitter Apple, *Citrullus Colocynthis*. *Genetic Resources and Crop Evolution*, 54:327-336. <https://doi.org/10.1007/s10722-005-4897-2>.
- Darwish, R.S., Abdulmunem, O.A., Khairy, A., Ghareeb, D.A., Yassin, A.M., Abdulmalek, S.A. and Shawky, E. (2021). Comparative metabolomics reveals the cytotoxic and anti-inflammatory discriminatory chemical markers of raw and roasted colocynth fruit (*Citrullus colocynthis* L.). *RSC Advances*, 11, 37049–37062. <https://doi.org/10.1039/D1RA07751A>.
- Dhaka, V. and Meena, K.L. (2018). Seasonal variation in free proline content in some species of family **Euphorbiaceae** of the **Rajasthan**, India. *Journal of Experimental Biology and Agricultural Sciences*, 6(1):249-252. DOI:10.18006/2018.6(1).249.252.
- Do, P.T., Degenkolbe T., Erban, A., Heyer, A.G., Kopka, J., Köhl, K.I., Hinch, D.K. and Zuther, E. (2013). Dissecting rice polyamine metabolism under controlled

- long-term drought stress. PLOS ONE 8: e60325. <https://doi.org/10.1371/journal.pone.0060325>.
- Duke, J.A. (1978). The quest for tolerant germplasm Proceedings of the 32nd ASA Special Symposium, Crop Tolerance to Suboptimal Land Conditions American Society Agronomy, (ASA'78), Madison, WI., pp: 1-61. <https://doi.org/10.2134/asaspecpub32.c1>.
- El-Absy, K.M. (2021). Seasonal Changes of Some Metabolites in *Hyoscyamus boveanus* (Dunal) Asch. & Schweinf – Saint Katherine, South Sinai, Egypt. Asian Journal of Biology, 13(2):49-66. <https://doi.org/10.9734/ajob/2021/v13i230184>.
- El-Ghani, M.M.A. and Amer, W.M. (2003). Soil–vegetation relationships in a coastal desert plain of southern Sinai, Egypt. Journal of Arid Environments, 55(4):607-628. [https://doi.org/10.1016/S0140-1963\(02\)00318-X](https://doi.org/10.1016/S0140-1963(02)00318-X).
- El-Keblawy, A., Shabana, H.A., Navarro, T. and Soliman, S. (2017). Effect of maturation time on dormancy and germination of *Citrullus colocynthis* (Cucurbitaceae) seeds from the Arabian hyper-arid deserts. BMC Plant Biology, 17(1):263. <https://doi.org/10.1186/s12870-017-1209-x>.
- Erdei, L., Trivedi, K. and Matsumoto, H. (1990). Effect of osmotic and salt stress on the accumulation of polyamines in varieties differing in salt and drought tolerance. Journal of Plant Physiology, 137:165-168. [https://doi.org/10.1016/S0176-1617\(11\)80075-1](https://doi.org/10.1016/S0176-1617(11)80075-1).
- Estevez, J.A., Landete-Castillejos, T., GarcíaB, A.J., Ceacero, F., Martínez, A., Gaspar-López, E., Calatayud, A. and Gallego, L. (2010). Seasonal variations in plant mineral content and free-choice minerals consumed by deer. Animal Production Science, 50(3):177-185. <https://doi.org/10.1071/AN09012>.
- Fayed, A. and Shaltout, K. (2004). Conservation and sustainable use of medicinal plants in arid and semiarid eco-systems project, Egypt (GEF, UNDP) (project no: 12347/12348), Flora of Saint Katherine Protectorate, and Floristic Survey of the Mountainous Southern Sinai: Saint Katherine Protectorate, final report, 13(3):13. Available:[http:// www.idosi.org/aejaes/ jaes](http://www.idosi.org/aejaes/jaes).
- Ferraz, G.A.S., PFerra, P F.P., Martins, F.B., Silva, F.M., Damasceno, F.A., Barbari, M. (2019). Principal components in the study of soil and plant properties in precision coffee farming. Agronomy Research, 17(2):418-429. <https://doi.org/10.15159/AR.19.114>.
- Gil, R., Lull, C., Boscaiu, M., Bautista, I., Lidón, A. and Vicente, O. (2011). Soluble carbohydrates as osmolytes in several halophytes from a Mediterranean salt marsh. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 39(2):09-17. <https://doi.org/10.15835/nbha3927176>.
- Gil, R., Bautista, I., Boscaiu, M., Lidon, A., Wankhade, S., Sa´nchez, H., Llinares, J. and Vicente, O. (2014). Responses of five Mediterranean halophytes to seasonal changes in environmental conditions. AoB Plants, 6: plu049; <https://doi.org/10.1093/aobpla/plu049>.
- Gilbert, F. and Zalat, S. (2021). A practical guide to discovering the natural, cultural and historical faces of South Sinai. PART II. – Fauna and flora of South Sinai. available at <https://discoversinai.net/downloads/fauna-flora.pdf>.
- Giwa, S., Abdullah, L.C. and Adam, N.M. (2010). Investigating “Egusi” (*Citrullus colocynthis* L.) seed oil as potential biodiesel feedstock. Energies, 3:607-18. doi: 10.3390/en3040607.
- Gonzalez-Hernandez, M.P., Starkey, E.E. and Karchesy, J. (2000). Seasonal variation in concentrations of fiber, crude protein, and phenolic compounds in leaves of



- red alder (*Alnus rubra*): nutritional implications for cervids. *Journal of Chemical Ecology*, 26(1):293-301. DOI: 10.1023/A:1005462100010.
- Gul, B. and Khan, M.A. (2008). Role of Calcium in Alleviating Salinity Effects in Coastal Halophytes. In: Khan M.A., Weber D.J. (eds) *Ecophysiology of High Salinity Tolerant Plants. – Tasks for Vegetation Science*, vol 40. Springer, Dordrecht. [https://doi.org/10.1007/1-4020-4018-0\\_6](https://doi.org/10.1007/1-4020-4018-0_6).
- Grigore, M.N., Boscaiu, M., Llinares, J. and Vicente, O. (2012). Mitigation of salt stress-induced inhibition of *Plantago crassifolia* reproductive development by supplemental calcium or magnesium. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 40:58-66. <https://doi.org/10.15835/nbha4028246>.
- Haggag, A.A. (2015). An Annotated Checklist of the endemic and Sub-endemic Grasshoppers (Orthoptera: Caelifera) of Egypt. *Egyptian Academic Journal of Biological Sciences*, 8(3):13-29. DOI:10.21608/EAJBSA.2015.12864
- Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., and Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant signaling & behavior*, 7(11):1456-1466. <https://doi.org/10.4161/psb.21949>
- Heath, R.L. and Packer, I. (1968). Photoperoxidation in isolated chloroplasts. 1-Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125:189-198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1).
- Hendry, G.A.F. and Price, A.H. (1993). Stress Indicators: Chlorophylls and Carotenoids. In: Hendry, G.A.F. and Grime, J.P., Eds., *Methods in Comparative Plant Ecology*, Chapman Hall, London 148-152. <http://dx.doi.org/10.1007/978-94-011-1494-3>.
- Huang, Z., Liu, Q., An, B., Wu, X., Sun, L., Wu, P., Liu, B. and Ma, X. (2021). Effects of planting density on morphological and photosynthetic characteristics of leaves in different positions on *Cunninghamia lanceolata* saplings. – *Forests* 12: 853. <https://doi.org/10.3390/f12070853>.
- Huseini, H.F., Darvishzadeh, F., Heshmat, R., Jafariazar, Z., Raza, M. and Larijani, B. (2009). The clinical investigation of *Citrullus colocynthis* (L.) Schrad fruit in treatment of type II diabetic patients: a randomized, double blind, placebo-controlled clinical trial. *Phytother Research*, 2:1186-9. doi: 10.1002/ptr.2754.
- Hussain, A.I., Rathore, H.A., Sattar, M.Z.A., Chatha, S.A.S., Sarker S.D. and Gilani, A.H. (2014). *Ethnopharmacology*, 155:54-66.
- Iwara, A.I. (2011). Multivariate Analysis of Soil-Vegetation Interrelationships in a South-Southern Secondary Forest of Nigeria. *International Journal of Biology*, 3(3):73-82. DOI:10.5539/ijb.v3n3p73
- Jackson, M.L. (1962). *Soil chemical analysis*. Constable and co. Ltd. London.
- Jackson, M.L. (1967). *Soil chemical analysis*. Prentice Hall of India Private Ltd., New Delhi., India.
- Jin, X., Shi, C., Yu, C.Y., Yamada, T. and Sacks, E.J. (2017). Determination of leaf water content by visible and near-infrared spectrometry and multivariate calibration in *Miscanthus*. *Frontiers Plant Science*, 8:7-21. <https://doi.org/10.3389/fpls.2017.00721>.
- Kamel, A.M. and El-Absy, K.M. (2020). Seasonal variations in protein patterns and mineral contents of *Lycium showii* under different habitat conditions. – *Asian Plant Research Journal*, 6(4):91-103. <https://doi.org/10.9734/aprj/2020/v6i430141>
- Kasim, W.A., El-Shourbagy, M.N., Ahmed, A.M. and El-Absy, K.M. (2008). Physiological adjustment of *Arthrocnemum macrostachyum* and *Nitraria*

- retusa* to Saline Habitats in Sinai, Egypt. ■ Australian Journal of Basic and Applied Sciences, 2(3):418-428. Corpus ID: 56351907.
- Kaspary, T.E., Cutti, L., Bellé, C., Casarotto, G. and Ramos, R.F. (2020). Nondestructive analysis of photosynthetic pigments in forage radish and vetch. Crop Production Rev. Ceres, 67(6):424-431. <https://doi.org/10.1590/0034-737X202067060001>
- Kaspary, T.E., Lamego, F.P., Cutti, L., Aguiar, A.C.M. and Bellé, C. (2014). Determination of photosynthetic pigments in fleabane biotypes susceptible and resistant to the herbicide glyphosate. Planta Daninha, Viçosa-MG, 32(2):417-426. <http://dx.doi.org/10.1590/S0100-83582014000200020>
- Kato, M. and Shimizu, S. (1987). Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves: phenolic-dependent peroxidative degradation. Canadian Journal of Botany, 65:729-735. <https://doi.org/10.1139/b87-097>
- Khafagi, O.A., Sharaf, A.A., Hatab E.E. and Moursy, M.M. (2013). Vegetation Composition and Ecological Gradients in Saint Katherine Mountain, South Sinai, Egypt. American-Eurasian J. Agric. & Environment Science, 13(3):402-414. DOI: 10.5829/idosi.aejaes.2013.13.03.11313.
- Kim, C., Sharik, T. and Jurgensen, M. (1995). Canopy cover effects on soil nitrogen mineralization in northern red oak (*Quercus rubra*) stands in northern Lower Michigan. Forest Ecology and Management, 76(1-3):21-28. [http://doi.org/10.1016/0378-1127\(95\)03563-P](http://doi.org/10.1016/0378-1127(95)03563-P)
- Kooch, Y., Jalilvand, H., Bahmanyar, M.A. and Pormajidian, M.R. ■ (2008). The Use of Principal Component Analysis in Studying Physical, Chemical and Biological Soil Properties in Southern Caspian Forests (North of Iran). Pakistan Journal of Biological Sciences, 11:366-372. DOI: 10.3923/pjbs.2008.366.372
- Levi, A., Simmons, A.M., Massey, L., Coffey, J., Wechter, W. P., Jarret, R. L., Tadmor, Y., Nimmakayala, P. and Reddy, U.K. (2017). Genetic diversity in the desert watermelon *Citrullus colocynthis* and its relationship with *Citrullus* species as determined by high-frequency oligonucleotides-targeting active gene markers. – Journal of the American Society for Horticultural Science, 142(1):47-56.
- Levitt, J. ■ (1980) Responses of plants to environmental stress: chilling, freezing and high temperature stresses, 2<sup>nd</sup> Ed New York: Academic Press.
- Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Packer, L. ■ Douce, R., ed. Methods in enzymology. – London: Academic Press, 148:350-382. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
- Mariod, A.A., Elwathig, M., Mirghani, S., Hussein, I. (2017). Chapter 14 - Cucurbits, Cucurbita Species ■ as New Oil Sources, Editor(s): Abdalbasit Adam Mariod, Mohamed Elwathig Saeed Mirghani, Ismail Hussein, Unconventional Oilseeds and Oil Sources, Academic Press, 2017, Pages 81-84, <https://doi.org/10.1016/B978-0-12-809435-8.00014-7>
- \*\*Midhat, ■ L., Ouazzani, N., Hejjaj, A., Ouammou, A. and Mandi, L. (2021). Assisted Phytostabilization of Acidic Polymetallic Mine Tailings Using Marble Waste and Native Plant *Citrullus Colocynthis*: Effect on Soil, Plant, and Metal Uptake. Recent Progress in Materials, 3(3):16. doi:10.21926/rpm.2103030.

- Metwally, M. S., Shaddad, S. M., Liu, M., Yao, R. J., Abdo, A. I., Li, P., Jiao, J. and Chen, X. (2019). Soil properties spatial variability and delineation of site-specific management zones based on soil fertility using fuzzy clustering in a hilly field in Jianyang, Sichuan, China. *Sustainability*, 11(24):7084. <https://doi.org/10.3390/su11247084>.
- Mibei, E. K., Ambuko, J., Giovannoni, J. J., Onyango, A. N. and Owino, W.O. (2016). Carotenoid profiling of the leaves of selected African eggplant accessions subjected to drought stress. *Food Science & Nutrition*, 5(1):113-122. <https://doi.org/10.1002/fsn3.370>.
- \*\*Midhat,?????**
- Moawed, M.M. and Ansari, A.A. (2015). Wild plants diversity of Red Sea coastal region, Tabuk, Saudi Arabia. *Journal of Chemical and Pharmaceutical Research*, 7(10):220-227. Corpus ID: 56401762.
- Morsy, A.A., Youssef, A.M., Mosallam, H.A.M. and Hashem, A.M. (2008). Assessment of Selected Species along Al-Alamein-Alexandria International Desert Road, Egypt. *Journal of Applied Sciences Research*, 4(10):1276-1284.
- Neina, D. (2019). The Role of Soil pH in Plant Nutrition and Soil Remediation", *Applied and Environmental Soil Science*, vol. 2019, Article ID 5794869, 9 pages,9. <https://doi.org/10.1155/2019/5794869>.
- Nejat, N. and Sadeghi, H. (2016). Finding out relationships among some morpho-biochemical parameters of *Christ's thorn* (*Ziziphus spina-christi*) under drought and salinity stresses. *Planta Daninha, Viçosa-MG*, 34(4):667-674. doi: 10.1590/S0100-83582016340400006.
- Osuagwu, G.G.E. and Edeoga, H.O. (2012). The influence of water stress (drought) on the mineral and vitamin content of leaves of *Gongronema latifolium* (Benth). *International Journal of Medicinal and Aromatic Plants*, (2):301-309.
- \*\*Pradhan, A. K., Rehman, M., Saikia, D., Jyoti, S.Y., Poudel, J. and Tanti, B.** (2020). Biochemical and molecular mechanism of abiotic stress tolerance in plants. *Plant ecophysiology and adaptation under climate change: Mechanisms and Perspectives I*. Springer; 825-853. <https://doi.org/10.1007/978-981-15-2156-0>.
- \*\*\*Pouris, J., Meletioui-Christou, M.S., Chimona, C. and Rhizopoulou, S.** (2020). Seasonal functional partitioning of carbohydrates and proline among plant parts of the sand daffodil. *Agronomy*, 10(4):539. <https://doi.org/10.3390/agronomy10040539>.
- Rai, K.B. (2001). *Laboratory Manual of Organic Chemistry*, 4 Edition, New Age International, New Delhi, 160-162.
- Rowell, D.L. (1994). *Soil Science Methods and Applications*. Longman Publishers, Singapore. 229.
- Saberi, M., Niknahad-Gharmakher, H., Heshmati, G., Barani, H. and Shahriari, A. (2017). Effects of Different Drought and Salinity Levels on Seed Germination of *Citrullus colocynthis*. *ECOPERSIA* (2017) Vol. 5(3):1903-1917. DOI: 10.18869/modares.Ecopersia.5.3.1903.
- Sadeghi, H. and Robati, Z. (2015). Response of *Cichorium intybus* L. to eight seed priming methods under osmotic stress conditions. *Biocatalysis and Agricultural Biotechnology*, 4(4):443-448. <https://doi.org/10.1016/j.bcab.2015.08.003>.
- Salama, F.M., Abd El-Ghani, M.M., El-Naggar, S.M. and Aljarroushi, M.M. (2013). Vegetation analysis and species diversity in the desert ecosystem of coastal

- wadis of South Sinai, Egypt. Journal of Biology and Earth Sciences, (3)2:B214-B227.
- Salama, F., Sayed, S. and Abd EL\_Gelil, A. (2015). Ecophysiological responses of *Calligonum polygonoides* and *Artemisia judaica* plants to severe desert aridity. Turk Journal Botany, 39(2):253-266. DOI:10.3906/BOT-1404-15.
- Salama, F.M., El-Ghani, M.M., El-Tayeh, N.A., Amro, A.A., and El-Naggar, S. (2017). Some aspects of drought resistance in *Citrullus colocynthis* L. in the Egyptian deserts. Taekholmia, 37(1):52-66. DOI:10.21608/TAEC.2017.11935.
- Salama, F.M, Abd El-Ghani , M. M. Gaafar , A. E., Hasanin D. M. Abd El- Wahab, D. A. (2021). Adaptive eco-physiological mechanisms of *Alhagi graecorum* in response to severe aridity in the Western desert of Egypt. Plant Biosystems. An International Journal Dealing with all Aspects of Plant Biology, <https://doi.org/10.1080/11263504.2021.1887957>.
- Sarker, U. and Oba, S. (2018). Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. Science Rep, 8:16496. <https://doi.org/10.1038/s41598-018-34944-0>.
- Sayed, S.A., Gadallah, M.A.A. and Salama, F.M. (2013). Ecophysiological studies on three desert plants growing in Wadi Natash, Eastern Desert, Egypt. Journal of Biology and Earth Sciences, 3(1):B135-B143.
- Sgarbossa, J., Schmidt, D., Schwerz, F., Schwer, Z L., Prochnow, D. and Caron, B.O. (2019). Effect of season and irrigation on the chemical composition of *Aloysia triphylla* essential oil. Revista Ceres, 66(2):85-93. <https://dx.doi.org/10.1590/0034-737x201966020002>.
- Shahid, M. and Rao, N.K. (2014). Diversity of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae) in the United Arab Emirates. Journal on New Biological Reports, 3:145-50.
- Shaltout, K.H., Al-Sodany, Y.M., Eid, E.M., Heneidy, S.Z. and Taher, M.A. (2020). Vegetation diversity along the altitudinal and environmental gradients in the main wadi beds in the mountainous region of South Sinai, Egypt. Journal of Mountain Science, 17(10). <https://doi.org/10.1007/s11629-020-6153-9>.
- Shaltout, K.; Heneidy, S.; Al-Sodany, Y.; Marie, A.; Eid, E.; Hatim, M. and El-Gharaib, A. (2004). Floristic Survey of the Mountainous Southern Sinai: Saint Katherine Protectorate, Conservation and Sustainable Use of Medicinal Plants in Arid and Semiarid Eco systems Project, Egypt, Final Report (GEF and UNDP) (Project No:12347/12348).
- Singwane, S.S., and Malinga, P.S. (2012). Impacts of Pine And Eucalyptus Forest Plantations on Soil Organic Matter Content in Swaziland - Case of Shiselweni Forests. Journal of Sustainable Development in Africa, 14(1):137-151. Corpus ID: 53406634.
- Steel, R.G.D. and Torrie, J.H. (1997). Dickey DA principles and procedures of statistics: a biometrical approach. 3rd ed. New York: McGraw Hill.
- Tannin-Spitz, T., Grossman, S., Dovrat, S., Gottlieb, H.E. and Bergman, M. (2007). Growth inhibitory activity of cucurbitacin glucosides isolated from *Citrullus colocynthis* on human breast cancer cells. Biochem Pharmacol, 73:56-67. <https://doi.org/10.1016/j.bcp.2006.09.012>.
- Thomas, J., Basahi, R., Al-Ansari, A.E., Sivadasan, M., El-Sheikh, M.A., Alfarhan, A.H. and Al-Atar, A.A. (2015). Additions to the Flora of Saudi Arabia: Two new generic records from the Southern Tihama of Saudi Arabia. National Academy Science Letters, 38:513-516.

- Torkey, H.M., Abou-Yousef, H.M., Abdel Azeiz, A.Z. and Farid, E.A. (2009). Insecticidal effect of cucurbitacin E glycoside isolated from *Citrullus colocynthis* against *Aphis craccivora*. – Australian Journal of Basic and Applied Sciences, 3(4):4060-4066.
- Tuzuner, A. (1990). Soil and water laboratory analysis guide. Ankara: General Directorate of Rural Services Publications.
- Uvalle Saucedo, J.I., Gonzalez Rodriguez, H., Ramirez Lozano, R.G., Silva, I.C. and Gomez Meza, M.V. (2008). Seasonal trends of chlorophylls a and b and carotenoids in native trees and shrubs of Northeastern Mexico. Journal of Biological Sciences, 8:258-267. DOI: 10.3923/jbs.2008.258.267.
- Verslues, P.E. and Sharma, S. (2010). Proline metabolism and its implications for plant-environment interaction. Arabidopsis Book. 8:e0140. <https://doi.org/10.1199/tab.0140>.
- Wang, Z., Hu, H., Goertzen, L.R., McElroy, J.S. and Dane, F. (2014). Analysis of the *Citrullus colocynthis* Transcriptome during Water Deficit Stress. PLoS ONE, 9(8):e104657. <https://doi.org/10.1371/journal.pone.0104657>.
- Wyn Jones, R. (1981). Salt tolerance. In *Physiological Processes Limiting Plant Productivity*. Ed. C.B. Johnson. Pp. 271-292. Butterworth, London Full
- Yinping, L., Tian, H., He, Q., Geng, Z., Yan, S., Tuniyazi, G. and Bai, X. (2021). Investigation of the geographical environment impact on the chemical components of *Peganum harmala* L. through a Combined Analytical Method. ACS Omega, 6 (39):25497-25505. <https://doi.org/10.1021/acsomega.1c03420>.
- Zalat, S., Gilbert, F., Fadel, H., El-Hawagry, Saleh, M., Kamel, S.M. and Gilbert, J. (2008). Biological explorations of Sinai: flora and fauna of Wadi Isla and Hebron, St Katherine Protectorate, Egypt. Egyptian Journal of Natural History, (5):6-15. DOI:10.4314/EJNH.V5I1.70969.
- Zheng, H., Zhao, H., Liu, H., Wang, J. and Zou, D. (2014). QTL analysis of Na<sup>+</sup> and K<sup>+</sup> concentrations in shoots and roots under NaCl stress based on linkage and association analysis in japonica rice. Euphytica, 201:109-121. <https://doi.org/10.1007/s10681-014-1192-3>.