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Ecophysiological studies of *Citrullus colocynthis* in response to spatial and seasonal changes in Wadi Al-Akhder, Tabuk Region, Saudi Arabia

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Abstract. *Al-Qahtani SM. 2023. Ecophysiological studies of* Citrullus colocynthis *in response to spatial and seasonal changes in Wadi Al-Akhder, Tabuk Region, Saudi Arabia. Biodiversitas 24: 3292-3299.* In order to achieve stress tolerance of *Citrullus colocynthis* (*C. colocynthis*), the soil physicochemical properties and chemical composition of plants in response to spatial and seasonal changes were investigated in Wadi Al-Akhder, Tabuk region, Saudi Arabia. The ANOVA results showed significant differences in studied factors for soil physicochemical properties and chemical composition of *C. colocynthis*. Soil pH was observed to range between neutral and slightly alkaline in the study region. Most soil physicochemical properties and most chemical composition extracted from *C. colocynthis* plants collected from Wadi Al-Akhder in the upstream and midstream sites were significantly increased compared with other stream sites, respectively. Most physicochemical properties of the soil supporting *C. colocynthis* significantly increased during the upstream sites 20-40 depth. In winter, *C. colocynthis* showed higher contents of mineral compositions (nitrogen, calcium, magnesium, sodium and potassium), photosynthetic pigments, moisture content percentage, and chemical compositions including ash, crude fiber, crude protein, and carbohydrate contents compared to those in summer while the opposite was found in the case of phosphorus and proline contents. Most chemical compositions extracted from *C. colocynthis* were greater in the winter season than in the summer season in the downstream site. During the desert environment, *C. colocynthis* plants tend to metabolic adaptation and resistance to external stress by accumulating osmolytes such as phosphorus, calcium, total pigment, and proline.

Keywords: Chemical composition, *C. colocynthis*, photosynthetic, soil physicochemical

INTRODUCTION

Plant species are extremely sensitive to climatic and environmental changes, and diverse abiotic stresses including salinity, drought, cold, excess water, and so on (Semenov et al. 2014; Koua et al. 2021). Amongst these environmental stresses, drought stress (water scarcity) had a negative effect on plant species establishment, then growth and development during the growing season in most arid and semi-arid areas (Chowdhury et al. 2021). Drought affects the plant-water relations at all levels from the organ, cellular and molecular, to the whole plant levels (Oyiga et al. 2020), chloroplast oxidative damage, and plant uptake by nutrients (Farooq et al. 2014). Also, drought resulted in a number of physiological and biochemical changes in plants, including alterations in photosynthesis, proteins, and enzymes that control osmotic pressure (Yang et al. 2021).

Kingdom of Saudi Arabia (KSA) is known for its many habitats and climatic regimes, including valleys, mountains, rocky deserts, sand dunes, plateaus, plains, and salt pans (El-Sheikh et al. 2021). There are approximately 2,290 species of plants in the KSA, of which 9 are Gymnosperms and 27 are Pteridophytes. These species are divided into 131 families and 855 genera. Each species represents one of these 33 families (Thomas et al. 2015). The floristic diversity in the Tabuk region was astounding (Al-Mutairi et al. 2016). A total of 82 plant species from 66 genera and 30 families were identified, according to Moawed and Ansari (2015). On the other hand, 96 species were discovered by Al-Mutairi et al. (2016), and they belonged to 75 genera and 38 families consisting of 34 dicots and 4 monocots. The Tabuk area flora showed that most plant species belonged to Saharo-Arabian elements, followed by 44 Irano-Turanian and Sudanean elements, responsible for almost 60% of all plant species (Al-Mutairi et al. 2016).

The Cucurbitaceae family includes *Citrullus colocynthis* (L.) Schrad. *C. colocynthis* is an annual herb that climbs or crawls and is native to arid and sandy soil (Grover et al. 2023). It is widely grown throughout the world's desert regions including the Mediterranean, tropical Africa, Arabia, west Asia and India (Kumar et al. 2021). According to Khan et al. (2023), the seeds and fruits of *C. colocynthis* hold enormous potential for creating phytopharmaceuticals with various biological activities, including food products and other commercial entities. It is well-known in many nations as a traditional medicine for the treatment of some chronic diseases, diabetes, edema, and bacterial infections, as well as against gastrointestinal tract tumors, joint pain, anti-leukemia, and as an anti-cancerous drug (Al-Snafi 2016; Othman et al. 2022). *C. colocynthis* is highly resistant to salinity, drought stress, and desert extremes (Darwish et al. 2021).

Therefore, to develop stress-resistant plants, botany scientists have been interested in understanding the physiological mechanism of the stress-tolerant plant across different environmental conditions (Singh et al. 2018). Metabolomics is a crucial part of a systems biology approach to studying plant defense because different metabolic profiles indicate changes in metabolic pathways (Ma and Qi 2021). According to Fernie and Tohge (2017), the plant kingdom has around 200,000 metabolites, which can be further broken down into primary and secondary metabolites. Numerous ecophysiological investigations have successfully clarified plant function and pinpoint adaptive features in certain environmental situations (Puglielli et al. 2023). During the piling up of organic and inorganic solutes, plants frequently adjust their internal osmotic pressure, which results in the plants overcoming external stress in the arid environment (Sayed et al. 2013). Furthermore, when plants experience drought stress, they develop a lot of metabolites to help them cope with the situation, which causes various physical and chemical changes (Mibei et al. 2017).

Due to climatic and environmental changes, the current study was executed to investigate the soil physicochemical and chemical properties of *C. colocynthis* plants in response to spatial and seasonal changes in Wadi Al-Akhder, Tabuk region, Saudi Arabia, to understand the possibility of osmotic adjustment and the adaptive mechanism by *C. colocynthis* to stress tolerance in the desert environment.

MATERIALS AND METHODS

Geographic and climatic data of the study area

The Tabuk area lies in the KSA Northern region covering 117,000 km^2 (Al-Mutairi et al. 2016). Wadi Al-Akhder (also known as Haydar or Aqabat) is located in the southeast of Tabuk and about 120 km the Tabuk city, and it extends between 28.5045°N and 36.6654°E (https://mapcarta.com/12566004). Thus, it has a unique plant species community (Alghanem et al. 2020). Wadi Al-Akhder is 68 km long, it is formed by the Ghawanim mountains in the east and linked to Wadi Mishash Bani Atiyah and Tuus Al Arqanah in the upland, and it does not run directly through Tabuk city (Abushandi and Alatawi 2015). Wadi Al-Akhder has a low annual precipitation rate of less than 200mm/year, like most regions in KSA. The temperature of Wadi Al-Akhder ranges from 43°C during the summer to less than 7°C during the winter (Alghanem et al. 2020).

The average temperature (°C), relative humidity (%), and rainfall (mm) for Wadi Al-Akhder, Tabuk area, KSA, is given in Figure 1. In Wadi Al-Akhder, the average temperature in August, relative humidity, and rainfall in January have registered the highest values. At the same time, the lowest were recorded for average temperature in January, relative humidity in May and June, and rainfall in July and September.

Collecting and analyzing soil samples

Along the Wadi Al-Akhder, the soil sample plots were collected at 5 random points from the rhizosphere of collected *C. colocynthis* plants in each site (upstream, midstream and downstream parts) as a profile (composite samples) at two depths of 0-15 and 15-30 cm. From each sample, three replicates were chosen at random. After packaging in plastic bags with labels, soil samples were delivered to the lab. Each soil sample was air-dried and cracked in the lab before being put through a 2 mm mesh. The hydrometer method was used to determine the soil texture for all samples collected (Wufem et al. 2014). According to Wilde et al. (1979), the soil extracts were prepared and determined as 1:5 dilutions (50 g of each soil and 250 mL of deionized water), then used to measure the electrical conductivity (EC) and pH. Calcium, magnesium, sodium, and potassium concentrations were measured using a flame photometer (Jenway, PFP-7) in a 1:5 soil extract described by Williams and Twine (1960). The concentration of chlorides was determined by direct titration against $AgNO_3$ solution using 5% potassium chromate as an indicator (Richards 1954). Sulfur was estimated by using saturation paste described by Allen (1986).

Collecting and analyzing plant samples

The aerial parts of *C. colocynthis* were collected manually from upstream, midstream and downstream sites at Wadi Al-Akhder in the winter (January) and summer (August) seasons of 2020. From each sample, three replicates were chosen at random. Plant samples that had just been harvested were cleaned and dried in the shade. In this study, they were crushed into fine powders using a crushing machine and stored in dark, airtight bottles for further measures. Determining total nitrogen (N) content was estimated by the method described by Kjeldahl (1983). The content of crude protein was computed as N×6.25 (Egan et al. 1981). Calcium and magnesium contents by atomic absorption spectrophotometer, sodium and potassium contents by flame photometer, and phosphorus content by colorimetric were determined according to AOAC (1990) method.

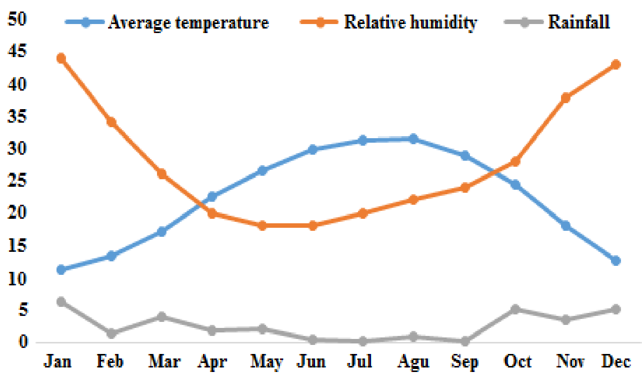

Figure 1. The average temperature (°C), relative humidity (%) and rainfall (mm) for Wadi Al-Akhder, Tabuk Region, KSA (https://www.weather-atlas.com/en/saudi-arabia/tabuk-climate#climate_text_1)

The parameters of photosynthetic pigments were quantified spectrophotometrically, and the chlorophyll a (ChA), chlorophyll b (ChB), and total carotenoids were computed using Nagata and Yamashita (1992) equations at wavelengths of 663, 645, and 470 nm, respectively. The contents of moisture, crude fiber, ash, and carbohydrate were determined by AOAC (1990) method. The free proline content was estimated using the technique of Bates et al. (1973).

Statistical analysis

Two-way analysis of variance (ANOVA) was performed on the data to assess the significance of the effect of studied factors and their interactions using a computer software program IBM SPSS Statistics for Windows, Version 27. The results obtained from ANOVA are expressed as mean ± standard error (SE). The multiple comparisons at P<0.05 for data using the least significant difference test (LSD) were calculated.

RESULTS AND DISCUSSION

Soil analysis

Soil physical properties

Table 1 depicts the soil's physical properties percentage (%) supporting *C. colocynthis* at various sites and depths in Wadi Al-Akhder. The physical properties of the soil supporting *C. colocynthis* differed significantly between the three sites and two depths, as well as their interaction, except for coarse and fine sands percentage by interaction and depths, respectively, which was non-significant. Similar results were confirmed in *C. colocynthis* by Salama et al. (2017). The opposite results were found by Sayed et al. (2013) on the soil of *C. colocynthis*. Moustafa et al. (2021) claimed that the sand, silt, and clay percentages (%) in soils collected from various locations varied remarkably. Soil physical properties revealed significant differences between soil samples collected from the various study sites at different times of the year (Al-Qahtani et al. 2020). Soil texture refers to the relative percentage of soil particles, i.e., sand, silt, and clay, in the soil (Alharbi et al. 2017). The coarse and medium sands upstream and other physical proprieties downstream provided a significantly higher percentage in the Tabouk region. Significantly highest values of coarse sand percentage (%) were observed in 0-20 depth. On the other hand, the values of other physical proprieties % were highest in 20-40 depth. The coarse and medium sands in upstream and midstream sites, and the fine sand, clay and silt in downstream, were recorded the highest percentage at both depths of *C. colocynthis* soil in the Tabouk region. Coarse sand percentage (%) was highest in both depths across three sites compared with other soil particles supporting *C. colocynthis*. Likewise, the highest values of sand content percentage (%) in the soil were observed by Alharbi et al. (2017). According to the results of soil particle analysis, the soil supporting *C. colocynthis* in Wadi Al-Akhder had a sandy texture. Similar results were confirmed by Al-Zahrani and Al-Amer (2006), where *C. colocynthis* grows quickly in the sandy soils of Saudi Arabia. Clay is a large component of heavy soils, whereas sand makes up a large portion of light soils. In addition to farming difficulty, soil texture strongly correlates with soil moisture, nutrient concentration, pH, salt level, and aeration (Wang et al. 2021).

Table 1. Main and interaction effects of sites and depths on physical proprieties of the soil supporting *C. colocynthis*

Factors	Sand			Clay and silt	Texture
	Coarse	Medium	Fine		
Single Factor					
1. Sites					
Up	76.43±1.46a	16.26±0.91a	5.12±0.75c	2.19±0.21c	Sandy
Mid	75.11±0.76a	15.34±0.36b	6.11±0.72b	3.44±0.43b	Sandy
Down	44.03±0.94b	14.30±0.39c	23.23±0.76a	18.44±0.41a	Sandy
LSD at 0.05	1.65*	0.54*	0.19*	0.02*	
2. Depths					
0-20 (1st)	66.12±5.48a	14.94±0.40b	11.44±2.97a	7.50±2.55b	Sandy
20-40 (2nd)	64.26±5.28b	15.66±0.65a	11.54±3.02a	8.54±2.69a	Sandy
LSD at 0.05	1.35*	0.44*	0.16ns	0.01*	
Two-way interaction					
Up x 1st	78.15±2.52a	14.61±0.95c	5.00±1.25e	2.24±0.33e	Sandy
Up x 2nd	74.70 ±1.15a	17.91±0.63a	5.25±1.13e	2.14±0.29f	Sandy
Mid x 1st	75.57±1.16a	15.58±0.50b	6.24±1.12cd	2.61±0.41d	Sandy
Mid x 2nd	74.65 ±1.17a	15.09±0.60bc	5.98±1.15d	4.28±0.32c	Sandy
Down x 1st	44.64±1.33a	14.63±0.49c	23.07±1.13b	17.66±0.42b	Sandy
Down x 2nd	43.41±1.53a	13.98±0.53c	23.40±1.27a	19.21±0.35a	Sandy
LSD at 0.05	2.33ns	0.77*	0.27*	0.02*	

Note: NS: non-significant; *Significant at p≤0.05. The same and different letters in a single column indicate statistical non-significance and significance at p≤0.05 according to the LSD test, respectively

Soil chemical properties

The three sites, depths, and their interaction significantly influenced the chemical properties of *C. colocynthis* soil in Wadi Al-Akhder, except for the interaction on calcium content (Table 2). Moustafa et al. (2021) discovered a similar pattern for these results. Al-Mutairi (2017) found a significant impact on pH and calcium and a non-significant impact on electrical conductivity (EC), magnesium, sodium, potassium and chloride among the four studied sites. In the study by Sayed et al. (2013) on the soil of *C. colocynthis* plants, Mg and Cl had a significant effect, but other chemical properties were non-significant. Soil chemical properties were significantly affected by the different sites, seasons and their interaction (Al-Qahtani et al. 2020). Significantly increased electrical conductivity (EC), pH and all chemical properties (except sulfur) of the soil supporting *C. colocynthis* upstream were found compared with midstream and downstream sites at Wadi Al-Akhder. The same previously mentioned soil properties (except chloride) were significantly higher at the second depth (20-40 cm) than at the first depth (0-20 cm). The highest increase in EC, pH, calcium, magnesium, sodium and potassium were noticed by interaction upstream x second depth (sandy soil). While the interactions upstream x first depth and midstream x second depth registered the highest chloride and sulfur values, respectively. The variation in pH values among different sites might be due to the site concerning the distance from the sea and the surrounding mountain types, both of which play an important role in soil properties (Moustafa et al. 2021).

The soil supporting *C. colocynthis* of Wadi Al-Akhder is neutral in the first depth downstream and slightly alkaline in the second depth at the other sites. The same finding was published by Al-Ghamdi (2015). Marschner (1995) reported that alkaline pH values greater than 7.5 reduce the solubility of magnesium, calcium, potassium, and other elements due to chemical reactions with some compounds such as HPO_4 and $CaCO_3$. The soils in the *C. colocynthis* sampling location have high calcium content but relatively low levels of sodium and chlorine, indicating that the soils are not very saline, as Procter et al. (2022) reported.

Soil EC (Electronic Conductivity) is probably the most commonly used measure for determining soil salinity (Richards 1954). The soil in our study is sandy, thus it is less saline because salts do not attach to sand particles and are easily leached through the soils. In arid locations, low precipitation and high temperatures cause high evaporation, which causes a rise in salts on the ground surface, therefore a high EC (Naorem et al. 2023). Karim et al. (2009) state that potassium is considered a macronutrient, whereas sodium is considered toxic at higher concentrations. They added that plants require moderate amounts of calcium and magnesium but typically low amounts of chloride; additionally, the sulfur reduction is more in soils with pH values greater than 8. Potassium decrease is common in sandy soils due to excessive leaching (Alharbi et al. 2017). Plant associations and speciation show significant differences in EC, pH and the contents of soil minerals (Alghanem et al. 2020). As a result, the obtained results support the notion that there is a clear variation in soil physicochemical properties depending on location (Moustafa et al. 2021), and their relationship to the topography and the site's proximity to coastal areas (Al-Mutairi 2017). In the Tabuk region soil, soil texture, chloride, sodium and EC are key variables controlling species composition (Al-Mutairi 2017).

Table 2. Main and interaction effects of sites and depths on chemical properties of the soil supporting *C. colocynthis*

Factors	EC (ds/m)	pH	Cation (meq/L)				Anion (meq/L)	
			Calcium	Magnesium	Sodium	Potassium	Chloride	Sulfur
Single factor								
1. Sites								
Up	1.53±0.22a	7.46±0.02a	5.21±0.20a	90.39±2.22a	6.28±1.26a	0.74±0.14a	8.72±0.12a	1.46±0.22b
Mid	0.72±0.07c	7.29±0.08b	3.12±0.15b	81.37±1.38c	3.49±0.33b	0.72±0.11b	2.97±0.36c	1.73±0.41a
Down	1.39±0.27b	7.12±0.09c	2.94±0.18b	88.60±1.70b	3.35±0.68c	0.60±0.10c	3.70±0.24b	1.49±0.42b
LSD at 0.05	0.02*	0.05*	0.32*	0.25*	0.06*	0.05*	0.05*	0.04*
2. Depths								
0-20 (1st)	0.80±0.07b	7.16±0.08b	3.43±0.33b	82.95±1.18b	2.68±0.24b	0.43±0.02b	5.67±0.83a	0.78±0.06b
20-40 (2nd)	1.63±0.19a	7.42±0.03a	4.08±0.41a	90.63±1.67a	6.06±0.77a	0.94±0.03a	4.59±0.98b	2.34±0.10a
LSD at 0.05	0.02*	0.04*	0.26*	0.20*	0.05*	0.04*	0.04*	0.03*
Two-way interaction								
Up x 1st	1.03±0.01c	7.45±0.03a	4.77±0.02b	85.52±0.73c	3.47±0.03d	0.43±0.02dc	8.98±0.01a	0.96±0.01d
Up x 2nd	2.02±0.02a	7.48±0.04a	5.64±0.04a	95.27±0.59a	9.10±0.04a	1.05±0.02a	8.45±0.02b	1.96±0.02c
Mid x 1st	0.56±0.01f	7.11±0.02c	2.78±0.03d	78.43±0.68f	2.75±0.02e	0.48±0.01d	3.78±0.04d	0.82±0.01e
Mid x 2nd	0.87±0.03d	7.47±0.01a	3.46±0.02c	84.31±0.65e	4.22±0.01c	0.96±0.03b	2.17±0.03f	2.65±0.03a
Down x 1st	0.79±0.01e	6.92±0.03d	2.74±0.01d	84.90±0.60d	1.83±0.03f	0.38±0.01d	4.25±0.04c	0.55±0.02f
Down x 2nd	1.99±0.02b	7.31±0.02b	3.13±0.35cd	92.31±0.66b	4.87±0.04b	0.82±0.02c	3.16±0.05e	2.42±0.01b
LSD at 0.05	0.03*	0.07*	0.45ns	0.35*	0.08*	0.06*	0.06*	0.05*

Note: NS: non-significant; *Significant at $p \leq 0.05$. The same and different letters in a single column indicate statistical non-significance and significance at $p \leq 0.05$ according to the LSD test, respectively

Plant analysis

Mineral compositions

Table 3 summarizes the status of the mineral compositions percentage (%) of *C. colocynthis* across sites, seasons and their interaction at Wadi Al-Akhder. The results of two-way ANOVA for mineral compositions percentage (%) of *C. colocynthis* indicated that the differences among sites, seasons and their interaction were significant. Likewise, Salama et al. (2017) stated the mineral compositions of *C. colocynthis* were significantly affected by seasons. Sodium, potassium and calcium have a significant influence, but magnesium had a non-significant of *C. colocynthis* plants (Sayed et al. 2013). Al-Qahtani et al. (2020) stated that all macronutrient compositions were significantly affected by the main effects and interaction for the sites and seasons, except for phosphorus, which was unaffected by season.

Nitrogen, calcium and phosphorus contents in midstream, and magnesium and potassium upstream, and sodium content in downstream have significantly increased than other streams at the Wadi Al-Akhder, Tabouk region. Except for phosphorus, all mineral compositions of *C. colocynthis* had significantly higher values during the winter than the summer. Similar results for phosphorus content and opposite results for other micronutrient contents were found by Al-Qahtani et al. (2020) in *Zilla spinosa* plants. Midstream x summer interaction significantly increased calcium and phosphorus contents, while, downstream x winter interaction significantly increased other mineral compositions studied of *C. colocynthis*.

Calcium was largely accumulated in *C. colocynthis* throughout three sites and two seasons. Salama et al. (2017) discovered a similar pattern in *C. colocynthis*. According to Malyukova et al. (2022), calcium is involved in the signaling processes (like proline buildup brought on by drought stress), and many plants have demonstrated the significance of calcium in the detection and induction of responses to stress. The potassium accumulated in the shoots of *C. colocynthis* and other studied plants from various stands may be to avoid Sodium toxicity (Sayed et al. 2013). According to Al-Qahtani et al. (2020), dry conditions with little rainfall frequently lead to a rise in soil salinity, which affects the amount of sodium in plants (relatively high levels).

Photosynthetic pigments

Sites and seasons and their interaction statistically affected the contents of chlorophyll a (ChA), chlorophyll b (ChB), total chlorophyll (ChT), chlorophyll a/b (ChA/B), carotenoids and total pigment (g/100g fr. wt.) of *C. colocynthis* growing Wadi Al-Akhder, as shown in Table 4. Seasons also significantly impacted the photosynthetic pigment contents of *C. colocynthis*, as observed by Salama et al. (2017). Furthermore, for *C. colocynthis* plants, the effects of collection stand changes on ChA and ChB contents were statistically significant (Sayed et al. 2013). The contents of ChA, ChB and ChT by midstream and other photosynthetic pigments up-stream increased significantly compared with downstream. As for seasons, the contents of photosynthetic pigments were significantly higher during the winter than during the summer. The concentration of some carotenoids and photosynthetic pigments is significantly affected by water scarcity (Mibei et al. 2017). Therefore, water scarcity decreased chlorophyll, carotenoids, and the ChA/B ratio of *C. colocynthis* (Bikdeloo et al. 2021). Environmental influences impact the watermelon (*C. lanatus*) photosynthetic system and pigment production (Gebremeskel et al. 2023). Under the sites x seasons interactions, the downstream with winter season significantly increased contents of ChA, ChB, and ChT, while, ChA/B, carotenoids and total pigment contents significantly increased upstream with the summer season, as compared to the values of the other interactions. Because of an increase in ChA relative to ChlB, the ChA/B ratio in *C. colocynthis* was greater than one at different sites (upstream and midstream sites) and seasons. A similar trend has been reported for *C. colocynthis* by Salama et al. (2017). The slightly lower ChA/B ratio values found in *C. colocynthis* indicate less damage to ChB under drought stress conditions (Bikdeloo et al. 2021). ChB is converted to ChA in higher plants as part of a ChT inter-conversion cycle that allows plants to adapt to changing light conditions, according to Ito et al. (1996). Ali et al. (2019) reported significant increases in chlorophyll content in *C. colocynthis* may be due to increased root vigor and absorption of water and other nutrients required for chlorophyll biosynthesis, such as nitrogen and magnesium.

Moisture and chemical compositions

Moisture content percentage (%) and investigated chemical compositions of *C. colocynthis* were significantly influenced by sites and seasons, and their interaction in Wadi Al-Akhder (Table 5). As Salama et al. (2017) and Al-Nablsi et al. (2022) have already stated, the seasons significantly affected the moisture content and metabolic compositions of *C. colocynthis*. Al-Qahtani et al. (2020) mentioned that the locations, seasons, and their interaction significantly impacted carbohydrates and moisture; but showed no significant impacts on crude protein and ash contents. The contents of moisture, crude protein, crude fiber, and ash of *C. colocynthis* were significantly increased in the midstream site than in the downstream and upstream sites in Wadi Al-Akhder. In contrast, the carbohydrate and proline contents were significantly higher in upstream site. The seasons results indicated that the contents of all chemical compositions studied in *C. colocynthis* during the winter increased, except proline content was increased in the summer across three sites in Wadi Al-Akhder. The carbohydrates and ash contents increased during the summer than during the winter season; the opposite was found for crude protein and moisture contents, as Al-Qahtani et al. (2020) mentioned in *Zilla spinosa* plants. The midstream x winter interaction resulted in the significantly highest moisture content percentage (%) of *C. colocynthis*. The upstream x summer interaction had the significantly highest carbohydrate and proline contents. But, the crude protein, crude fiber, and ash contents of *C. colocynthis* were significantly higher in the downstream x winter interaction n the Wadi Al-Akhder. Huang et al. (2020) stated that plants protein amount decreased with high temperatures. In our findings and similar to Ali et al. (2019) proline accumulation increased significantly in response to water scarcity conditions.

Drought conditions significantly decreased the percentage of water content in *C. colocynthis* leaves (Ali et al. 2019; Bikdeloo et al. 2021). The water content of *C. colocynthis* decreased in summer compared to winter, due to high summer temperatures and salinity (Salama et al. 2017), which led to vigor reduction. To ensure the biological processes of desert plants, the water status and cell turgidity are the most important characteristics of these plants (Sayed et al. 2013). According to Jeschke (1984), increased water flow interferes with processes that control the equilibrium between ion accumulation in root cell vacuoles and transport to the shoot and ion flow through root cell membranes at several sites.

Proline is an osmolyte that aids in the stabilization of subcellular structures (like membranes and proteins), the scavenging of free radicals, the maintenance of cell water balance, contributes to drought stress tolerance by reactive oxygen species scavenging, protecting crucial cellular macromolecules, and keeps up redox balance in adverse situations (Meena et al. 2019). Higher levels of soluble sugars, soluble proteins, potassium, and calcium frequently help *C. colocynthis* and other plants maintain turgor and increase drought tolerance (Sayed et al. 2013).

Table 3. Main and interaction effects of sites and seasons on mineral compositions percentage (%) of *C. colocynthis*

Factors	Nitrogen	Calcium	Magnesium	Sodium	Potassium	Phosphorus
Single factor						
1. Sites						
Up	0.76±0.09b	1.92±0.17b	2.06±0.06a	0.78±0.07b	1.48±0.24a	0.33±0.05b
Mid	1.05±0.06a	2.85±0.20a	0.82±0.03c	0.76±0.06c	1.36±0.20b	0.66±0.11a
Down	0.70±0.31c	1.48±0.66c	1.43±0.64b	0.82±0.37a	1.47±0.66a	0.07±0.03c
LSD at 0.05	0.05*	0.09*	0.09*	0.02*	0.06*	0.01*
2. Seasons						
Winter	1.17±0.07a	2.55±0.10a	1.94±0.31a	1.16±0.12a	2.26±0.18a	0.26±0.04b
Summer	0.50±0.14b	1.61±0.48b	0.94±0.28b	0.42±0.11b	0.62±0.16b	0.45±0.13a
LSD at 0.05	0.04*	0.08*	0.07*	0.01*	0.05*	0.01*
Two-way interaction						
Up x Winter	0.95±0.02c	2.31±0.05c	2.18±0.06b	0.93±0.01b	2.03±0.05b	0.22±0.02d
Up x Summer	0.56±0.03d	1.54±0.03d	1.94±0.01c	0.64±0.02d	0.94±0.03c	0.44±0.04b
Mid x Winter	1.18±0.06b	2.40±0.06c	0.77±0.02d	0.90±0.03c	1.80±0.01d	0.41±0.03c
Mid x Summer	0.93±0.01c	3.30±0.07a	0.87±0.03d	0.63±0.01d	0.92±0.01c	0.91±0.05a
Down x Winter	1.40±0.02a	2.95±0.05b	2.87±0.06a	1.64±0.01a	2.95±0.03a	0.14±0.01e
Down x Summer	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
LSD at 0.05	0.07*	0.13*	0.12*	0.02*	0.08*	0.02*

Note: NS: non-significant; *Significant at p≤0.05. The same and different letters in a single column indicate statistical non-significance and significance at p≤0.05 according to the LSD test, respectively

Table 4. Main and interaction effects of sites and seasons on photosynthetic pigments contents (g/100g fr. wt.) of *C. colocynthis*

Factors	Chlorophyll a (ChA)	Chlorophyll b (ChB)	ChT	ChA/B	Carotenoids	Total Pigment
Single factor						
1. Sites						
Up	8.54±0.46b	5.38±0.82b	13.92±1.28b	1.73±0.18a	474.63±9.37a	488.54±8.12a
Mid	9.23±0.47a	7.77±0.21a	17.00±0.27a	1.20±0.09b	398.52±8.01b	415.52±7.77b
Down	5.60±2.50c	4.17±1.87c	9.77±4.38c	0.67±0.30c	202.59±90.60c	212.36±94.97c
LSD at 0.05	0.17*	0.24*	0.39*	0.07*	2.06*	2.27*
2. Seasons						
Winter	10.33±0.24a	7.62±0.19a	17.95±0.42a	1.36±0.01a	413.26±10.76a	431.21±10.58a
Summer	5.24±1.32b	3.93±1.19b	9.17±2.43b	1.04±0.31b	303.89±76.83b	313.06±78.99b
LSD at 0.05	0.14*	0.19*	0.32*	0.06*	1.68*	1.86*
Two-way interaction						
Up x Winter	9.55±0.10c	7.20±0.10b	16.75±0.25c	1.33±0.01b	453.83±1.66b	470.58±1.86b
Up x Summer	7.53±0.14e	3.55±0.18c	11.08±0.36d	2.13±0.07a	495.42±1.79a	506.50±1.92a
Mid x Winter	10.25±0.05b	7.32±0.12b	17.57±0.17b	1.40±0.02b	380.77±1.63e	398.35±1.77e
Mid x Summer	8.21±0.07d	8.22±0.11a	16.43±0.18c	1.00±0.02c	416.26±1.90c	432.69±1.98c
Down x Winter	11.20±0.10a	8.34±0.13a	19.54±0.26a	1.34±0.01b	405.18±0.78d	424.72±1.01d
Down x Summer	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
LSD at 0.05	0.24*	0.34*	0.55*	0.10*	2.92*	3.21*

Note: ChT: chlorophyll a+b or total chlorophyll; ChA/B: chlorophyll a/b; NS: non-significant; *Significant at p≤0.05. The same and different letters in a single column indicate statistical non-significance and significance at p≤0.05 according to the LSD test, respectively

Table 5. Main and interaction effects of sites and seasons on moisture content and some chemical compositions of *C. colocynthis*

Factors	Moisture content %	Crude protein	Crude fiber	Ash	Carbohydrate	Proline
Single factor						
1. Sites						
Up	51.12±2.68b	4.74±0.52b	24.07±0.38b	20.49±0.49b	36.57±1.33a	6.96±1.06a
Mid	59.31±3.32a	6.48±0.30a	28.28±1.21a	21.95±0.40a	30.82±0.55b	4.43±0.53b
Down	29.87±13.36c	4.35±1.95c	15.50±6.93c	12.85±5.75c	15.09±6.75c	2.66±1.19c
LSD at 0.05	0.81*	0.35*	0.92*	0.51*	0.86*	0.30*
2. Seasons						
Winter	61.18±1.45a	7.25±0.42a	26.83±1.09a	23.25±0.67a	31.85±0.61a	4.40±0.30b
Summer	32.36±8.15b	3.13±0.85b	18.41±4.71b	13.61±3.41b	23.13±5.95b	4.97±1.36a
LSD at 0.05	0.67*	0.29*	0.75*	0.41*	0.70*	0.25*
Two-way interaction						
Up x Winter	57.10±0.28c	5.90±0.10c	23.76±0.34c	21.43±0.38c	33.71±0.36b	4.60±0.10c
Up x Summer	45.15±0.33e	3.57±0.04d	24.39±0.70c	19.56±0.40d	39.43±0.72a	9.33±0.17a
Mid x Winter	66.69±0.71a	7.14±0.03b	25.72±0.37b	22.63±0.45b	31.68±0.82c	3.27±0.13d
Mid x Summer	51.93±0.28d	5.82±0.12c	30.85±0.77a	21.26±0.34c	29.96±0.28d	5.58±0.19b
Down x Winter	59.75±0.48b	8.70±0.32a	31.01±0.07a	25.71±0.36a	30.17±0.72d	5.32±0.10b
Down x Summer	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
LSD at 0.05	1.15*	0.50*	1.30*	0.72*	1.21*	0.43*

Note: NS: non-significant; *Significant at p≤0.05. The same and different letters in a single column indicate statistical non-significance and significance at p≤0.05 according to the LSD test, respectively

In conclusion, the ANOVA results reflect the soil texture and properties variation and chemical composition of *C. colocynthis* plants at the in Tabouk region. The physicochemical properties of the soil supporting *C. colocynthis* were significantly influenced by the sites, depths, and their interactions. Also, sites, seasons, and their interaction have significantly affected the chemical composition of *C. colocynthis* plants. The *C. colocynthis* plants tend to re-adjust their internal osmotic pressure in response to stress by accumulating osmolytes such as phosphorus, calcium, total pigment, and proline, resulting in metabolic adaptation and resistance to external stress in the desert environment.

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