Original Research Article

Ecophysiological studies on *Salvadora persica*

Abstract

The objective of this work was to study the ecophysiological response of *Salvadora persica* (*S. persica*) growing in Wadi El Gemal, to know the extent of the adaptation mechanism of the arak plant in its natural environment across the winter and summer seasons. Most physical and chemical characteristics of the soil supporting *S. persica* were significantly higher during 20-40 depth than during the 0-20 depth, but no significant differences were evident for pH and Mg^{2+} content between the two depths. Total chlorophyll, elements and chemical compositions studied of *S. persica* in the winter season were significantly higher than in the summer season, the opposite is true for total carotenoids, Mg^{2+}, S, P, total carbohydrate and proline contents. SDS-PAGE method was used to evaluate protein patterns in *S. persica*, which displayed different molecular weights of protein pattern across the winter and summer seasons. *S. persica* plants during the winter season showed a higher number of bands compared with those of the summer season. The number of polymorphic and unique bands was ten and three for *S. persica* leaves in both seasons, respectively. The differences in chemical compositions of *S. persica* due to seasonal changes led to the protein patterns changing and the activation of plant physiological stress tolerance mechanisms, indicating the protective role of these compositions and protein patterns in *S. persica*.

Key words: *soil characteristics, phytochemical compositions, protein patterns, SDS-PAGE, S. persica.*

1. Introduction

Ecophysiology is an experimental science to study the complex relationship between a plant's internal and external environments. Ecophysiologists work to understand how organisms react to and make up for distinct external environmental stressors as well as to pinpoint physiological processes that serve as environmental adaptations (Ferry-Graham and Gibb, 2008). Plant ecophysiology, also known as physiological plant ecology, is the study of how plants function and perform while subjected to environmental restrictions (Sonti, 2021). Pant ecophysiology provides a mechanistic understanding underlying current advances in the study of ecosystem fluxes (Menzer and McFadden, 2017) and plant community ecology (Kowarik and von der Lippe, 2018). Ecophysiological studies have been powerful in elucidating plant function and identifying traits that are adaptive in specific environmental conditions (Ackerly et al. 2000). Plant ecophysiology deserves further study, particularly in light of projected changes in climatic conditions influencing ecophysiological function (Sonti, 2021).

Desertification is an important issue in semi-arid climates, where there is a rapid increase in soil erosion and a steady decline in vegetation cover. Climate change is an important factor for sustainable water resource management in arid and semi-arid countries (Alghanem et al. 2020), and for modifying species distribution in desert areas controlling environmental heterogeneity and disturbances effects on plant species diversity (Khafagi et al., 2013). To cope with drastic environmental changes, plant species have evolved a variety of coping mechanisms that enable them to thrive and adapt in a variety of climates and terrains (Wang et al. 2014). Abiotic stressors have had a significant impact on the morphological, biological, and biochemical systems of plants. Two important environmental factors that affect plant productivity and spread are drought and salinity (Bray et al., 2000). In order to find and comprehend the mechanisms underlying drought resistance, there is growing interest in investigating the physiological behavior of different plant species (Martınez et al. 2005). Osmotic adjustment, antioxidants, and scavenger chemicals are among the physiological and molecular strategies used to achieve drought tolerance (Bartels and Sunkars, 2005). While, decreased stomatal conductance, reduced leaf area, and extensive root systems are morphological changes used to achieve drought avoidance in plants (Levitt, 1980).

Salvadora persica L. (*S. persica*) is classified into the kingdom Plantae, division Magnoliophyta, class Magnoliopsida, subclass Dilleniidae, order Capparales and family Salvadoraceae (Aljarbou et al. 2022). *S. persica* is frequently known also as Miswak, Siwak or Arak (Mekhemar et al. 2021). It is one of the most commonly widely used for teeth cleaning, oral hygiene (especially among the global Muslim community) and other medicinal properties, with great ethnobotanical importance (Kumari et al. 2017; Ghoneim et al. 2019). In Islam, there has been an ascent in prophetic medicine, which derives its principles from all deeds and recommendations made or carried out by the prophet Muhammad, peace be upon him, and *S. persica* has been directed toward promoting health (Nordin et al. 2012). S. persica plant received WHO approval in 2000 as a practical, inexpensive toothbrush that may be used on a regular basis (WHO, 2000). Pharmacological studies showed that the *S. persica* plant possesses alexiteric, analgesic, anti-inflammatory, anti-microbial, anti-plaque, astringent, diuretic aphrodisiac anti-pyretic, bitter stomachic activities, hypoglycemic, antiplasmodial, antiseptic and antiplaque actions (Balto et al. 2013; Mervat et al. 2017; Mekhemar et al. 2021).

Because soil and plants are interdependent, the pH, amount of organic matter in the soil as well as the type and nature of vegetation affect its physical, chemical, and biological characteristics (Kim et al.,1995; Al-Mujahidy et al., 2013; El-Absy, K. M. 2022). The variations in the measured element concentrations are not explained by the nature of the soil in which the plants are grown, but rather by the interactions between the elements or the genotype of the plants (Sayed et al. 2013). Key physiological functions might be hampered and cellular architecture can be disrupted by environmental stress such as drought, salinity, and temperature (Larcher, 2003), which can lead to turgor loss of plant species (El-Absy et al. 2015). The organism's or the community's ability to adapt to its surroundings depends on all environmental influences acting on it at once, not just one of them. Additionally, the degree to which a certain component is present in the habitat and restricting plant growth affects how an organism or the physiognomy of the vegetation adapts (Misra, 1980). To respond to stressful environmental conditions, the plants synthesize a vast array of metabolites (Mibei et al., 2016; El-Absy, 2021). El-Absy (2021) noted that seasonal fluctuations have a considerable impact on the majority of the chemical components in the plant. This may be due to seasonal variations in physiological demands and effort rather than plant content availability (Estevez et al., 2010). Recent phytochemical investigations of *S. persica* different parts afforded different classes of compounds metabolites (Farag et al. 2021). Many researchers such as Yadav and Saini (2007); Mohamed et al. (2014); Rangani et al. (2016); Rafey et al. (2022) have used the method of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis analysis (SDS-PAGE) in *S. persica*.

In this study, the main objective of research on the ecophysiology of *S. persica* plant is to know the extent of the adaptation mechanism of the arak plant in its natural environment and to the possibility of osmotic adjustment adopted to a tolerance of harsh environmental conditions during the winter and summer seasons.

2. Materials and methods

2.1. Study area

The largest wadi along the Egyptian Red Sea coast is Wadi El Gemal. Wadi El Gemal National Park spans 4,770 km^2 of land and 2,000 km^2 of marine waters, including more than 70 km of breathtaking shoreline. The Park extends from the Eastern Desert's sharp hills via a web of protected wadis to the Red Sea's emerald-colored depths. In Wadi El Gemal, 140 plant species were discovered, many of which are useful medicinally. The environmental conditions in Wadi El Gemal, which stretches from west to east, are the most diverse, including the types of soil, plant communities, and animal species assemblages (Mikhail et al. 2012; Milto et al. 2019). The study location (24°33′45.9″ N and 34°51′21.1″ E) was visited from August 2019 (summer season) to January 2020 (winter season).

2.2. Distribution and Description of S. persica:

S. persica is a desert perennial evergreen facultative halophytic plant (Parida et al. 2016), capable of growing under extreme conditions, from very dry environments to highly saline soils (Maggio et al. 2000). It is mainly distributed in dry and subtropical regions of Africa and the Middle East, as well as the Indian subcontinent (Mansour et al. 2020), such as Saudi Arabia, Yemen, Iran, Iraq, Egypt, India, Pakistan, Malaysia, Sudan, Ethiopia, and Mauritania as well as countries in Central Africa, Southwestern Africa, and South America (Maggio et al. 2000).

S. persica is a small, soft, light-yellow woody tree that branches to form a large leafy bush with a widely spreading crown, with a life span of 25 years (Aljarbou et al. 2022), and its height is up to 10 m, with a diameter up to three feet (Farag et al. 2021). Branches are numerous, drooping, glabrous, terete, finely striate, shining, and almost white. Leaves are somewhat fleshy, glaucous, 3.8–6.3 by 2–3.2 cm in size, elliptic-lanceolate or ovate, obtuse, and often mucronate at the apex, the base is usually acute, less commonly rounded, main nerves are in 5–6 pairs. The flowers are greenish yellow in color, in axillary and terminal compound lax panicles 5–12.5 cm long, numerous in the upper axils, pedicels 1.5–3 mm long, bracts beneath the pedicels, ovate and very caducous (Khatak et al. 2010). Fruits are drupes with persistent calyx and corolla. They are fleshy, globose, single-seeded, smooth, 5–10 mm in diameter and spherical in shape. The fruits are pink to scarlet in color when mature (Sher et al., 2010).

2.3. Soil physical and chemical properties

Soil samples were collected from the three random points at the two depths 0-20 cm and 20-40 cm in the Wadi El Gemal. 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. In this study, 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^{-1}. The soil soluble cations (meq/L) including sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), as well as the soil soluble anions (meq/L) including chlorides (Cl^-) and bicarbonate (HCO_3^-) were determined using a saturation paste described by Tuzuner (1990).

2.4. Plant analysis

The *S. persica* samples were manually taken in triplicates at random from the location under investigation in the winter and summer seasons of 2020 year. 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 the oven at 70°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 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. Atomic absorption spectrophotometry (GBC Avanta E, Victoria, Australia) was used to measure the concentrations of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), sulfur (S), Silica (Si) and phosphorus (P) [Chapman, 1965]. The micro-Kjeldahl method was used to determine the total nitrogen (N) level (Bremner, 1965).

The equation described by Jin et al., (2017) was used to determine the plant water content. According to Allen (1989), the total protein percentage was calculated by multiplying the total nitrogen by 6.25. AOAC (2000) procedures were used to calculate crude fiber. Based on the techniques of Chaplin and Kennedy (1994) and Bates et al. (1972), the contents of total available carbohydrates and proline were determined, respectively. The degree of succulence of the shoot systems of plants was calculated as the initial fresh weight / dry weight ratio (Fr.wt / Dry wt.) according to Dehan and Tal (1978). Samples of the shoot were weighed for fresh weight determination, then, washed with distilled water and dried in an aerated oven at 60 ˚C until constant weight. Values of fresh weight (fr.wt.) and dry weight (dry wt.) were expressed in gram (g).

2.5.Protein Gel Electrophoretic:

The protein from four samples of *L. Shawii* leaves during both studied seasons and locations were isolated using a modified sequential extraction standard procedure developed by Curioni et al. (2000).

- ***SDS-PAGE Procedure***: Each sample was subjected to Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis analysis (SDS-PAGE) by following the basic method developed by Laemmli [30] and modified by Singh and Shepherd [31]. The dried protein pellets were solubilized in 250 µL of a sample buffer. The electrophoresis was carried out using a 10% gel concentration [30]. A 10-well, 0.75 mm comb was used in a Bio-Rad Mini Protein 3 System having gel size 8.3–7.3 cm. The SDS-gels contained 4% polyacrylamide stacking gel and a resolving gel of 10% polyacrylamide. Samples (30 µL aliquots from sample [5 mg] extracted with 250 µl of sample buffer) were applied into precast application slots.
- ***Detection of protein Bands and Gel Imaging***: Upon the completion of electrophoresis, the proteins were fixed in methanol/acetic acid/water (40/10/50). Then staining with Coomassie Blue R-250. 200 ml of the destaining solution was used to destain the gel. The gel was gently agitated on a shaker for 2 hours. This

destaining procedure was repeated several times until the background color of tile gel was removed. Total bands for each species were scored and their molecular weight (Mol. Wt.) calculated using the protein marker as standard. The gel scanning was done on Helena Jonior 24 photo scanner and the data were integrated using the scanner software. On the first well of each gel, the proteins were employed as the molecular weight (Daltons) markers ranging from 10–250 KDa.

2.6. Statistical analysis:

The Komolgorov-Smirnov test was used to confirm the normality of the data distribution. Then, the measured data were subjected to a two-way ANOVA test (Steel and Torrie, 1997) and Student's t-test (McDonald, 2008) to determine the statistically significant differences ($p \leq 0.05$ and $p \leq 0.01$) of the effect of soil depths and seasonal variations. The data of soil and plant analysis are expressed as the mean (± standard error) of three replicates. For a better understanding of the relationships between the qualities tested across experimental conditions, principal component analysis (PCA) was used. To perform statistical analyses in this study, the computer software program OriginPro 2018 b9.5.0.193 was used.

3. Results and Discussion

3.1. Climatic conditions

The Wadi el Gemal region has a hot desert climate, as defined by the Köppen-Geiger classification. In Wadi El Gemal, the annual maximum temperature ranges from 23°C in January to 36°C in August. Rainfall totals 21 mm annually, with minimum amounts of 1 mm in July and maximum amounts of 5 mm in November. According to standards set by World Meteorological Organization (1988), Wadi El Gemal is characterized by arid climates (< 150 mm). The lowest and highest percentage of relative humidity were registered in November and June of the 2020 year, respectively. Temperature, as well as the strength and direction of the wind, have an impact on how much rain falls in the study region. One millimeter of rain every minute is the minimal quantity of rainfall that can result in runoff and flash floods, or around 10 millimeters overall (Cook et al., 1985).

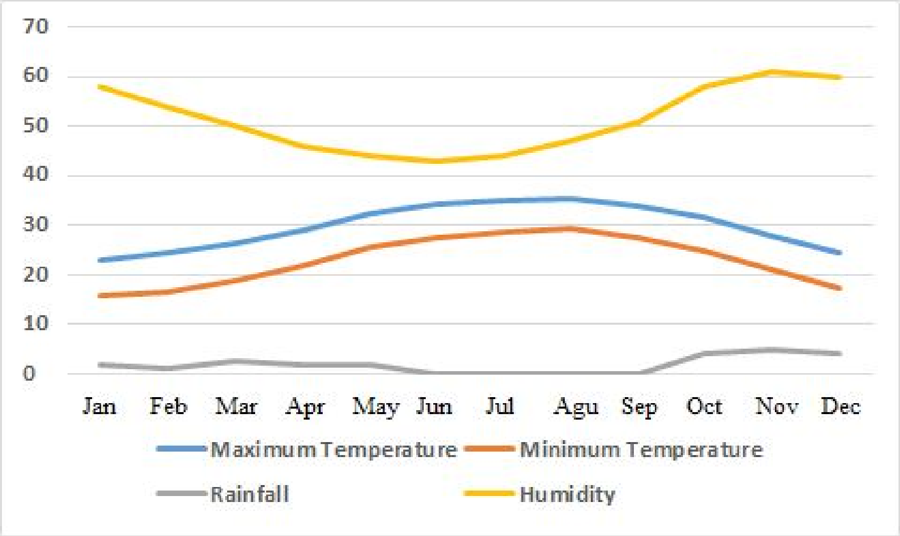

Fig. 1. Monthly average temperature (°C), relative humidity (%) and rainfall (mm) in Wadi El Gemal during the 2020 year.

3.2. Soil variables

The physical characteristics of the soil supporting *S. persica* in the research location are shown in Table 1. All physical characteristics of the soil supporting *S. persica* differed significantly at probability 0.05 level between the two depths studied. The same finding was published by Salama et al. (2014). The ratios of sand, silt, and clay in soils taken from diverse environments reportedly varied significantly (Al-Ghamdi and El-Zohri, 2017; Moustafa et al. 2021). Significantly highest values of sand % were observed in 0-20 depth, while the clay and silt % showed the highest values in 20-40 cm depth at adjoining soil of *S. persica*. Based on the overall mean, the sand % was highest across the two soil depths compared with other soil particles, thus the soil of the studied location had a sandy texture. *S. persica* plants are found on clays, loam, black soils, and sand (Kumar et al. 2012), and it is adapted to alkaline, non-saline, or very saline soils (Sujata, 2015). The texture of soil adjacent to *S. persica* varies from coarse (sandy) to fine (sandy-clay-loam) depending on the sampling site (Tounekti et al. 2018). Sandy soil, also known as light-textured soil, has low water holding capacity due to rapid water infiltration and percolation, as well as high soil aeration (USDA, 2002).

Table 1. Mean values and standard error of mean (±SEM) for the mechanical properties % of the soil adjacent to *S. persica* across two depths.

Depths	Sand	Clay	Silt	Texture
0 – 20	92.00±2.12	5.17±1.15	2.83±0.48	Sandy
20 – 40	90.42±0.88	6.39±0.73	3.19±0.62	
Difference	1.58±2.30	-1.22±1.36	-0.36±0.78	
Overall	91.21±1.09	5.78±0.67	3.01±0.36	
P–Values	0.03*	0.02*	0.07*	

Statistically significant differences at $^*p \leq 0.05$ between the two depths, according to Student's t-test.

In Egypt, the highest humid period of the year extended from November to April which was associated with low temperatures and evaporation; therefore, the soil had much favorable moisture (Bedair et al. 2020). As can be seen in Fig. 2, the results of the two-way ANOVA test showed that the depths, seasons, and their interaction had a highly significant impact ($P < 0.01$) on the water content % in the adjoining soil of *S. persica*. The same tendency was observed by El-Lamey (2020) and El-Absy (2021) who reported that the water content % in the soil of plant species was significantly affected by depths and seasons during different habitat conditions. Of course, the water content % is significantly higher during the winter season than during the summer season, due to increase rainfall. The seasonal effect can result in favorable changes to the soil's water content, such as an increase in the availability of phosphorus needed for plant sustenance (Misra and Tyler, 1999). Additionally, it is significantly increased in 20 - 40 depth compared to 0 - 20 depth. Compared with the other interaction between depths and seasons, the interaction of 20-40 depth in the winter season recorded a significantly higher water content %, while, a significantly lower water content % was found in 0-20 depth in the summer season. Generally, the rhizospheric community can be affected directly or indirectly by soil characteristics including texture, pH, the presence of microaggregates, major cations, and organic matter (Garbeva et al. 2004). The *S. persica* plant can live in harsh conditions and can tolerate soils ranging from intensely drought to highly saline (Haque et al. 2015; Mekhemar et al. 2021). The moisture gradient is complicated and linked to numerous environmental elements, including elevation, slope, climatic drought, soil texture, and character of the soil surface, according to Moustafa and Zayed (1996).

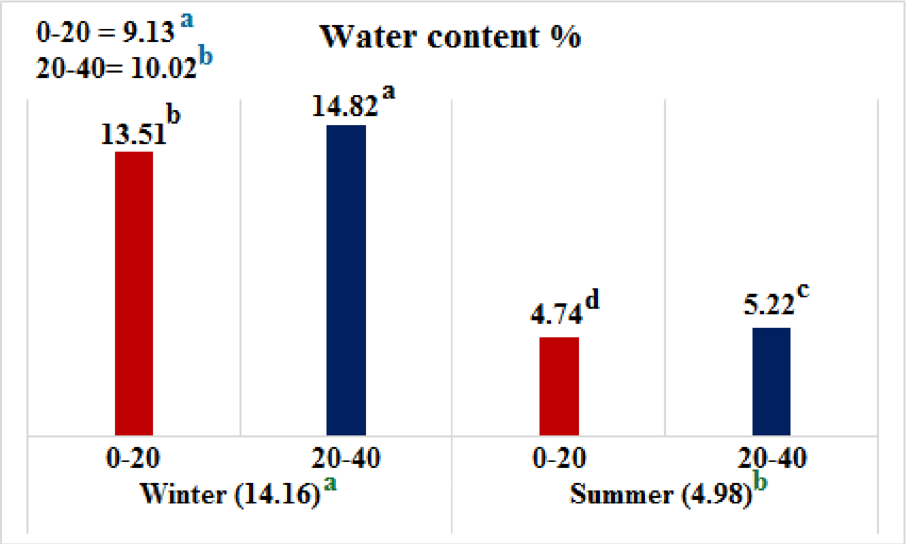

Fig.2. Water content % at the soil associated with *S. persica* in the two depths and the two seasons during Wadi El-Gemal. Different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test.

The contents of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Cl^{-} and other elements usually determine soil fertility. One of the most trustworthy and popular metrics, especially to describe field variability, is soil EC (Aljeddani and Ahmed, 2020). Statistically, the Student's t-test showed that all chemical characteristics of the soil associated with *S. persica* have significant differences ($P < 0.05$ or $P < 0.01$) between the 0-20 and 20-40 depths, except pH and Mg^{2+} properties, as shown in Table 2. In the study by Salama et al. (2014), the measured chemical properties in the soil of *S. persica* showed significant differences ($p< 0.05, 0.01$). Significantly increased electrical conductivity (EC), Na^{+}, K^{+}, Ca^{2+}, Cl^{-} and HCO$_3^{-}$ of the soil in the 20-40 cm depth were found compared with the 0-20 cm depth soil. While the increased pH and Mg^{2+} were noticed in the 0-20 and the 20-40 cm depths, respectively. The Na^+, HCO_3^- and Ca^{2+} contents showed higher values relative to the other chemical properties in the soil associated with *S. persica* at the two depths in the location studied, according to the overall mean. These results are similar to the analysis of adjoining soil of S. persica described by Aljeddani and Ahmed (2020). The variation in values of soil chemical properties between various environments may be due to location in relation to the distance from the sea and the types of surrounding mountains, both of which play a significant effect on soil characteristics (Moustafa et al. 2021). The soil in the study area connected to *S. persica* tended to be alkaline based on the pH readings. At alkaline pH levels greater than 7.5, chemical interactions with some compounds, such as HPO_4 and $CaCO_3$, cause the solubility of magnesium, calcium, and potassium as well as other elements to drop (Marschner, 1995). The soil adjacent to *S. persica* contains a large amount of carbonates that reflect its alkaline pH (Aljeddani and Ahmed, 2020). Decrease values of EC may have a substantial impact on plant health and nutrient availability because soluble salts are more likely to build in arid soils due to low precipitation and temperature rise, thus a high value of EC (Smith and Doran, 1996; Corwin and Lesch, 2005). The values of EC range from 19.5 to 25.7 ds m^{-1} in the soil adjacent to *S. persica* (Tounekti et al. 2018). The soil associated with *S. persica* in Wadi Gimal and its tributaries was soil rich in fine sand, pH and K^+ and poor in Mg^{+2} and water contents (Salama et al. 2014). Significant variations in environmental factors are reflected in the variety of soil properties and texture (Al-Mutairi, 2017). The plants are able to adjust and flourish in environments with various soil characteristics (Comole et al., 2021).

Table 2. Mean values and standard error of mean (±SEM) for the chemical properties % of the soil adjacent to *S. persica* across two depths.

Depths	Ec (ds/m^{-1})	pH	Na^+ (meq/L)	K^+ (meq/L)	Ca^{2+} (meq/L)	Mg^{2+} (meq/L)	Cl^- (meq/L)	HCO_3^- (meq/L)
0 – 20	14.09±0.58	8.21±0.12	8.63±0.17	0.69±0.02	6.38±0.12	5.49±0.06	4.35±0.12	7.64±0.08
20 – 40	15.38±0.64	8.17±0.10	10.37±0.21	0.74±0.03	6.47±0.13	5.69±0.08	4.78±0.08	7.89±0.05
Difference	-1.29±0.86	0.04±0.16	-1.74±0.28	-0.05±0.03	-0.09±0.13	-0.20±0.09	-0.43±0.14	-0.25±0.10
Overall	14.74±0.48	8.19±0.07	9.5±0.41	0.72±0.02	6.43±0.08	5.59±0.06	4.57±0.11	7.77±0.07
P–Values	0.01^*	0.81^{ns}	0.00^{**}	0.02^*	0.07^*	0.11^{ns}	0.04^*	0.06^*

Statistically significant differences at $*p \leq 0.05$ and $**p \leq 0.01$; ns: indicate the non-significant difference between the two depths, according to Student's t-test.

3.3. Plant variables

The alterations in photosynthetic pigments affect plant metabolism significantly (Rangani et al. 2016), because they are vital components of the energy metabolism of plants (Parida et al. 2016). Table 3 displays the photosynthetic pigment contents in *S. persica* during the winter and summer seasons. The photosynthetic pigment contents in *S. persica* were affected significantly ($P < 0.05$ or 0.01) by the two seasons. These photosynthetic pigment contents in *S. persica* plants indicate significant differences in response to changes in plant habitat (Tounekti et al. 2018). Significant differences in all photosynthetic pigments in plant species between seasons were observed by Uvalle Sauceda et al. (2008), Devi et al. (2015) and El-Absy, K.M. (2021). The winter season showed the highest levels of Chl.a, Chl.b, and Chl.a+b contents in *S. persica* plants. While, the highest values for Chl.a/b, total carotenoids and total pigment contents were observed in the summer season. The Chl.a content was greater than Chl.b content in *S. persica* plants, thus the values of

Chl.a/b were more than 1 during the two seasons. Similar results were recorded for *S. persica* by Malhotra and Madan (2017), and other species plants by Huang et al. (2021) and El-Absy (2021 and 2022). During the two research seasons, *S. persica* plants had the greatest total carotenoids content, followed by contents of Chl.b and Chl.a, accoeding to overall mean.

The increased concentrations of chlorophyll and carotenoids that desert plants achieved during dry conditions allowed the plants to adjust to variations in light conditions and stresses (Morsy et al., 2008). According to Ait Said et al. (2013), a decline in Chl.a can be viewed as a defensive adaptation mechanism that prevents increasing photon absorption. Each leaf area's chlorophyll content demonstrates how various plant species have adapted to the local environmental conditions (Ivanov et al. 2020). Chlorophyll can substantiate the tolerance of plants to high pH conditions (Cimen et al. 2014).

Table 3. Mean values and standard error of mean (±SEM) for the photosynthetic pigments contents (g/100g fr. wt.) of *S. persica* under winter and summer seasons.

Seasons	Chlorophyll a (Chl.a)	Chlorophyll b (Chl.b)	Total Chlorophyll	Chl.a/b	Total Carotenoids	Total Pigment
Winter	5.99±0.06	3.89±0.05	9.88±0.11	1.54±0.01	435.00±2.89	444.88±3.00
Summer	4.68±0.05	2.36±0.02	7.04±0.02	1.98±0.04	441.00±3.46	448.04±3.44
Difference	1.31±0.07	1.53±0.06	2.84±0.11	-0.44±0.04	-6.00±4.51	-3.16±4.56
Overall	5.34±0.29	3.13±0.34	8.46±0.64	1.76±0.10	438.00±2.42	446.46±2.16
P–Values	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.05^{*}	0.03^{*}

Statistically significant differences at $*p \leq 0.05$ and $**p \leq 0.01$ between the two seasons, according to Student's t-test.

Using statistical analysis by Student's t-test, significant effects (P < 0.05 or 0.01) of seasons on all micro and macro elements compositions were found, except Ca^{2+} only (Table 4). Accumulation of Na^{+} and K^{+} in *S. persica* plants showed significant differences in response to changes in plant habitat (Tounekti et al. 2018). A significant seasonal trend was found in elements compositions of *S. persica* by Joshi et al. (1993) and of other plant species by Al-Qahtani et al. (2020), Kamel and El-Absy (2020) and El-Absy (2021 and 2022). This may reflect seasonal changes in physiological needs and effort, rather than availability in plant content (Estevez et al. 2010). The contents of Na^{+}, Ca^{2+}, Cl^{-}, Si, N and K^{+} in *S. persica* plants in the winter season were higher than in the summer season, the opposite is true for other elements contents including Mg^{2+}, S and P. The change in mineral accumulation at various stands suggests that the plants have the capacity to control the uptake and accumulation of the elements from the external source in accordance with their adjustment needs, consequently, ions play a significant role in the production of osmotic potential in the xerophytes plants (Sayed et al. 2013). According to the overall mean during the two seasons, Cl^{-} and K^{+} contents of *S. persica* plants registered the highest values compared with other micro and macro elements, followed by Ca^{2+} and levels, respectively. Similar to our results, Aljeddani and Ahmed (2020) reported that the highest divalent component found is Ca^{2+} as well as the contents of K^{+} and Na^{+} are in equilibrium in *S. persica*.

The amount of nitrogen is the main factor that affects the leaf area, photosynthesis rate, growth and development of the plant. The Na^{+} maintains the osmotic potential in the cells (Blumwald et al. 2000), and it is also related to physiological demands (Ceacero et al. 2009). K^{+} is a major plant macronutrient that plays important roles related to drought resistance (Elumalai et al. 2002). *S. persica* depends highly on inorganic ions including Na^+, Cl^-, and K^+ for osmotic adjustment and maintenance of cell turgor (Tounekti et al. 2018). In *S. persica* plants, the effects of Ca^{2+} and Mg^{2+} contents on Na^+ efflux are transient, while they cause a prolonged shift toward K^+ uptake (Parida et al. 2016). According to Salama et al. (2015), stressed plants can modify their osmotic adjustment by absorbing and removing inorganic osmoregulatory ions including K^+, Na^+, Ca^{+2}, and Mg^{+2}. Si alleviates the toxic effects caused by salt stress, drought, and heavy metals (Luyckx et al. 2017).

Table 4. Mean values and standard error of mean (±SEM) for the micro and macro elements compositions of *S. persica* under winter and summer seasons.

Seasons	Micro elements (μg g^{-1} dry wt.)						Macro elements (%)		
	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	S	Si	N	P	K
Winter	1.74±0.02	1.48±0.05	0.99±0.05	2.45±0.03	1.00±0.01	0.52±0.01	2.18±0.06	0.31±0.01	2.53±0.02
Summer	0.89±0.03	1.40±0.01	1.53±0.02	1.25±0.04	1.21±0.03	0.49±0.01	1.36±0.05	0.37±0.02	1.85±0.03
Difference	0.85±0.04	0.08±0.05	-0.54±0.05	1.20±0.05	-0.21±0.03	0.03±0.02	0.82±0.08	-0.06±0.02	0.68±0.02
Overall	1.32±0.19	1.44±0.03	1.26±0.12	1.85±0.27	1.11±0.05	0.51±0.01	1.77±0.19	0.34±0.02	2.19±0.15
P–Values	0.00^{**}	0.17^{ns}	0.00^{**}	0.00^{**}	0.00^{**}	0.09^{*}	0.00^{**}	0.04^{*}	0.00^{**}

Statistically significant differences at $*p \leq 0.05$ and $**p \leq 0.01$; ns: indicate the non-significant difference between the two seasons, according to Student's t-test.

The seasonal variations differences were statistically significant for levels of water content %, total protein, crude fiber ($P<0.01$), total carbohydrate, proline and succulence degree ($P<0.05$), as shown in Table 5. These results agreed with the results of Salama et al., (2017) for water content, El-Absy et al. (2015) for crude fiber and succulence degree, El-Absy (2021) for total protein, Al-Qahtani et al., (2020) for total carbohydrates, Tounekti et al. (2018) for proline. The winter season of *S. persica* showed significantly higher levels of water content%, total protein, crude fiber, and succulence degree than the summer season. In contrast to the winter season, the summer season had higher levels of proline and total carbohydrates in *S. persica*. The proline and total carbohydrates can be used as an indicator of disturbed physiological conditions as drought and salinity stresses in most plant species (Dhaka and Meena 2018; Al-Qahtani et al. 2020; El-Absy 2021). Regarding the overall mean, the highest values had noticed for crude fiber, followed by total protein and total carbohydrate levels during the two seasons. Proline content in *S. persica* plants showed significant differences in response to changes in plant habitat (Tounekti et al. 2018).

To maintain the water content % in the mesophyll tissue of *S. persica*, the increase in epidermal thickness might be an adaptation of these plants to minimize the transpiration rate (Parida et al. 2016). Thus, plants reduce their internal water potential under abiotic stresses (Erdei et al., 1990). Total soluble nitrogen may be contributing partially to the building up of the osmotic potential of plants (Youssef, 1988), which may be accompanied by protein accumulation to improve the plant species to drought stress tolerance (Salama et al, 2021).

Under stress conditions, the total available carbohydrates are converted to soluble sugars that lead to decreasing osmotic potential, and variations in water potential gradient are responsible for water, organic and inorganic solute translocations in the plant cells (Stocker, 1960). Proline is an important osmolyte produced at a high cost of energy as compared to the uptake of Na^+ into the cytoplasm, and it is increased under stress conditions (Parida et al. 2016). Proline accumulation in the leaves and roots of *S. persica* seems to play a role in osmotic adjustment and osmo-protection (Tounekti et al. 2018). Succulence degree is one of the most common features of many halophytes as well as xerophytes which can be increased by univalent more than by divalent or trivalent ions (El-Absy et al. 2015). The increased leaf succulence in *S. persica* might be the plant's attempt to increase the size of its vacuole, and to counter the increased Na$^+$ ion content from the cytosol of the leaf tissue, where sequester large quantities of Na$^+$ away from metabolic active compartments of the cell (Parida et al. 2016).

The plant species seek to modify their internal osmotic pressure by accumulating inorganic and organic solutes in order to counteract the external stress in the arid environment (Sayed et al. 2013). The ability of plants to accumulate inorganic ions in high quantities inside their tissues is the most important mechanism to maintain the plant water potential more negative than the external medium in order to maintain the water uptake (Mile et al., 2002). A combination of physiological (accumulation of Na+ and K+ in the leaves) adjustments and increased osmo- (accumulation of proline) and photo- (reduction in chlorophyll content) protection helped the *S. persica* plants overcome salinity, hypoxia, and their combination in its native habitats (Tounekti et al. 2018). Diverse mechanisms to adapt are supported by rich and complex metabolic networks that enable the plant to synthesize a wide range of compounds (Wang et al. 2014).

Table 5. Mean values and standard error of mean (±SEM) for some chemical compositions of *S. persica* under winter and summer seasons.

Seasons	Water Content %	Total Protein	Crude Fiber	Total Carbohydrate(g%)	Proline (mg/g)	Succulence degree
Winter	54.69±0.64	13.65±0.38	39.28±1.80	9.58±0.07	5.62±0.07	1.78±0.03
Summer	40.73±1.15	8.52±0.31	28.16±1.10	10.81±0.55	7.60±0.49	1.57±0.04
Difference	13.96±1.32	5.13±0.49	11.12±2.11	-1.23±0.55	-1.98±0.49	0.21±0.05
Overall	47.71±3.18	11.08±1.17	33.72±2.66	10.20±0.37	6.61±0.49	1.68±0.05
P–Values	0.00**	0.00**	0.00**	0.09*	0.02*	0.01*

Statistically significant differences at $*p \leq 0.05$ and $**p \leq 0.01$; ns: indicate the non-significant difference between the two seasons, according to Student's t-test.

3.4. Protein Gel Electrophoretic:

The protein profiles and relative mobility of bands (RF) extracted from *S. persica* leaves during the winter and summer seasons are presented in Table 6 and figure 3. The results indicated that the number of the total bands in *S. persica* was 13 band as well as the molecular weight ranged from 17 to 97 kDa across the two seasons. Also during both seasons, the RF values of *S. persica* plants ranged from 0.18 to 0.92. According to the protein profiles using SDS-PAGE, the seasonal variations led to changes in the protein patterns, where the number of bands present are twelve and eleven in the winter and summer seasons, respectively. While the number of bands absent are one and two in the winter and summer seasons, respectively. Twelve protein bands were observed in extracts of *S. persica* leaves, which ranged from 24 to 67 kDa (Yadav and Saini, 2007). While molecular weights ranged from 14 to 20 kDa for five α-amylases from the *S. persica* (Mohamed et al. 2014). The salt stress induces alterations in the protein profile in plants (Galal, 2017), where the high molecular weight proteins may be responsible for reducing Na+ inflow and hence raising salt stress tolerance (Schachtman et al. 1997).

The all persistent bands and RF appeared in both seasons except the bands number 7, 8 and 12. In contrast to the summer season, *S. persica* plants in the winter season were distinguished with two 7 and 12 bands with molecular weight 49 and 29 kDa as well as with 0.51 and 0.85 RF values, respectively. While, the band No. 8 was found only in the summer season with molecular weigh 46 kDa and 0.57 RF value. The bands No. 1 and 13 had recorded the highest and lowest molecular mass with values 97 and 17 kDa of *S. persica* in both seasons, respectively. These results indicate that different molecular weights of *S. persica* in both seasons. *S. persica* showed high genetic diversity due to 85 loci showing polymorphism out of 106 loci (Monfared et al. 2018). Out of thirteen bands, the *S. persica* plants were characterized by the presence of three unique and 10 polymorphic bands in this study. The average of polymorphic bands No. and monomorphic bands No. were 9.4 and 2.22, they also added no unique bands were observed for either the accessions of *S. persica* (Monfared et al. 2018). According to Kamel and El-Absy (2020), *lycium showii* plants demonstrated the appearance of the majority of low molecular weight proteins is unique, in agreement with our results of *S. persica* plants.

Table 6. SDS-PAGE of protein bands extracted from *S. persica* leaves growing in Wadi El Gemal during the winter and summer seasons.

Marker M.W(kDa)	Bands No.	RF	M.W (kDa)	Winter	Summer	Polymorphism
120	1	0.18	97	1	1	Polymorphic
	2	0.24	85	1	1	Polymorphic
85	3	0.31	76	1	1	Polymorphic
	4	0.35	73	1	1	Polymorphic
50	5	0.41	65	1	1	Polymorphic
	6	0.47	56	1	1	Polymorphic
	7	0.51	49	1	0	Unique
35	8	0.57	46	0	1	Unique
	9	0.59	39	1	1	Polymorphic
25	10	0.61	37	1	1	Polymorphic
	11	0.69	35	1	1	Polymorphic
	12	0.85	29	1	0	Unique
20	13	0.92	17	1	1	Polymorphic
			1	12	11	
			0	1	2	

kDa: kilo Dalton; RF = Relative mobility of bands; (1): Presence of bands; (0): absence of bands.

In this study, PCA analysis was used to identify the similarities and dissimilarities relationships among protein bands of *S. persica* leaves during the two seasons (Fig. 3). The first (PC1) and second (PC2) principal components explained 56.15%, and 43.85% of the total variance of protein bands, respectively. The PC1 and PC2 mainly distributed and distinguished protein bands of *S. persica* across the two seasons into three groups. These results are in harmony with Kamel and El-Absy (2020), who reported that protein bands were divided into different groups based on the differences among them. The first group was related to PC1 and includes the protein bands number 7 and 12, which are strongly positively associated with the winter season. The second group is related to PC2, which includes protein band number 8 across the summer season. While the third group comprised the other protein bands of *S. persica* leaves in both seasons. Each group contained protein bands that were highly similar across both seasons, the opposite is true. These findings show variations in the protein patterns of *S. persica* leaves in both seasons. According to Kamel and El-Absy (2020), seasonal changes have a considerable impact on controlling the expression of protein patterns in plants, which could be evidence of the adaptation of the plant to different stresses, also the protein patterns may be considered as key genetic markers of stress tolerance. In both favorable and unfavorable growth conditions, stress proteins, which make up a large portion of molecular chaperones, play a crucial role in maintaining cellular homeostasis (Jung et al. 2014). Plant responses to abiotic stresses involve interactions and crosstalk between many molecular pathways (Wang et al. 2014). Genetic identities are influenced both by polymorphic loci and the number of monomorphic loci (Monfared et al. 2018).

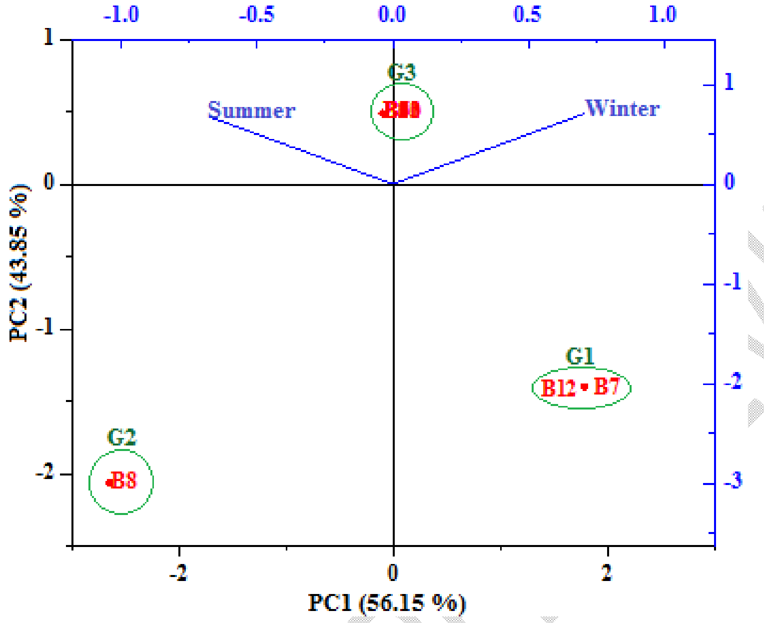

Fig.3. Biplot diagram between PC1 and PC2 shows similarities and dissimilarities relationships between bands of *S. persica* during the two seasons. B1-B13: The bands No.1 – No. 13.

5. Conclusions

Significant differences in the chemical compositions of *S. persica* during seasonal changes indicate the maintenance of cellular osmotic balance to protect the plant during different stress conditions. Based on SDS-PAGE method, the protein patterns reflect variations of behavior and adaptation of *S. persica* under harsh environmental conditions. Thus, *S. persica* plants are favorable to the conditions of the arid desert. Generally, our results can provide information on the ecophysiological function and molecular characteristics of *S. persica* plants across seasonal changes.

6. References

Ackerly DD, Dudley SA, Sulton SE, Schmitt J, Coleman JS, Linder CR. The evolution of plant ecophysiological traits: recent advances and future directions. Bioscience 2000; 50: 979-995.

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

Al-Ghamdi, A. A. M. and El-Zohri, M. (2017). Effect of two different habitats on some primary and secondary phytochemicals of Miswak (Salvadora persica

L.). African Journal of Biotechnology, 16(11), 517-527. https://doi.org/10.5897/AJB2017.15894

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.

Aljarbou F., A. Almobarak, A. Binrayes, and H.M. Alamri (2022). Salvadora persica's Biological Properties and Applications in Different Dental Specialties: A Narrative Review. Evidence-Based Complementary and Alternative Medicine, 9 pages, https://doi.org/10.1155/2022/8667687

Aljeddani G.S. and H.E. Ahmed, 2020. Autecology and Physiological Features of Salvadora persica Plants Grown under Dry Conditions. American Journal of Plant Physiology, 15: 14-22. DOI:10.3923/ajpp.2020.14.22

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-Qahtani H, Alfarhan AH, Al-Othman ZM. 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. 2020;27(10):2756-2769, https://doi.org/10.1016/j.sjbs.2020.06.035

AOAC, (2000). Official Methods of Analysis,17th Ed. Association of official analytical Chemists (Washington D.C.,U.S.A, 2000).

Balto, H.; Al-Howiriny, T.; Al-Somily, A.; Siddiqui, Y.; Al-Sowygh, Z.; Halawany, H.; Al-Hadlaq, S. Screening for the antimicrobial activity of Salvadora persica extracts against Enterococcus faecalis and Candida albicans. Int. J. Phytomed 2013, 5, 486–492.

Bartels D. and R. Sunkar (2005) Drought and Salt Tolerance in Plants, Critical Reviews in Plant Sciences, 24:1, 23-58, https://doi.org/10.1080/07352680590910410

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.

Bedair H., K. Shaltout, D. Ahmed, A. Sharaf El-Din, R. El-Fahhar (2020). Characterization of the Wild Trees and Shrubs in the Egyptian Flora. Egyptian Journal of Botany, 60(1):147-168. DOI: 10.21608/ejbo.2019.6982.1276

Blumwald, E., G.S. Aharon and M.P. Apse, 2000. Sodium transport in plant cells. Biochim. Biophys. Acta (BBA)-Biomembr., 1465: 140-151.

Bray EA, Bailey-Serres J, Weretilnyk E. 2000. Responses to abiotic stresses. In: Biochemistry and Molecular Biology of Plants. Gruissem, W., Buchnnan, B., and Jones, R. eds. American Society of Plant Physiologists, Rockville, MD, 1158-1249.

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.

Ceacero F, Landete-Castillejos T, Garcia AJ, Estevez JA, Gaspar-López E, Gallego L (2009) Effects of ad libitum mineral consumption in Iberian red deer hinds and calves. Animal Production Science 50, 37–44. doi:10.1071/AN09078

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.

Cimen, B., T. Yesiloglu, M. Incesu and B. Yilmaz, 2014. Growth and photosynthetic response of young 'Navelina' trees budded on to eight citrus rootstocks in response to iron deficiency. N. Z. J. Crop Hortic. Sci., 42: 170-182.

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

Cook, R.U., D. Brunsden, J.C. Doornkamp, and D.K. Jones. 1985. Urban geomorphology in dry lands Oxford University Press, 212-215.

Corwin, D.L. & Lesch, S.M.(2005). Apparent soil electrical conductivity measurements in agriculture. Computers and Electronics in Agriculture, 46(1-3),11-43. https://doi.org/10.1016/j.compag.2004.10.005

Curioni A, Ponga NE, Pasini G, Spettoli P, Voltarel M, peruffo ADB. characterisation of the glutenin fraction from eikorn wheat (Triticum monococum SSP.Mononcoccum) with different bread making qualities. Italian J of Food Sci. 2000;12:91-102.

Dehan, K., Tal, M. (1978). Salt tolerance in the wild relatives of the cultivated tomato: responses of Solanum pennellii to high salinity. Irrigation Science, 1, 71–76. https://doi.org/10.1007/BF00269009

Devi K, Kapila S, Rao A. Seasonal variations in photosynthetic pigments of three species of Marchantiaceae. International Journal of Advances in Pharmacy, Biology and Chemistry. 2015;4(3): 713-718.

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

El-Absy K.M., W.A. Kasim, H.F. El-Kady, M.N. El-Shourbagy (2015). Physiological Studies on Achillea fragrantissima and Artemisia judaica in Saint Katherine, South Sinai, Egypt. International Journal of Scientific Research in Agricultural Sciences, 2(Proceedings), pp. 127-136.

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-Absy, K. M. (2022). Effect of Different Habitats Conditions on *Citrullus colocynthis* (L.) Schrad. Growing Naturally in Egypt and Kingdom of Saudi Arabia. Journal of Advances in Biology & Biotechnology, 25(2), 8-29. https://doi.org/10.9734/jabb/2022/v25i230265

El-Lamey TM. Changes in some chemical compounds of *Retama raetam* (Forssk.) Webb & Berthel. in response to different environmental conditions. Journal of Biodiversity and Environmental Sciences. 2020;16(2):78-91.

Elumalai, R.P., P. Nagpal and J.W. Reed, 2002. A mutation in the arabidopsis KT2/KUP2 potassium transporter gene affects shoot cell expansion. Plant Cell, 14: 119-131.

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

Farag M., W.M. Abdel-Mageed, A.A. El Gamal, O.A. Basudan, (2021). Salvadora persica L.: Toothbrush tree with health benefits and industrial applications – An updated evidence-based review, Saudi Pharmaceutical Journal, 29(7):751-763, https://doi.org/10.1016/j.jsps.2021.05.007

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

Ferry-Graham L.A., A.C. Gibb, (2008). Ecophysiology, Editor(s): Brian Fath, Encyclopedia of Ecology (Second Edition), Elsevier, Pages 346-349, https://doi.org/10.1016/B978-0-444-63768-0.00531-X.

Galal A. (2017). Physico-chemical changes in karkade (Hibiscus sabdariffa L.) seedlings responding to salt stress. Acta Biologica Hungarica 68(1), 73–87. DOI: 10.1556/018.68.2017.1.7

Garbeva P, Veen JA, ElsasJD (2004). Microbial diversity in soil: Selection microbial populations by plant and soil type and implications for disease suppressiveness. Annu. Rev. Phytopathol. 42:243-270

Ghoneim MM, Afifi WM, Ibrahim M, Elagawany M, Khayat MT, Aboutaleb MH, et al. Biological evaluation and molecular docking study of metabolites from Salvadora persica L. Growing in Egypt. Pharmacognosy Magazine, 2019;15:232-7.

Haque, M.M.; Alsareii, S.A. A review of the therapeutic effects of using miswak (Salvadora persica) on oral health. Saudi Med. J. 2015, 36, 530–543.

Huang, Z., Liu, Q., An, B., Wu, X., Sun, L., Wu, P., Liu, B., 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

Ivanov LA, Ronzhina DA, Yudina PK, Zolotareva NV, I. V. Kalashnikova IV, Ivanova LA. Seasonal dynamics of the chlorophyll and carotenoid content in the leaves of steppe and forest plants on species and community level. Russ Journal Plant Physiology. 2020;67:453-462. https://doi.org/10.1134/S1021443720030115

Jackson, M.L. (1962). Soil chemical analysis constable and co. Ltd. London.

Jackson, M.L. (1967). Soil chemical analysis. Pritice Hall of India Private., 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

Joshi AJ, Krishankumar M, Mali BS. (1993). Seasonal changes in proteins,amino acids and minerals in Salvadora persica Linn.with reference to saline habitats. Ind. J. Plant Physiol. 34:202-204

Jung YJ, Nou S, Kang KK. Overexpression of Oshsp16.9 gene encoding small heat shock protein enhances tolerance to abiotic stresses in rice. Plant Breed Biotech. 2014;2(4):370-379.

Kamel AM, El-Absy KM. Seasonal variations in protein patterns and mineral contents of Lycium showii under different habitat conditions. Asian Plant Research Journal. 2020;6(4): 91-103. https://doi.org/10.9734/aprj/2020/v6i430141

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.

Khatak, M., Khatak, S., Siddqui, A. A., Vasudeva, N., Aggarwal, A., & Aggarwal, P. (2010). Salvadora persica. Pharmacognosy reviews, 4(8), 209–214. https://doi.org/10.4103/0973-7847.70920

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

Kowarik, I. and von der Lippe, M. (201 8) Plant population success across urban ecosystems: a framework to inform biodiversity conservation in cities. The Journal of Applied Ecology 55, 2354–2361.

Kumar S, Rani C, Mangal M (2012). A Critical review on Salvadora persica: An important medicinal plant of arid zone. Int. J. Phytomed. 4:292-303

Kumari A, Parida AK, Rangani J and Panda A (2017) Antioxidant Activities, Metabolic Profiling, Proximate Analysis, Mineral Nutrient Composition of *Salvadora persica* Fruit Unravel a Potential Functional Food and a Natural Source of Pharmaceuticals. Front. Pharmacol. 8:61. doi: 10.3389/fphar.2017.00061

Larcher W (2003). Physiological plant ecology. Springer-Verlag, Berlin. https://doi.org/10.1007/978-0-387-78341-3

Levitt J (1980) Responses of plants to environmental stress: chilling, freezing and high temperature stresses, 2nd 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

Luyckx M, Hausman J-F, Lutts S and Guerriero G (2017) Silicon and Plants: Current Knowledge and Technological Perspectives. Front. Plant Sci. 8:411. doi: 10.3389/fpls.2017.00411

Maggio A., M. P. Reddy, and R. J. Joly, “Leaf gas exchange and solute accumulation in the halophyte *Salvadora persica* grown at moderate salinity,” Environmental and Experimental Botany, vol. 44, 2000.

Malhotra, G.S. and Madan, J. (2017). Trends of pigmentation in foliar tissue of some arid and Sub-arid zone plants of Northern Rajasthan. ESSENCE - International Journal for Environmental Rehabilitation and Conservation, VIII(1):224 – 231

Mansour, H.; Alsamadany, H.; Al-Hasawi, Z.M. Genetic diversity and genetic structure of Salvadora persica L., rare plant species in Rabigh province, Saudi Arabia: Implications for conservation. J. Taibah Univ. Sci. 2020, 14, 881–888.

Marschner, H. (1995). Mineral nutrition of higher plants. second edition. London: Academic Press. pp 889. https://doi.org/10.1016/C2009-0-63043-9

Martınez J, Kinet J, Bajji M, Lutts S. 2005. NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L. Journal of Experimental Botany 56(419), 2421-2431.

McDonald JH. Handbook of Biological Statistics Sparky House Publishing, Baltimore. 2008.

Mekhemar, M.; Geib, M.; Kumar, M.; Radha; Hassan, Y.; Dörfer, C. *Salvadora persica*: Nature’s Gift for Periodontal Health. Antioxidants 2021, 10, 712. https://doi.org/10.3390/antiox10050712

Menzer, O. and McFadden, J.P. (2017) Statistical partitioning of a three-year time series of direct urban net CO2 flux measurements into biogenic and anthropogenic components. Atmospheric Environment, 170, 319–333.

Mervat, E.-H.; Ali, H.M.; Ashmawy, N.A.; Salem, M.Z.M. Chemical Composition and Bioactivity of Salvadora persica Extracts against Some Potato Bacterial Pathogens. BioResources 2017, 12, 1835–1849.

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

Mikhail G. (2012). WADI EL GEMAL NATIONAL PARK, Integrated Development Plan. Available in: https://www.eeaa.gov.eg/portals/0/eeaaReports/EPASP/FSPA%20studies/13-%20WGPA%20Development%20Plan.pdf

Mile O, Meszaros I, Verses S, Lakatos G. 2002. Ecophysiological study on the salt tolerance of a pannonian endemism (Lepidium crassifolium (W. et K.)) in inland saline area. Acta Biologicae Szegediensis 46(3-4), 249-250.

Milto K.D., S.A. Saber, A.M. Nagy, R.A. Nazarov, D.A. Melnikov and N.B. Ananjeva (2019). First report on the reptile diversity of Wadi El Gemal National Park, eastern desert, Egypt. Russian Journal of Herpetology, 26(3):175 – 184. DOI: 10.30906/1026-2296-2019-26-3-175-184

Misra KC (1980). Manual of Plant Ecology (2n. Ed) Oxford and IBH Publishing Co. New Delhi, Bombay, Calcutta.

Misra A, Tyler G. Influence of soil moisture on soil solution chemistry and concentrations of minerals in the Calcicoles Phleum phleoides and Veronica spicata grown on a limestone soil. Annals of Botany. 1999;84:401-410. https://doi.org/10.1006/anbo.1999.0941

Mohamed, S.A., Almulaiky, Y.Q., Ahmed, Y.M. Al-Bar, O.A.M. & Ibrahim I.H. (2014). Purification and characterization of α-Amylase from Miswak Salvadora persica . BMC Complement Altern Med 14, 119 (2014). https://doi.org/10.1186/1472-6882-14-119

Monfared M.A., D. Samsampour, G.R. Sharifi-Sirchi, F. Sadeghi (2018). Assessment of genetic diversity in Salvadora persica L. based on inter simple sequence repeat (ISSR) genetic marker, Journal of Genetic Engineering and Biotechnology, 16(2), 661-667, https://doi.org/10.1016/j.jgeb.2018.04.005.

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.

Moustafa, M., Alamri, S., Al‑Emam, A., Alghamdi, H., Shati, A., Alrumman, S., Sulayli, A., Al‑Khatani, M., & A. Abbas, A. (2021). Biological, Physical and Chemical Properties of Nanosilver Particles Collected from Soil in Asir, Saudi

Arabia. Arabian Journal for Science and Engineering, 46, 129–140. https://doi.org/10.1007/s13369-020-04833-8

Moustafa AM, Zayed A. Effect of environmental factors on the flora of alluvial fans in southern Sinai, Journal of Arid Environments. 1996;32(4):431-443. https://doi.org/10.1006/jare.1996.0036

Nordin, F.N. (2012). "A review on the sunnah of miswak (*Salvadora Persica*) and its potentiality to improve oral health," Revel Science, 2(01).

Parida AK, Veerabathini SK, Kumari A and Agarwal PK (2016) Physiological, Anatomical and Metabolic Implications of Salt Tolerance in the Halophyte *Salvadora persica* under Hydroponic Culture Condition. Front. Plant Sci. 7:351. doi:10.3389/fpls.2016.00351

Rafey, A.; Amin, A.; Kamran, M.; Aziz, M.I.; Athar, V.; Niaz, S.I.; Pieters, L. Evaluation of Major Constituents of Medicinally Important Plants for Anti-Inflammatory, Antidiabetic and AGEs Inhibiting Properties: In Vitro and Simulatory Evidence. Molecules 2022, 27, 6715. https://doi.org/10.3390/molecules27196715

Rangani J, Parida AK, Panda A and Kumari A (2016) Coordinated Changes in Antioxidative Enzymes Protect the Photosynthetic Machinery from Salinity Induced Oxidative Damage and Confer Salt Tolerance in an Extreme Halophyte Salvadora persica L. Frontiers Plant Science, 7:50. doi: 10.3389/fpls.2016.00050

Rowell, D.L. (1994). Soil science methods and applications. Longman Publishers, Singapors. 229.

Salama, F., El-Ghani, M. A., Gadallah, M., El-Naggar, S., and Amro, A. (2014). Variations in Vegetation Structure, Species Dominance and Plant Communities in South of the Eastern Desert-Egypt. Notulae Scientia Biologicae, 6(1), 41-58. https://doi.org/10.15835/nsb619191

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. Taeckholmia, 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

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.

Schachtman DP, Kumar R, Schroeder JI, Marsh EL. Molecular and functional characterisation of a novel low-affinity cation transporter (LCT1) in higher plants. Proceedings of the National Academy of Sciences of the USA. 1997;94:11079-11084.

Sher, H., Al-Yemeni, M. N.,Masrahi, Y. S., and Shah, A. H. (2010). Ethnomedicinal and ethnoecological evaluation of *Salvadora persica* L.: a threatened medicinal plant in Arabian Peninsula. J. Med. Plants Res. 4, 1209–1215. doi: 10.5897/JMPR10.230

Smith, J.L. & Doran, J.W. (1996). Measurement and use of pH and electrical conductivity for soil quality analysis. In Methods for assessing soil quality. Soil Science Society of America Special Publication, 49:169-185. https://doi.org/10.2136/sssaspecpub49.c10

Sonti, N.F. (2021). Urban Plant Ecophysiology. CAB International. Urban Ecology: Its Nature and Challenges (ed. P. Barbosa), Pages 67-84.

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.

Stocker, O. (1960). Physiological and Morphological Changes in Plants due to Water Deficiency in Plant – Water Relationships in Arid and Semi-Arid Conditions. UNESCO. Paris, Pp. 63 – 94.

Sujata M (2015). Medicinally potent and highly salt tolerant plant of arid zone- Salvadora persica L. (Meswak): A Review. J. Plant Sci. 3(1-1):45-49.

Tounekti, T., M. Mahdhi, T.A. Al-Turki and H. Khemira, 2018. Physiological responses of the halophyte Salvadora persica to the combined effect of salinity and flooding. International Journal of Agriculture & Biology, 20(10): 2211–2220. DOI: 10.17957/IJAB/15.0764

Tuzuner, A. (1990). Soil and water laboratory analysis guide. Ankara: General Directorate of Rural Services Publications.

USDA, (2002). Natural Resources Conservation Service. Soil Conservationists. Salinity Management Guide - Salt Management. Available at http://www.launionsweb.org/salinity.htm.

Uvalle Sauceda JI, Gonzalez Rodriguez H, Ramirez Lozano RG, Silva IC, Gomez Meza MV. Seasonal trends of chlorophylls a and b and carotenoids in native trees and shrubs of Northeastern Mexico. Journal of Biological Sciences. 2008;8: 258-267. DOI: 10.3923/jbs.2008.258.267

Wang Z, Hu H, Goertzen LR, McElroy JS, Dane F (2014) Analysis of the *Citrullus colocynthis* Transcriptome during Water Deficit Stress. PLoS ONE 9(8): e104657. doi:10.1371/journal.pone.0104657

WHO. Consensus statement on oral hygiene. Int. Dent. J. 2000, 50, 139.

World Meteorological Organisation. 1988. Manual on water quality monitoring, Hydrology Report No. 27, Geneva.

Yadav J.P. and S. Saini (2007). Genetic characterization of salvadora species using SDS-PAGE. Plant Archives 7(1):83-85.

Youssef AM (1988). Ecological studies on the plant communities of the desert area South Ismailia. M.Sc. Thesis, Bot.Dep., Fac. Sci., Ain Shams Univ., Cairo, Egypt.