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Ecophysiological responses of Moringaperegrina (Forssk.) Fiori growing naturally under different habitat conditions of Eastern Desert and Fieran Oasis, Egypt

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Abstract*:Moringaperegrina (Forssk.) Fioriis one of the most valuable and economically important medicinal species in the Egyptian desert.Owing to its high curing value and wild occurrence in diverse environments. The present study aims toidentifythe plant metabolites, which enact drought-resistance mechanism and enable this species to exhibit good growth under stress conditions,in order toimprove the tolerance of crops against such environmental stresses using phytochemical genomics and crop breedingMoringaperegrina was collected from OasisFieranin South Sinai and Wadi Abu Halfaya in Eastern Desert in wet and dry seasons. This study indicated that M.peregrina is a drought resistant tree and responses to stress by accumulation of carbohydrates, soluble sugars and free amino acids contents, as compatible organic solutes and also indicated a decrease in the contents of lipids and proteins. This alternation in protein synthesis is one of the fundamental metabolic processes that may influence water stress tolerance. The antioxidant activity of M. peregrina was studied using total phenolic content,DPPH radical scavenging activity and Trollox equivalent antioxidant capacity (TEAC)assays. The results showed that M. peregrina is a good source of natural antioxidants. All M. peregrina extracts showed high scavenging ability and antioxidant activity and there is a strong correlation between total phenolic content and antioxidant activity. Under stress conditions, the antioxidant capacity ofleaves extract of M. peregrinewas increased to avoid the effect of free radical reactive oxygen species (ROS).On the other hand, this study reveals that the content of total amino acids and the oil yield of M. peregrina seeds were affected by growing conditions and geological conditions of the regions. Since at OasisFieran, the content of total amino acids in seeds was 25.43 mg/g and its yield from oil was 45.79 % while at Wadi Abu Halfaya, the content of total amino acids was 32.62 mg/g and the oil yield was 38.42%. The survival and good growth of M. peregrina under arid and semi-arid conditions, indicate that this species hasa great potential for planting in arid degraded sites and semi-arid regions because it is highly tolerant to drought and thus,M. peregrinacan be used as a donor to transfer stress tolerance gene to other economical plants to increase their tolerance abilityto drought.*

Keywords:*Amino acids, antioxidant, Moringaperegrina, phenolic compounds,radical scavenging, stress.*

I. Introduction

Arid environment occupy almost a third of the total land area of the world and zones classified as hyper-arid cover about 4%of the total [1].Recent climatic models predict that the aridity will increase in several areas of the world during the 21th century in association with global warming [2][3][4]. Drought conditions affect a wide variety of physiological and metabolic processes in plants, leading to growth reduction [5][6].Under droughtand/or saline conditions stomatal conductance decreases.This decrease in gas exchange lowers the intrinsic CO_2 concentrations. Because of this water-conserving response, photosynthesis and many aspects of the plant metabolism are affected. Loss of coordination between different metabolic pathways causes electrons with high energy state to be accepted by molecular O_2[7][8]. As a result, reactive oxygen species like singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), super oxide (O_2^-), and hydroxyl ($OH^\bullet$) are produced in different compartments of the plant cell such as the chloroplast, mitochondria, and peroxisomes. These effects caused by drought and salinity retard the yield of crops up to 50% [9]which makes it necessary to understand adaptive mechanism by which trees in arid and hyper-arid areas overcome this issue.Moringa peregrina(Forssk.) Fioriis one of the most valuable and economically important medicinal species in the Egyptiandesert.This plantbelongs to a monogeneric family of shrubs and tree,Moringaceae.It has a wide geographic range,growing from the dead Sea area sporadically along the red Sea to northen Somalia and around the Arabian Peninsula to the mouth of the Arabian (Persian) Gulf,Red sea coast Sinai mountains [10] [11].In Egypt,M. peregrine is restricted to the mountains of the Red Sea region, an area of hyper-arid environmental conditions and South Sinai Peninsula. It is known in Arabic as HabbaGhalia [12].

The plant has been used by Egyptians since Old and Middle Kingdoms (3000-2000 B.C) [13]. Ethnobotanical studies indicate that itis used to treat head ache, fever, abdominal pains, constipation, burns, back and muscle pains, and during labour in child birth. In Egypt , the seeds of Moringa trees were used as a

good source ofedible oil in theeast cost of Red Sea [14].The extracted oil is composed of 14.7% saturated and 84.7% unsaturated fatty acids including Palmitic (9.3%),Palmitoleic (2.4%),Stearic(3.5%),Olic (78.0%),Linoleic (0.6%), Araachidic (1.8%) and Behenic (2.6%) [15].

M. peregrina tree isan extremely fast growing small tree, it commonlyreaches aboutthree meters in height just 10 months after theseedis planted [16]. It has a grayish – green bark long alternate leaves , and bisexual yellowish white to pink, showy,fragrant flowers [10] [11].Moringa species are one of the most useful tropical trees with a multiple of uses.Theirleaves, fruits and seeds have been reported as rich source of protein, essential elements (Ca, Mg, K and Fe and vitamins; vitamins A, C and E) [17] [18]. They are a good source of B vitamins[19]. Leaves of Moringaspecieswere traditionally reported to have various biological activities, including antitumor,antioxidant and hypoglycemic actions [20].Also root, flowers and seeds are extensively used for treating inflammation, cardiovascular and liver diseases[21]. Thus, all parts of thetree are edible and form a part of traditional diets in many countries of the tropics and sub- tropics [14]. Owing to their high curing value and wild occurrence in diverse environments, they have been considered to be promising plants for marginal lands, new reclaimed-soils and semi-arid regions. So a better understanding of the stress response is critically important for agricultural and economic performance to improve the tolerance of crops against such environmental stresses using phytochemical genomics and crop breeding [22].The present investigation was carried out to study the physiological responses and changes in chemical composition of Moringaperegrina under different habitat conditions during winter and summer seasons.

II. Materials And Methods

2.1. Plant species

Moringaperegrina is a desert tree (Fig.1&2).This tree has a tuberous rootstock that starts to develop at seedling stage [23].The leaves have several tiny leaflets that drop when the leaf matures .However,the naked leaf axis remains and represents the main photosynthetic organ of the tree. The flowering season spans two menthes from March to April and the fruiting period Lasts for up to three months Flowers are 1-1.5cm long and pinkish white or pale yellow.Fruits are relatively large pendulous capsules (10-25 x1-1.5cm^2) each containing 5-15 large ovoid-trigonous seeds (1.2-1.5x0.8-1cm^2)[24].

Figure1. Moringaperegrina tree growing inOasisFieran (South Sinai)

Figure 2.Moringa peregrina tree growing in Wadi Abu Halfaya (Eastern Desert) (a) flowers and pods (b)

2.2. Ecological studies

Study area:

Description of study area

The study was carried out in Oasis Fieran (South Sinai) and WadiabuHalfaya (eastern desert) inwinter(February2010) and in summer (July 2010).The first area,WadiFieran(28°30′-47′N, 33°33′-34°00′E) is, with a length of c.59 km,the longest and also the broadest wadi in South Sinai. It rises from the mountains surrounding the monastery of St. Catherine at about 2500 m. At an elevation of 750 m and 43 km E of the mouth of the wadi lies Fieran Oasis [25].It is a small depression, which appears as a deep, fertile extension of the wadi, surrounded by high red mountains of igneous and metamorphic rocks [26], and extends over a distance of 10 km with a dense growth of date palms. Fieran Oasis lies in the hyper arid zone with a hot summer, a mild winter with rainfall [27].Meteorological information (2009-2010) of the St. Catherine station shows that rains occur only in March during 2009 and in January, August, September and December during 2010, the mean annual rainfall was 3.56 mm and 60.45 mm during 2009 and 2010, respectively. The mean monthly temperature ranges from 6^{o}C to 33^{o}C and 6^{o}C to 31^{o}C during 2009 and 2010, respectively, with the minimum in January and February and the maximum in July and August. The least humid month of 2009 was June with an average daily low humidity of 16%, and the most humid month was December with an average daily low humidity of 37%. During 2010,the least humid month was May with an average daily low humidity of 15%, and the most humid month was January with an average daily low humidity of 36%.

During the past century, annual mean temperature has increased by 0.75^{o}C and precipitation has shown marked variation throughout the Mediterranean basin [28]. This change affected the wild vegetation of South Sinai in general and resulted in rarity of trees and change in vegetation composition [29].

The second studied area, Wadi AbuHalfaia (25°40′-45′N, 34°10′-15′-0″E), tributary from WadiEseland bounded by Gabel Sabai from the west and Gabal Abu El Tiyur from the east,it is located southwest Quseir town, Central Eastern Desert .The Eastern Desert of Egypt occupies the area extended from the Nile Valley eastward to the Gulf of Suez and the Red Sea,which is about 223,000 Km^{2},i.e. 22.3% of the total area of Egypt.It traversed by numerous canyon-like depressions (wadis) running to the Red Sea or to the Nile Valley .It consists of a high backbone of high rugged mountains that run parallel to and at relatively short distance from the coast, these mountains are flanked to the north and west by intensively dissected sedimentary plateau [30].The Moringashrub is represented by patches that cover limited areas of the upstream runnels of the drainage systems .These runnels collect water at the foot of the higher mountains. This plant is confined to the foot of the mountains that are higher than 1300m, where the ground is usually covered with coarse rock detritus, a character typical of the upstream runnels at the foot of the mountains [31]. Quseir, The area of the present study is situated within a region of dry climate. The mean annual rainfall was 1.02 and 13.21mm during 2009 and 2010, respectively. The rainy season stretches from October to May and the maximum precipitation occurs in December. The mean monthly air temperature ranges between 20.5^{o}C during January to 27.8^{o}C during August (2009) and 22.1^{o}C to 29.4^{o}C (2010). The mean monthly relative humidity varies between 51% (2009) and 53.85% (2010) during June and 55% during October.

Soil analysis

Soil samples supporting the investigated plant were taken at one depth from (0 - 20 cm) at OasisFieranand two depth,(0 – 20 cm) and (20 – 40 cm) at WadiAbuHalfaia

a)Soil physical properties

Soil texture (granulometric analysis)was determined through mechanical analysis by the sieve method [32].

b) Soil chemical analysis

Sample preparation was carried according toPag (1987) [33].The hydrogen ion concentration (pH) and Electrical conductivity (EC) measurements.The pH of soil extract was measured using pH meter instrument and the EC was measured by electrical conductivity meter.

Cations determination

The concentration of potassium and sodium was determined by a flame photometer (Jenway PFP7) and calcium and magnesium contents were determined by titration with ethylene diamine tetra-acetic acid (EDTA) according to the method of Rowell (1994)[34]. Available K and P extracted from soil samples using NH_4HCO_3-DTPA [35], while available P was colorimetrically determined using ammonium molybdate and ascorbic acid [34] using spectrophotometer (Unicom UV-300),where absorbance was read at 880 nm.

Anions determination

Sulphate ions were precipitated as barium sulphates according to the turbidimetric method [34]. The resulting turbidity was measured by using a spectrophotometer (Unicom UV-300) and absorbance was read at 480 nm, and then compared with a standard curve prepared from serial sulphatesolutions.While the concentration of chloride was determined by titrating a known volume of the soil extract against silver nitrate (0.5N) using 1% potassium chromate as an indicator as described by Jackson (1967)[32].

2.3.Plantchemical analysis

Plant materials were dried in the oven at 70 ̊Cto a constant weight and ground to fine powder, then ash and crude fiber were determined by AOAC (2000) methods [36]. Nitrogen(N) content of samples were estimated by the method described by Kjeldahl (1983) [37] and crude protein was calculated as N×6.25 [38]. Total lipids from plants and seeds were extracted with petroleum ether (40-60 ̊C)in a Soxhelt extractor for 18h and quantified gravimetrically after elimination of residual ether.

Mineral analysis

The dried samples (0.50) were taken and digested with 10ml concentrated nitric acid. After adding (2-4ml) of perchloric acid, the contents were heated gently on a hot plate and used for mineral analysis [39].The concentrations of calcium, magnesium, Potassium and sodium were determined according to the method of Rowell (1994) [34]. Plant dry matter was ashed , part of ashed powder was dissolved in HCl to measure sulphur and phosphorus contents in plant by using turbidimetic and phosphomolybdat methods [34], respectively,while the other part was dissolved in diluted nitric acid (0.01N) to extract chloride from ashed powder samples and titrated the extracted chloride with standard silver nitrate [40].The concentrations of manganese ,copper, zinc and iron were determined by using ICP emission spectroscopy[41].

Determination of total carbohydrates

Total carbohydrates determination was based on the phenol–sulfuric acid method [42].

Determination of total soluble sugars, reducing and non-reducing sugar

The plant sample was extracted twice in 40 ml of boiling water and twice in 40 ml of aqueous boiling ethanol (80%v/v) and clarified using saturated neutral lead acetate solution [36].The total soluble sugar were estimated using the general phenol–sulfuric acid method [42] while reducing sugar was estimated according to Nelson –Somogimethod [43].non reducing was calculated by the difference between total and reducing sugars.

Determination free and total amino acids

The investigation of free amino acids and protein amino acids in leaves and seeds of M. peregrina were determined by using Amino Acid Analyzer apparatus model (LC 3000 Eppendorf, Central Lab. of Desert Research Center) according to the method of Pellet and Young (1980) [44].

Analysis of phenolic compounds

Phenolic compounds were determined by HPLC according to the method of Coupy et al.,(1999)[45].1ml of sample was diluted by 10ml water and 100µl were taken for injection into HPLC Hewllet Packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet detector set at 280 nm and quaternary HP pump series1100) . Packed column Hypesil BDS- C18, 4.0 x 250mm was used to separate phenolic compounds. Thecolumn temperature was maintained at 35 °C. Gradientseparation was carried out with methanol andacetonitrile as a mobile phase at flow rate of 1 ml/min. phenolic acid standards from sigma Co. were dissolvedin mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by data analysis of Hewllet Packard software, Germany.

Determination of total phenol

Total phenolic contents of the different methanolic extracts of Moringa were determined using Folin-Ciocalteu (FC) reagent according to the method of Slinkard and Singleton (1997)[46]. An aliquot of 40 µL was pipetted into separate cuvettes, and to each, 3.16 mL of double distilled water andFolin - Ciocalteu reagent (200 µL) wereadded and mixed well. After 8 min, 600 µL of sodium carbonate solution was mixed thoroughly in the solution. The solution was allowed to stand at 40°C for 30 min and absorbance of each solution was determined at 765 nm against blank. A concentration versus absorbance linear plot was thus obtained. The concentration of total phenolic compounds of each fraction was expressed as tannic acid equivalents.

Antioxidant activity and antioxidant capacity

Radical scavenging activity of plants against stable DPPH (2,2-diphenyl-2-picrylhdrazyl hydrate,Sigma-Aldrich Chemie,Steinheim,Germany) was determined spectrophotometrically. When DPPHreacts with an antioxidant compound,which can donate hydrogen,it is reduced .The changes in colour (from deep-violet to light-yellow) were measured at 515 nm on a Shimadzu spectrophotometer (UV-1601 PC).Radical scavenging activities of plants were measured by method of Brand-Williams et al.,(1995)[47].Butylated hydroxyl anisol (BHA) andtert-butylated hydroxyl qunione (TBHQ) were used as reference compounds.Percent inhibitions vs.sample volume (µL) curves were used to determine the concentration at which 50% radical scavenging occurred (EC_{50}).

Finally,the antioxidant capacity of the plants were compared to that of a synthetic antioxidant 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and expressed as Troloxequivalent antioxidant capacity values (TEAC)

2.4. Statisticalanalysis

The data, except data of amino acids, phenolic compounds and antioxidant activity and antioxidant capacity were statically analyzed using the statistical program (Costat Version 6.4).The significant differences between means were calculated by a split plot analysis of variance (ANOVA) using Duncan's multiple –range test. Differences at a $P < 0.05$ were considered statistically significant.

III. Results And Discussion

3.1.Soil analysis

Physicochemical characters of OasisFieran and Wadi Abu Halfaya soils were represented in Tables1 and 2. The soil texture was sandy clay loam at OasisFieran and sandy at Wadi Abu Halfaya. The percentages of silt and clay in OasisFieran soil were 24.9 and 14.2%, respectively, while in Wadi Abu Halfaya soil, their percentages were 1.68 and 1.37% inthe first depth (0-20) and 4.05 and 0.78 % inthe second depth (20-40), respectively. The pH values fluctuated in the basic range. Generally no significant differences in soil pH due to location changes were noticed. The lowest pH value of 7.80 was recorded inOasisFieran soiland the highest value of 7.87 in the first layer (0-20cm) ofWadi Abu Halfaya soil. Electric conductivity values ranged between 0.88 and 1.35dS/m at Wadi Abu Halfaya and 1.03dS/m at OasisFieran. The percentages of $CaCO_3$ were 1.25 % inOasisFieran soil and 0.25 and 0 .50% in the first and second depths of Wadi Abu Halfaya soil, respectively. The Soil moisturecontent atOasisFieran was 0.51% in summer and 1.49% in winter. While at Wadi Abu Halfaya, soil moisture content was decreased with depth from 0.035 to 0.014% in summer and from 0.77 to 0.62% in winter, respectively.The presence of high soil moisture content in OasisFieran soil maydue to the high value of annual total rainfall (60.45mm) in winter andhigh water-holding capacities as a result of high silt and clay contents.

Table1. Physical properties ofMoringaperegrina associated soils atOasisFieran and WadiAbu Halfaya

Locality	Soil depth (cm)	Soil particles distribution (%)			Soil texture class	Soil moisture content (%)	
		Sand	Silt	Clay		Summer	Winter
1) OasisFieran	0-20	60.81	24.99	14.20	Sandy Clay Loam	1.49	0.51
2) Wadi AbuHalfaya	0-20 20-40	90.95 95.17	1.68 4.05	1.37 0.78	Sand Sand	0.77 0.62	0.035 0.014

Table 2. Chemical properties of M. peregrina associated soils at OasisFieran(1) and WadiHalfaya(2)

Locality	Soil depth (cm)	pH at 1:2.5	EC dS/m	Cation milliequivalent/Liter				Anion milliequivalent/ Liter		$CaCO_3$ %	P ppm	K ppm
				Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}			
1	0-20	7.80	1.03	4.90	3.60	1.29	0.45	3.25	4.55	1.25	12.5	85.5
2	0-20 20-40	7.87 7.82	1.35 0.88	6.90 3.60	2.95 3.35	2.34 0.79	2.68 0.68	4.85 2.20	6.05 5.15	0.25 0.50	24.5 12.9	97.2 60.6

The Contents of soluble cations and anions and available phosphorus (P) andpotassium (K) in the soil were illustrated in Table 2. The concentrations of calcium (Ca^{2+}), sodium (Na^{+}), potassium (K^{+}) and chloride (Cl^{-}) were higher in the first depth(0-20)of Abu Halfaya soil than in the soil of OasisFieran habitat. According to their concentrations, the mineral elements were arranged in the following order: $Ca^{2+}>Mg^{2+}>Cl^{-}>Na^{+} > K^{+}$ in OasisFieran soil and in the second depth (20-40cm) of Wadi Abu Halfayasoil. While in the first depth(0-20) of Wadi Abu Halfayasoil they arranged in the following order:$Ca^{2+}>Cl^{-}>Mg^{2+}> K^{+}>Na^{+}$.Generally, Ca^{2+} was the dominant anion at two habitats. The maximum value of Ca^{2+} (6.90meq^{-1}) was recorded in the first depth (0-20cm) of Wadi Abu Halfaya and the minimum value of 3.60meq^{-1} in its second depth (20-40cm). The contents of sodium and potassium in the soil of OasisFieran were 1.29 and 0.45meq^{-1}, respectively.While in the soil of Wadi Abu Halfaya,their concentrations were decreased gradually with depth from 2.34 and 2.68 $meq^{-1}$to 0.79 and 0.68 meq^{-1}, respectively. The highest content of Mg^{2+}(3.60meq^{-1}) was recorded in OasisFieran soil, but its minimum value of 2.95 was detected in Wadi Abu Halfaya soil. Moore [48] detected thata soil with an alkaline to strongly alkaline reaction can have a number of nutrient deficiencies including phosphorous, nitrogen, copper, zinc, manganese and iron.

3.2.Chemical Composition

As shown in Table 3,the minerals; K, Mg, P, andCl^{-} were found in higher concentrations (1.72, 0.27, 0.38 and 0.029 g%, respectively) in M. peregrina in summer season than in winter season (1.55, 0.18, 0.21 and 0.024 g %, respectively) at OasisFieran. While at Wadi Abu Halfaya, their contents were slightly increased from 1.02, 0.13, 0.09 and 0.027 g% in summer to 0.95, 0.42, 0.13 and 0.037 g% in winter, respectively.The concentration of sulphate (SO_4^{2})also increased from 0.72 and 0.74g% in wet season to 0.89 and 0.91g% in dry season atOasisFieran andWadi Abu Halfaya, respectively.

The obtained results showed that M. peregrinaunder stress conditions tended to accumulate certain compound termed compatible solutes, which do not interfere with normal biochemical reactions [49].These compatible solutes include carbohydrates, total soluble sugars (reducing and non –reducing sugars). Their major functions have been reported to be osmotic adjustment, carbon storage, and radical scavenging [50].There was a highly significant difference between seasons in the content of reducingsugar,its value increased with stress from 0.99 and 1.15g% to 1.37 and 1.71g% at OasisFieran andWadi Abu Halfaya, respectively.Also, there was a significantincrease in the content of non-reducing sugar at OasisFieran under stress conditions;its concentration was 1.11g% in winter and 1.63g% in summer.While at Wadi Abu Halfaya, the content of M. peregrina from non- reducing sugar was slightly increased from 0.87% in winter to 0.96 g% in summer .Soluble sugar has a protective role for chloroplast from damage under water deficit conditions [51]. The accumulation of potassium (K^{+}),Sulphate (SO_4^{2}),carbohydrates, soluble sugars at higher concentration in M. peregrina under stress condition may assist in turgor maintenance and help to enhance drought tolerance [52]. The most important adaptation mechanism in desert plants to maintain the plant water potential more negative than the external medium to insure the water uptake, is the ability of plants to accumulate the inorganic solutes in high quantities inside their tissues [53] [54].

Ash contents were significantly higher ($p < 0.05$) in summer sample when compared with winter sample, collected from OasisFieran which may due to their richness in minerals; potassium (K), magnesium (Mg), phosphorous (P),sulphate(SO_4^{2})and manganese (Mn).

Table3. Determination of some chemical compositions inM. peregrina at two different habitats.Results are means ±S.D(n=3) Different letters in a singleline show significant differences for $P < 0.05$

Locality		Oasis Fieran		Wadi Abu Halfaya		LSD
		Season		Season		
Parameters		Winter	Summer	Winter	Summer	
Minerals	Na^+ (g/100g DW)	0.50±0.04 ^{b}	0.52±0.07^b	0.98±0.08 ^{a}	0.99±0.07 ^{a}	0.095
	K^+ (g/100g DW)	1.55±0.13^b	1.72±0.06^a	0.95±0.10^c	1.02±0.10^c	0.143
	Ca(g/100g DW)	2.74±0.17^b	2.27±0.39^c	2.63±0.09^b	3.83±0.12^a	0.224
	Mg^{2+} (g/100g DW)	0.18±0.07^c	0.27±0.05^b	0.42±0.41^a	0.13±0.02^c	0.072
	SO_4^{2-}(g/100g DW)	0.72±0.05^b	0.89±0.08^a	0.74±0.06^b	0.91±0.07^a	0.011
	P (g/100g DW)	0.21±0.03^b	0.38±0.06^a	0.13±0.04^c	0.09±0.02^c	0.054
	Cl^-(g/100g DW)	0.024±0.003^c	0.029±0.002^b	0.037±0.005^a	0.027±0.005^b	0.003
	N (g/100g DW)	1.42±0.06^b	0.95±0.100^d	1.55±0.12^c	1.18±0.28^c	0.119
	Fe (mg/100g DW)	23.16±0.41^b	20.60±0.40^c	30.3±0.40^a	19.9±0.34^d	0.430
	Mn (mg/100g DW)	8.63±0.15^b	8.92±0.25^{ab}	8.20±0.40^c	9.23±0.39^a	0.343
	Zn (mg/100g DW)	2.22±0.21^a	1.17±0.39^b	1.93±0.33^a	2.27±0.29^a	0.328
	Cu (mg/100g DW)	12.4±0.35^b	10.9±4.79^b	17.6±0.08^a	7.87±0.20^c	2.536
Ash content(g/100g DW)		5.39±0.37^d	5.92±0.12^c	6.35±0.13^a	6.07±0.18^{ab}	0.299
T.Carbohydrate (g/100g DW)		5.06±0.43^c	5.95±0.55^b	6.30±0.66^b	7.90±0.74^a	0.392
T.Soluble sugar (g/100g DW)		2.03±0.12^b	2.67±0.25^a	2.1±0.20^b	3.0±0.40^a	0.366
Reducing sugar (g/100g DW)		0.99±0.034^d	1.37±0.08^b	1.15±0.02^c	1.71±0.30^a	0.030
Non-reducing sugar(g/100g DW)		1.11±0.20^b	1.63±0.48^a	0.87±0.14^b	0.96±0.22^b	0.388
Total lipids(g/100g DW)		8.79±0.77^b	6.17±0.48^c	10.3±0.60^a	5.57±0.39^c	0.869
Protein (g/100g DW)		8.87±0.37^b	5.97±0.63^d	9.73±0.73^a	7.37±1.76^c	0.740

The obtained results indicated the existence of highly significant difference between seasons in the contents of lipid and protein,since their values increasedat OasisFieranfrom 6.17 and 5.97g% in summer to 8.79 and 8.87g% in winter, respectively.Also at WadiAbu Halfaya,their values increased from 5.57 and 7.37g% in summer to 10.3 and 9.73 g% in winter, respectively. The decrease in membrane lipid content under water stress is correlated to an inhibition of lipid biosynthesis [55] and a stimulation of lipolytic andperoxidative activities [56],which resulted from inducing of degradativeprocesses in the membrane by drought. Reduced the rate of protein synthesis or protein level also occur during stress. Several studies have shown that under stress conditions, the level of protein was decreased.Vyas et al. (1996) [57] observed that water stress causes both reductions in the rate of protein synthesis as well as changes in the type of proteins produced. Also Al-Jebory (2012) [58] reported that the content of protein in Pisumsativum decreased with increasing drought stress. The alternation in protein synthesis or degradation is one of the fundamental metabolic processes that may influence water stress tolerance [59].

Amino Acids

As shown in Table (4), under stress conditions,M. peregrinatended to accumulate freeamino acids:aspartic acid, threonine, serine, glycine, valine,isoleucine, leucine,tyrosine phenylalanine and histidine in summer season at OasisFieran and aspartic acid, serine,glutamic, glycine,alanine, tyrosine, phenylalanine and histidine at Wadi Abu Halfaya in the same season**.**The accumulation of glutamic in response to stress at Wadi Abu Halfaya,may be attributed to the activation of its biosynthesis from glutamate or inactivation of its degradation [60]. While, the accumulationof Aspartic acid (2-fold) under stress at WadiFieran, is considered the most commonly accumulating nitrogen containing compounds in a number of stress tolerant species (reviewed by Rabe (1990) [61].

The concentration of phenylalanine increased 5-fold and 7-fold in summer at OasisFieran and Wadi Abu Halfaya, respectively.Also the level of tyrosine increased 2-fold and 9-fold in summer as compared to its concentration in winter at OasisFieran and Wadi Abu Halfaya, respectively. The concentration of total detected amino acids increased by 37.17and22.82% at OasisFieran and Wadi Abu Halfaya under stress condition, respectively. This result supports the hypothesis that accumulation of amino acids is one of the adaptive mechanisms for stress condition. These compounds could contribute to the tolerance of the plant to water deficit and have been reported to function in osmotic adjustment, protection of cellular macromolecules, storage of nitrogen, maintenance of cellular pH, detoxification of the cells and scavenging of free radicals [62].Therefore, most researches showed that the levels of total and free amino acids increase remarkably during water stress. Gzik(1996) [63] reported that the total of 18 amino acids including phenylalanine and tyrosine increased in sugar beet leaf during water stress, while Zagdanska (1984) [64] reported an increase in aspartate, alanine and

glutamate in stressed leaves of wheat, and suggested that this could help maintain energy fluxes of the chloroplast. Stewart and Larher(1980) [65] found an accumulation of amino acids in the presence of water deficit, leading to a dynamic adjustment of N metabolism. Amino acids are precursors for the synthesis of secondary metabolites such as alkaloids, which provide chemical defense for plants that confer beneficial physiological effects in consumers. Alkaloids provide protection to plants from a variety of herbivores, and some of them possess significant pharmacological activity such as analgesic, antibacterial and antibiotic [66].

Under stress conditions, the accumulation of amino acids may be actually a part of an adaptive process contributing to osmotic adjustment and has been taken as an index for drought tolerant potential of many plants [67] [68]. Also, they could be accumulated due to an increase in breakdown and degradation [69] of protein or inhibition of protein synthesis [70],or decrease in amino acids amide export [71].

This study supports the hypothesis that accumulation of amino acids is one of the adaptive mechanisms for drought stress condition.

Table4. Free amino acids in leaves of M. peregrina and total amino acids in seeds at two different habitats

Locality	**Oasis Fieran**			**Wadi Abu Halfaya**		
Plant parts	**Leaves**		**Seeds**	**Leaves**		**Seeds**
AminoAcids	**Winter**	**Summer**	**T.Amino Acids** (g/100DW)	**Winter**	**Summer**	**T.Amino Acids** g/100DW)
	F.Amino Acids (g/100DW)	**F.Amino Acids** (g/100DW)		**F.Amino Acids** (g/100DW)	**F.Amino Acids** (g/100DW)	
Aspartic acid	0.059	0.124	1.803	0.035	0.036	2.311
Threonine	0.045	0.048	0.956	0.154	0.008	1.133
Serine	0.011	0.074	0.975	0.040	0.047	1.090
Glutamic	0.540	0.347	4.578	0.005	0.253	6.591
Glycine	0.023	0.065	0.813	-	0.011	0.996
Alanine	0.079	-	1.444	0.049	0.056	1.715
Cystine	0.010	-	0.773	0.017	-	0.327
Valine	0.053	0.078	1.097	0.034	0.031	1.327
Methionine	0.013	-	0.593	0.056	0.054	0.677
Isoleucine	0.026	0.035	0.888	0.242	0.010	1.196
Leucine	0.049	0.102	1.973	0.163	0.054	2.211
Tyrosine	0.182	0.440	1.242	0.013	0.127	1.392
Phenylalanine	0.089	0.473	1.777	0.009	0.069	1.846
Histidine	0.067	0.107	1.480	0.057	0.042	1.857
Lysine	0.048	0.038	0.817	0.036	0.383	0.670
Arginine	0.144	0.053	4.218	0.154	0.128	7.282
Total	1.433	1.984	25.427	1.309	1.065	32.621

From Table 4, it could be detected the difference in the concentration of different amino acids in M. peregrinaseeds which collected from two different habitates.Since, the concentration of total amino acids inM. peregrina seeds was 25.42 mg/g at OasisFieranand 32.62 mg/g atWadi Abu Halfaya. The most abundant essential amino acid is arginine,its highest concentration (7.28g %) was detected in the seeds of M. peregrina at Wadi Abu Halfaya. Also, the highest contents of other essential amino acids, threonine,valine, isoleucine, Leucine, phenylalanine and histidine were detected in the seed ofMoringacollected fromthe same wadi. However, the oil yield of M. peregrinaseed was 45.79 % at OasisFieran and 38.42% at Wadi Abu Halfaya, thisyield can be improved by proper propagation and cultivation since higher values (54.29 - 57.25%) were reported by Osman and Abohassan(2012) [72] for M. peregrina kernel seed oil from Saudi Arabia. According to Ibrahim et al.((1974) [73], content and properties of Moringaseed oil depend on the species and the environmental condition. Generally, the variation in the yield of oil and chemical characteristics might be attributed to the differences in growing conditions and geological conditions of the regions [74]. In Egypt,the oil extracted from seed of Moringa trees were used as edible sources in south coast of Red Sea region.It contains significant amounts of oleic oil and linoleic and showed high growth inhibition against three human cancer cell lines,breast adenocarcinoma (MCF-7),hepatocellular carcinoma (Hep G2) and colon carcinoma (HCT-116) [75]. Many vegetable seed oils rich in linoleic acid have a beneficial effect on blood lipids, cholesterol and lowering blood pressure [76].

Phenolic Compounds

Phenolic compounds of M. peregrinawere represented in Table 5. Phenolic compounds and flavonoids are among the most influential and wide distributed secondary products in the plant kingdom. Many ofthese playimportant physiological and ecological roles, being involved in resistance to different types of stress [77] [78]. They are mainly synthetized from cinnamic acid, which is formed from phenylalanine by the action of L-phenylalanine ammonia-lyasePAL [79]. The obtained results indicated an increasein the levels of most phenolic compounds in winter season at both habitats. The concentrations of protocatechuic and vanillicacids were decreased from 5.42 and 6.79 mg/g in winter to 1and 4.07mg/g in summer and from 0.98 and 4.15 mg/g in winter to 0.10 and 2.84 in summer at OasisFieran and Wadi Abu Halfaya, respectively. Also, catechol ,caffeine, catechine, salicylic and cinnamicacids, their concentrations were decreased from 9.52,1.47,13.2, 0.40 and 0.28 mg/g in winter to 1.91,0.21,8.91,0.26 and 0.09mg/g in summer at OasisFieran, respectively, while, ferulic acid and coumarinwere detected onlyin winter sample at the same habitat. The analysis ofphenolic compounds ofM. peregrinasample, collected from WadiAbuHalfaya revealed the detection of chlorogenic acid (12.04 mg/g), catechin (4.22mg/g) and coumarin (2.32 mg/g) in winter samples only anda decrease in the levels of gallic ,ferulic, salicylic, cinnamic acids and chrysin, from 0.97 ,1.42 ,0.26,0.41 and 0.014mg/g in winter to 0.50 ,0.61,0.10,0.03 and 0.005mg/g in summer, respectively. Phenolics have several functions in plants as antibiotics, natural pesticides,attractant for pollinators, protective agents against ultraviolet (UV) light [80].Phenolic compounds can accumulate in plant cell during ontogeny, regulating development and germination of seeds [81]. The environmental stress can cause a decline [82] [83] or an increase [84] [85] in the content of phenolic compounds in a cell, when these substance protect plants from stresses.

Table 5. Phenolic compounds in Moringaperegrina at two different habitats

Location	**Oasis Fieran**		**Wadi Abu Halfaya**	
	Seasons		**Seasons**	
Phenolic Compounds	**Winter** (mg/g DW)	**Summer** (mg/g DW)	**Summer** (mg/g DW)	**Winter** (mg/g DW)
Gallic acid	0.40	0.75	0.97	0.50
Pyrogallol	-	-	-	-
Protocatechuic	5.42	1.00	0.98	0.10
Vanillic	6.79	4.07	4.15	2.84
Chlorogenic acid	-	-	12.04	-
Catechol	9.52	1.91	-	1.49
Caffeine	1.47	0.21	0.17	0.65
Catechine	13.2	8.91	4.22	-
Ferulic acid	3.84	-	1.42	0.61
Salicylic acid	0.40	0.26	0.26	0.10
Coumarine	14.8	-	2.32	-
Cinnamic acid	0.28	0.09	0.41	0.03
Chrysin	-	0.008	0.014	0.005
Benzoic acid	6.04	2.49	2.50	2.00

3.3.Antioxidant activities of methanolic extracts of Moringaperegrina

All environmental stresses have been reported to lead to the production of reactive oxygen species (ROS) that cause oxidative damage [86].Plants possess efficient systems for scavenging active oxygen species that protect against destructive oxidative reactions [87].

Thefree radical-scavengingactivity of methanolic extracts of Moringa was determined by measuring the decrease of absorbance of DPPH free radical at 516 nm in the presence of various concentrations. 2, 2-diphenyl -1-picrylhydrazyl (DPPH) is a commercial oxidizing stable free radical, which is readily reduced by antioxidants .Increased reduction of DPPH is related to the high scavenging activity given by particular samples [88].

In this investigation, DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH and the colour changes from purple to yellow after reduction. DPPH can be used to determine the proton radical scavenging action of extracts of the leaves of the selected plants, because it possesses phenolic compounds, a proton free radical. The more the polar capacity of the extract, the greater the antioxidant activity is.

As shown in Table 6, all M. peregrina extracts showed high scavenging ability and antioxidant activity and an increase in their scavenging ability when the concentration of extracts was increased and also in winter samples compared to that of summer. The antioxidant activity of M. peregrinaextracts at 500 and 700µg/ml

were 85.55 and 86.29% in winter and 54.40and 63.05% in summer at OasisFieran, respectively. While at Wadi AbuHalfaya, The antioxidant activity at 500 and 700 ppm were 63.08 and 66.90% in winterand 51.05 and 55.22% in summer, respectively.

Table6. Radical scavenging activity of M.peregrina extracts and standard antioxidants on DPPH freeradical

Locality	OasisFieran % Inhibation		Wadi Abu El-Halfaya % Inhibation		BHA (200µg/ml) %Inhibation	TBHQ (200µg/ml) %Inhibation
	Seasons		Seasons			
Conc. (µg/ml)	Winter	Summer	Winter	Summer		
300	62.97±0.42	39.25±0.54	47.78±0.12	42.21±0.14	72.3±0.85	86.3±0.22
500	85.55±0.36	54.40±0.67	63.08±0.35	51.05±0.05		
700	86.29±0.06	63.05±0.66	66.90±0.59	55.22±0.29		

Comparing antioxidant activity ofM. peregrina extracts with synthetic antioxidant, BHA (200µg/ml) and TBHQ (200µg/ml) indicated that, all plants extracts have potential antioxidant activity like BHA and TBHQ. The ability of reducing power of methanolic extracts of M. peregrina at 500 and 700 µg/ml, collected from OasisFieranin winter season showed almost similar with synthetic antioxidant TBHQ (200µg/ml) and higher than BHA (200µg/ml). The high antioxidant activity in DPPH assay is related to the amount of the total phenolic compounds of the plant [89] .Since, the amount of phenols increased in winter from 10.95 in summer to 19.90 and from 7.68 to 15.67 mg/g at OasisFieran and Wadi AbuHalfaya, respectively (Table7),which may beattributed to the high electron or hydrogen donating ability of the phenolic compounds contained in extracts [90].These results are in agreement with thefindings of many research groups who reported direct relationships between total phenolic content and antioxidant activity[91][92][93].

Table7. Total phenol content,Trolox equivalent antioxidant capacity (TEAC) of M.peregrinaextracts

Locations	OasisFieran		Wadi Abu Halfaya	
	Seasons		Seasons	
Parameters	Winter	Summer	Winter	Summer
T.Phenol(mg/g DW)	19.90±0.20	10.95±0.14	15.67±0.15	7.68±0.07
EC_{50}(g)	0.02	1.49	0.67	2.71
TEAC(nmol/g)	0.13	0.19	0.18	0.24

The EC_{50} values (the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50%) were calculated and listed in Table (7). The lower the EC_{50} value, the higher is the antioxidant activity. The EC_{50} value of M. peregrine extracts in winter samples shows less than that in summer samples. The lowest value of EC_{50} (0.02g) was recorded at OasisFieran in winter plantextracts, followed by the value of EC_{50} (0.67g)at Wadi Abu Halfayain the same season. Finally, the antioxidant capacity of the plants were compared to that of a synthetic antioxidant 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and expressed as Trolox equivalent antioxidant capacity values (TEAC).In the method for determination of antioxidant capacity, a colour solution of $ABTS^{+\bullet}$ free radical was used. Expressing plant extract's antioxidant capacity in **(**nmol/g) Trolox equivalent has the benefits that the antioxidant capacity is quantified and different plant extracts are comparable. It has been demonstrated that all the extracts from the tested M. peregrina are capable of scavenging $ABTS^{+\bullet}$ free radical, the plant samples, collected from OasisFieran in winter and summer had Trolox equivalent of 0.13 and 0.19 mol/g, respectively. While the other collected samples fromWadi Abu Halfaya had Trolox equivalent of 0.18 and 0.24mol/g in the same seasons, respectively. A study performed by Babbaret al., (2011) [94] showed that phenolic compounds alone are not fully responsible for the antioxidant activity of plant. Other constituents such as flavonoids, reducing carbohydrate, tocopherols, carotenoids, terpenes, and pigments as well as the synergistic effect among them could possibly contribute to the total antioxidant activity. Therefore, the observed antioxidant capacity of leaves extract of M. peregrina may be attributed to its phenolic content and also related to the flavonoid rutin which is isolated from this plant in previous study and are in agreement with several reports on flavonoids in Moringaceaefamily[95] [96]. Several flavonoids have been reported to quench active oxygen species and inhibit in vitro oxidation of low-density lipoproteins [97] [98].

Antioxidants play an important role in plant resistance to abiotic stresses [99]),as they protect cells from free radicals and reactive oxygen species (ROS),which are produced in response to various environmental stresses and cause peroxidation of lipids, denaturation of proteins and DNA damage[100] [101].Mechanism of action of antioxidants includes the suppression of ROS formation,the inhibition of enzymes or chelating of elements

involved in free-radical production.Furthermore, antioxidants scavenge reactive species, and upregulate antioxidant defences[102].

IV. Conclusion

Moringa peregrinais a drought resistant tree and has a wide capacity to tolerate period of drought.Therefore the plant has capability to minimize the stress effects and developed several adaptive mechanisms to cope with this stress.The physiological responses of M.peregrina to a deficit of water and stress conditions include xerophytic modificationin its leaves and stem following water deficit treatment[103].These modification include reduce surface to volume ratio of leaf and stem, leaf rolling and hair covering, and increased frequencies and indexes of stomata [104]. Another trait of the tree is that the root forms a tuber, which starts to take on form at the seedling phase [23].

This study reveals that the plant tended to accumulate carbohydrate, soluble sugars (reducing and non-reducing sugars) and free amino acids as adaptive response to stress conditions.The plants tended to accumulate the most compatible solutes in cytoplasm to balance the osmotic pressure inside the cells,enhance tolerance to drought and overcome stress conditions.

The results of phenolic compounds content in this study,demonstrated that M.peregrina is a good source of natural antioxidants such as the phenolic compounds. All M. peregrina extracts showed high scavenging ability and antioxidant activity and an increase in their scavenging ability when the concentration of extracts was increased.In addition, its antioxidant capacity was increased under stress conditions to avoid the effect of free radical reactive oxygen species (ROS) which are produced in response to various environmental stresses and cause peroxidation of lipids, denaturation of proteins and DNA damage. It was also a good source of minerals, potential catalysts of oxidative reactions.

As water scarcity is the major problem in arid and semi-arid regions, the drought resistant characteristic of Moringaperegrina make this tree very suitable to be planted in these region due to its economic and medicinal importance [15] coupled with its fast growing habit [105] and high seed germination rates [106].Also, it can be used as a donor to transfer stress tolerance gene to other economical plants to increase their tolerance abilityto drought.

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