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METABOLIC ADJUSTMENT STRATEGY IN TWO NON-SUCCULENT XEROPHYTIC SPECIES GROWING UNDER DROUGHT CONDITIONS

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ABSTRACT

The present work was designed to study ecophysiological responses of two non-succulent xerophytes, *Launaea spinosa* and *Leptadenia pyrotechnica* growing naturally in wadi Hagul, estern desert of Egypt during dry and wet seasons. The results revealed that organic constituents including, crude protein, free amino acids and nucleic acids were generally increased during wet season in the studied species. However, soluble protein, was significantly higher during dry season. Concentrations of protein amino acids, at both seasons of *Launaea spinosa* were greater than that of *Leptadenia pyrotechnica*. Electrophoretic protein fractions showed variations due to the differences in growing seasons.

INTRODUCTION

Plant growth and productivity is adversely affected by natures warth in the form of various abiotic and biotic stress factors. Plants are frequently exposed to many stress conditions such as low temperature, salt, drought, flooding, heat, oxidative stress and heavy metal toxicity (Jaleel *et al.,* 2007). Osmoregulation, osmotic compensation, or osmotic adjustment is the process to maintain the turgidity of plant cells by a sufficient increase in cell solutes to compensate for the external osmotic stress. The osmotic adjustment is considered an important mechanism developed by the plants to tolerat water deficiency (Costa, 1999), which promotes protection of the plant cell structures as plasma membranes and chloroplasts (Martinez *et al.,* 2004). Plants cope with the water deficit resulting from drought environment; xerophytic plant cells accumulate three kinds of osmotica: salts, small organic solutes and hydrophilic compounds. The accumulation of any osmoticum is a characteristic feature for the drought-tolerant species to survive in highly dry conditions. The enhanced tolerance of xerophytes to water stress can be associated with end accumulation of certain proteins. Accumulation of specific protein can be considered a stable characteristic linked to increase survival or growth of species under stress conditions (La Rosa *et al,* 1989). The induction of new proteins is not limited to heat shock (Vierling, 1990). Other stresses, including water deficit, salinity, anoxia and osmotic stress (induced artificially by polyethylene glycol solutions) result in the synthesis of new families of proteins. Although some of these stress proteins are similar to heat shock proteins (HSBs), the type of protein formed varies according to tissue type, growth conditions and plant species. Thus, the synthesis of new

proteins appears to be a common response to stress, but there is no universal set of stress proteins. The induction of stress proteins; dehydrins, osmotin and aquaporins were reported by many investigators (Langenkaneper *et al.,* 2001). The aim of the present work is to elucidate some of the physiological and biochemical mechanisms followed by some xerophytes to compensate the external osmotic stress to cope with their habitat conditions.

MATERIALS AND METHODS

The plant materials used in the present investigation growing naturally at wadi Hagul eastern desert of Egypt. The study sites were selected 15& 25 Km in the extension of the wadi east of the high way (where main habitats of the two studied species represented). *Launaea spinosa* (Forssk.) Sch. Bip. Ex Kuntze and *Leptadenia pyrotechnica* (Forssk.) Decne. are non succulent perennial xerophytes.

The climatic factors (temperature ^{o}C, relative humidity % , rainfall mm/Cm, wind velocity Km / h and evaporation mm / day of Suez were obtained from the Meteorological Office of Egypt in the period from (2000 to 2009).

Soil analyses

The soil samples were collected during summer (2008) and winter (2009) at two successive horizontal depths; upper depth (0-20) and lower depth (20-40) cm, and the physical and chemical analyses of the soil were carried out according to Piper (1947), Jackson and Thomas (1960), Jackson (1967) Wild *et al.* (1979) and Rowell (1994).

Plant analyses

Protein nitrogen was estimated by applying the modified microkjeldahel method as described by Peach and Tracey (1956). Percentage of crude protein was determined from dried sample by using the procedure described by Koch and McMeekin (1924). The results were expressed as g/100g dry weight of the plant material. Soluble proteins were determined according to Lowery *et al.* (1951). The free and protein amino acids were determined using GC-MS method according to the method of Mabbott (1990). Sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on vertical slab gels according to the method of Laemmli (1970) as modified by Studier (1973). Nucleic acids were extracted according to Guinn (1966) method. RNA and DNA were determined by the use of the U.V. spectrophotometer at 260 nm. (Ogur and Rosen, 1950).

Variance analysis of data was done using ANOVA program for statistical analysis. The differences among means for all treatments were tested for significance at 1% level by using Duncan (1955) and new multiple range tests as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Climatic factors serve as an important factor in determining the development, distribution and density of vegetation of plants on earth (Zahran, 1989), and also their survival (Larcher, 1995). The meteorological data (Table 1), indicated that plant species grown under climatic conditions of wadi Hagul, grow under less favourable conditions; more fluctuation in air temperature during all seasons of the year, less relative humidity during summer months, less rainfall, wind velocity show very narrow variation finally evaporation was varied in a narrow range.

Table 1: Some meteorological data of Suez during the period from 2000 to 2009 (average).

Month	Air temperature °C		RH %	Wind velocity (Km / h)	Evaporation mm / day	Rain fall mm / month
	Min.	Max.				
January	11.6	22	60.1	8.99	7.23	4.65
February	12.2	23.1	59.1	9.11	7.69	4.43
March	14.1	25	55.2	10.12	8.25	2.7
April	17.2	29	49.3	10.33	9.41	0.2
May	20	32.1	48.21	8.50	12.32	-
June	21.1	36.0	51.2	8.77	12.72	-
July	21.9	37.5	53.32	8.52	13.51	-
August	22.1	38.6	46.65	9.78	10.78	-
September	21.0	33.7	57.37	9.60	10.0	-
October	20.0	31.3	59.28	8.98	8.98	0.1
November	15.0	26.7	62.13	9.0	7.45	1.1
December	12.8	22.5	64.91	5.2	6.13	4.55
Total						**17.73**

Soil analyses:

Physical and chemical analyses of soil sample associated with the studied plants are shown in Tables 2&3. Physical analysis of soil samples was characterized by a higher proportion of fine and medium sand fractions compared to other textures. In addition, site 1 was characterized by a high percentage of fine gravel and clay at upper layer during dry and wet seasons. The results revealed that the soil moisture content attained higher values in the rainy season, results also revealed that there was a gradual increase in the soil moisture content with increasing soil depth (Table 2). Electrical conductivity was higher during wet season in soil associated with *Launaea spinosa,* while the opposite was recorded in soil associated with *Leptadenia pyrotechnica*, soil reaction was alkaline. Calcium and magnesium were the dominant cations, while sodium and potassium were the lowest ones (Table 3.) On the other hand, the sulphate anion was the dominant, while chloride and bicarbonate were the lowest anions (Table 3) .All studied anions and cations had the same trend, they increased in dry season, whereas they dropped to an apparently lower values in wet season, the reverse was attained with sodium and in some cases in bicarbonates.

2-3

In nature, vegetation zones are mainly governed by soil physical and chemical characteristics (Abo-Sitta, 1981 ; Youssef, 1988) and in turn, such properties are affected by both the climate and type of vegetation that develop on it.

Plant analyses:

Total protein

Data presented in Table 4 show that both species contained high significant protein values in wet season as compared with dry season.

Soluble protein

The results in Table 4 clearly show that soluble protein was significantly higher during dry season than in the wet one. An increase in the soluble protein concentration under drought stress could be related to an increase in the protein synthesis related to acclimation and reprogramming to new conditions as well as to cell protection against these stresses (Chen and Plant, 1999). The reduction in the total proteins showed in the plants under water stress is due to probable increase of the proteases activity, which breakdown the proteins (Debouba *et al.*, 2006). In inadequate conditions to the plant is active the pathway in protein breakdown, because the plant use the proteins to the synthesis of nitrogen compounds as amino acids that might auxiliary the plant osmotic adjustment (Sankar, 2007).

Table 4: Seasonal variation in total protein and soluble protein (%) of the studied plant species.

Plant species	Total protein %			Soluble protein %		
	Dry	Wet	$LSD_{1\%}$	Dry	Wet	$LSD_{1\%}$
Launaea spinosa	0.730 b	1.19 a	0.063	0.412 a	0.146 b	0.01
L. pyrotechnica	0.641 b	1.34 a	0.036	0.477 a	0.208 b	0.03

Protein amino acids

It is observed from Table 5 that *Launaea spinosa* possessed a considerable concentration of total protein amino acids as compared with *Leptadenia pyrotechnica*. It is also observed that sum of protein amino acids in dry season was greater than that in wet season in case of *Lanuaea spinosa,* while the opposite was found in *Leptadenia pyrotechnicea*. It is noticed that the sum of both acidic and basic amino acids (hydrophilic amino acids) represented 14%, 21.3% of the total amino acids in wet season and 15.3%, 19% in dry one for *Launaea spinosa* and *Leptadenia pyrotechnica,* respectively. Khidr *et al.* (2007) studied the changes in protein amino acids of *Anabasis articulate*, *Thymelaea hirsuta* and *Zygophyllum album* (sp. of wide ecological amplitude). They found that all the studied species contained more hydrophilic amino acids than hydrophobic ones under dry season. *Zygophyllum album* (inhabited dry saline habitat) was characterized by hydrophilic amino acid rich-protein.

The high accumulation of proline was recorded for *Launaea spinosa* in dry season. It constituted 4.15% of the total amino acid. However, this accumulation (in dry season) was concomitant with a significant reduction in free proline at the same season. There was an enhancement for specific

amino acids in dry season over that in wet one. Soluble proteien and free proline act as osmotic agents or osmoprotectors that play a major role in the osmotic adjustment of water deficit (Yang and Miao, 2010).

The accumulation of certain amino acids under salt stress has been reported in many literatures (EL.Shourbagy and Abdulla, 1975; Ebad *et al.*, 1991; Nour EL-Din, 1995and 2005). In this respect, El-Shourbagh *et al.* (1980) reported that the synthesis of protein rich in certain amino acids could be the key to the survival for the species. Plants accumulate proline during adaptation to various types of environmental stresses such as drought, salinity, and low temperatures, nutrient deficiency (Zaifnejad *et al.*, 1997 ; Hare *et al.*, 1999). Different roles have been proposed for proline accumulation as an adaptive response; it has been suggested that proline may function as an osmoticum (Wyn Jones *et al.*, 1977), a sink of energy and reducing power (Blum and Ebercon, 1976), a nitrogen storage compound (Ahmed and Hellebust, 1988), a hydroxyl-radical scavenger (Smirnoff and Cumbes, 1989) and a compatible solute that protects enzymes (Charest and Phan, 1990). It may also play a role in regulation of cellular redox potentials (Saradhi, 1991).

Table 5: Protein amino acids (mg/g dry wt.) of the studied plant species during the wet and dry seasons.

Amino acids mg/g		***Launaea spinosa***		***L. pyrotechnica***	
		Dry	**Wet**	**Dry**	**Wet**
Acidic	Aspartic	1.014	0.794	0.056	0.332
	Glutamic	0.285	0.108	0.009	0.018
	Glutamine	0.152	0.186	0.053	0.055
Basic	Histidine	0.030	0.082	0.026	0.019
	Arginine	0.069	0.056	0.022	0.015
	Lysine	0.042	0.109	0.004	0.012
Cyclic	Phenylalanine	0.337	0.230	0.083	0.039
	Tyrosine	0.248	1.009	0.097	0.093
	Proline	0.431	0.566	0.064	0.059
	Tryptophane	0.072	0.006	0.027	0.012
Aliphatic	Glycine	1.760	0.154	0.019	0.033
	Alanine	0.202	0.036	0.026	0.005
	Valine	0.257	0.678	0.042	0.555
	Leucine	1.003	2.840	0.038	0.39
	Isoleucine	1.050	0.962	0.080	0.021
	Serine	0.095	0.250	0.055	0.123
	Therionine	0.751	0.554	0.092	0.182
S-containig	Cysteine	0.414	0.177	0.043	0.055
	Cystine	0.024	0.006	0.002	0.002
	Methionine	2.140	0.680	0.056	0.099
	Total	**10.38**	**9.483**	**0.894**	**2.119**

Free amino acids

Data presented in Table 6 show that the studied species have the same number (twenty amino acids) in the two seasons but differ greatly in their concentrations.

It is shown from Table 6 that the sum of free amino acids in wet season was greater than that in dry one; it was 12.34-fold & 1.58-fold enhancement in *Launea spinosa* and *Leptadenia pyrotechnica*, respectively. It is noticed that the sum of both acidic and basic amino acids (hydrophilic amino acids) represented 17.7% and 21.2% of the total amino acids in wet season and 12.4% and 20 % in dry one for *Launea spinosa* and *Leptadenia pyrotechnica*, respectively one of the striking point is that , under dry conditions, phenyl alanine took the first and second order among all amino acid for *Launea spinosa and Leptadenia pyrotechnica* respectively. Leucine represented the highest concentration of all amino acids in *Leptadenia pyrotechnica* and *Launea spinosa* respectively.Cyclic amino acids represented 33.3 and 24.7 % of total free amino acids for *Launea spinosa* and *Leptadenia pyrotechnica*, respectively. Many plants cope with osmotic stress by synthesizing and accumulating some compatible solutes, which are termed as osmoprotectants or osmolytes. These compounds are small, electrically neutral molecules, while are non-toxic even at molar concentrations (Alonso *et al.*, 2001). During osmotic stress, plant cells accumulate solutes to prevent water loss and to re-establish cell turgor. Organic solutes that accumulate during the osmotic adjustment include proline and other amino acids, polyamines and quaternary ammonium compounds like glycine betaine (Tamura *et al.*, 2003).

Table 6: Free amino acids (µg/g dry wt.) of the studied plant species during the wet and dry seasons.

Amino acids µg/g		***Launaea spinosa***		***L. pyrotechnica***	
		Dry	**Wet**	**Dry**	**Wet**
Acidic	Aspartic	13.4	386	5.28	18.9
	Glutamic	5.28	33.9	3	5.53
	Glutamine	7.83	107	1.42	12.1
Basic	Histidine	5.34	20.4	1.24	9.19
	Arginine	2.21	46.5	0.71	388
	Lysine	1.18	17.3	0.37	8.77
Cyclic	Phenylalanine	49.5	98.7	14.3	7.07
	Tyrosine	24.6	226	4.18	23.6
	Proline	14.3	352	5.66	24.3
	Tryptophane	5.29	32.5	0.5	6.03
Aliphatic	Glycine	12.7	23	2.73	6.56
	Alanine	3.45	19.5	1.72	5.06
	Valine	8.34	133	2.69	26.7
	Leucine	32.7	39.2	33.5	23.1
	Isoleucine	15.9	71.2	2.5	24.7
	Serine	25.4	131	2.49	19.2
	Therionine	24.3	1297	3.24	14.7
S-containing	Cysteine	11.6	64.1	2.94	16.5
	Cystine	17	345	11.02	16.1
	Methionine	0.841	2.9	0.38	1.63
	Total	281.161	3446.2	99.87	273.62

Some amino compounds (betaine, glutamate, glutamine and asparagine) were also found at higher concentrations in stressed tissues (Andreas, 1995). The increase in free amino acids is due to high protein hydrolysis, in which the free amino acids are utilized by the plant to reduced the effects of the water deficit through organic solute accumulation and this way increased the water retention capacity (Sircelj *et al.*, 2005). Under water stress the free amino acids as proline and glycinebetaine are strongly influenced and consequently quickly accumulated (Carceller *et al.*, 1999 ; Nakamura *et al.*, 2001), as well as of secondary form occur the increase of aspartate, glutamate and alanine (Ramos *et al.*, 2005). As well as proline, other nitrogen compounds could be accumulated in plants in response to drought stress (Good and Zaplachinski, 1994). The amino acid metabolism may play an important role in plant stress tolerance, by osmotic adjustment through an accumulation of compatible osmolytes,by detoxification of active oxygen species, xenobiotics and heavy metals; and by intracellular pH regulation (Rhodes *et al.*, 1999 and Alia *et al.*, 2001). Proline may be regarded as a scavenger of hydroxyl and singlet oxygen radicals (Smirnoff and Cumbes, 1989 ; Alia *et al.*, 2001). It is known that not all species accumulate proline in response to water or salt stress (Schraml and Rennenberg, 2000) .

Protein pattern:

Protein banding pattern of the studied plant species during wet and dry seasons is shown in Table 7 and Photo 1. It is observed that all protein bands which synthesized during dry season for the first species *Launaea spinosa* differ than those found in wet season. Also,the studied species possessed greater number of protein bands during dry season than wet one. *Launaea spinosa*, possessed two protein bands only, in wet season, with molecular mass of 306 and 59.3 kD. Number of bands increased to 3 for *that species* during dry season. *Leptadenia pyrotechnica* was characterized by the presence of nine protein bands in dry season (two folds that found in wet season) which began with band no.2 with Mm of 306 kD and ended by band no. 16 with Mm of 24.5 kD. Some of these bands no. (7, 13,14) with molecular mass 50.9, 30.4 and 27 kD, respectively were found in the same plant during wet season. The enhanced increase in protein bands in dry season may accommodate plants to stress conditions. Nilson and Orcutt (2000) showed that osmotin (a type of responsive protein) may have a role in osmotic adjustment as an adaptive mechanism for salt stress either by stimulating rapid accumulation of proline and glycinbetaine in the cytoplasm as a non – toxic osmoticum resulting in osmotic adjustment without perturbing metabolic function. On the other hand, Almoguera, *et al.* (1993) showed that sunflower plants can accumulate small heat shock proteins in response to water stress and the expression was comparable with response to heat shock. Again, small heat shock proteins can be produced under oxidative stress which induces the expression of cytosolic (Sun *et al.,* 2001) and mitochondrial (Banzet *et al*., 1998) small heat shock proteins which may contribute to oxidative stress tolerance. The new synthesized protein bands may be induced for stress adaptation during dry season, while disappearance

of other bands may be attributed to the degradation of some proteins (Vierling, 1991).

Table 7: Seasonal variation in protein patterns of studied species using SDS-PAGE.

Mol. wt. of protein marker(kD)	Band No.	*Launaea spinosa*		*L. pyrotechnica*	
		Dry	Wet	Dry	Wet
311.3	1	+			
306.2	2		+	+	
59.3	3		+		+
58.1	4	+			
57.0	5				
56.9	6			+	
50.9	7			+	+
49.6	8				
46.9	9			+	
42.9	10			+	
42.6	11	+			
33.3	12			+	
30.4	13			+	+
27.0	14			+	+
25.0	15				
24.5	16			+	
Total		3	2	9	4

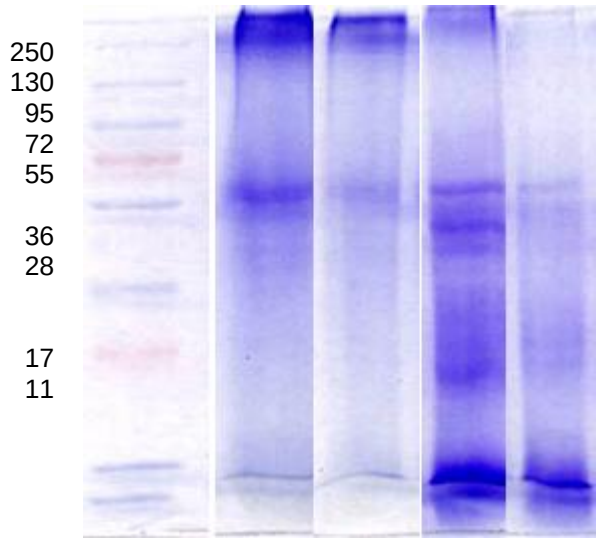

Photo 1: Seasonal variation in protein patterns of the studied species using SDS-PAGE.

It could be concluded that protein electrophoresis provides good marker for the identification and characterization of the studied plants in both the dry and wet seasons. Plants response to water deficit and salinity (Bray, 1993) as well as to high temperature (Schlesinger, 1990) manifests itself in gene expression and in de novo formation of specific macromolecules maintaining cell metabolism under stress. A chaperone-like activity of heat shock proteins (HSPs) was demonstrated by Schlesinger, (1990). Harrington and Alm (1988) provided preliminary evidence that salt shock result in the synthesis of several polypeptides which are similar in size on SDS gels to the HSPs.

Nucleic acid concentration:

It is observed from Table 8 that DNA and RNA concentrations in both plant species were significantly increased during wet season, on the other hand RNA/DNA was significantly increased during wet season for *Launaea spinosa,* while *Leptadenia pyrotechnica* showed an opposite result. The higher levels of DNA during the wet season can be due to active cell division (El-Shourbagy *et al.*, 1980). Abo Kassem *et al.* (2002) found that, the DNase activity was decreased with different anion concentration which showed significant correlation with the reduction in protein content associated with the increase in nucleic acid content in radish. The primary effect of abiotic stress is ion imbalance and hyperosmotic stresses. A direct result of these primary effects is the enhanced accumulation of reactive oxygen species (ROS) that are harmful to plant cells at high accumulation (Ahmed *et al.*, 2009). The Excess of ROC causes damage to protein, DNA and ultimately results in cell death (Ahmed *et al.*, 2008; Tuteja *et al.*, 2009). DNA can be modified by ROS in many different ways. HO is the most reactive, $^{1}O_{2}$ do not react at all (Wiseman and Halliwell, 1996). 8-hydroxyguanine is the most commonly observed modification. In addition to direct DNA oxidation, ROS can also indirectly modify DNA. (Jeong *et al.*, 2005). Jaleel *et al.*, (2008) found that drought stress lowered the DNA and RNA content to a large extent in *Catharanthus roseus* plant. The reduction may be due to increased activities of DNase and RNase (Tewari and Singh, 1991). The decreased DNA and RNA content can be correlated with the reduction in dry weight of the drought stressed plants and it also can be correlated with the reduction in number of cells per unit area of leaves of the drought stressed *C. roseus* plants. RNA/DNA ratio should be a good indicator of the condition of the individuals from wild population (Chicharo and Chicharo, 1994). Morsy (1996) concluded that in dry desert habitats true xerophytes attained higher concentrations of DNA& RNA and attributed this result to the adaptive mechanisms followed by plants of this group.

Table 8: Seasonal variation in nucleic acid concentration of the studied species.

Plant species	DNA mg/ g			RNA mg/ g			RNA/ DNA		
	Dry	Wet	$LSD_{1\%}$	Dry	Wet	$LSD_{1\%}$	Dry	Wet	$LSD_{1\%}$
L. spinosa	0.425 b	0.483 a	0.02	1.16 b	1.35 a	0.13	2.73 b	2.80 a	0.06
L. pyrotechnica	0.504 b	0.587 a	0.019	1.38 b	1.58 a	0.03	2.74 a	2.69 b	0.05

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استراتيجية الإنضباط الأيضى في نوعين من النباتات الصحراوية الغير عصيرية تحت ظروف الجفاف.

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استهدفت هذه الدراسة اختيار نوعين من النباتات الجفافية هما الكباث *Launaea spinosa*، و المرخ *Leptadenia pyrotechinca* والتى تنمو طبيعيا بوادى حجول، الصحراء الشرقية المصرية. حيث تم اختيار موقعى الدراسة بالكيلو 15، 25 شرق الطريق الرئيسى لامتداد الوادى حيث البيئات الرئيسية الممثلة لنباتى الدراسة خلال موسمى الرطوبة والجفاف وقد اسفرت الدراسة على النتائج التالية:-

أظهرت نسبة كل من البروتين الخام والأحماض الأمينية الحرة والأحماض النووية أعلى قيمة لها فى موسم الرطوبة. بينما كان أعلى تركيز للأحماض الأمينية البروتنية فى موسم الجفاف لنبات الكباث، فى حين تراكم الأحماض الأمينية البروتنية لنبات المرخ خلال موسم الرطوبة. وقد زادت نسبة البروتين الذائب فى موسم الجفاف للنباتين.

أوضحت دراسة التفريد الكهربى لانماط البروتين ظهور بعض البروتينات الخاصة بالنباتات محل الدراسة والتى تعكس سلوك هذه النباتات تحت ظروف الجفاف.

قام بتحكيم البحث

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Table 2: Physical analyses of soil depth profiles associated with the studied species.

Site & depth	Season	Gravel	Fine gravel	Coarse sand	Medium sand	Fine sand	Silt	Clay	Soil texture	Soil moisture %
1(0-20)	Dry	4.010	24.90	17.39	13.81	10.32	11.80	17.77	Fine gravel	0.23 d
	Wet	_	6.98	13.22	7.46	12.34	19.97	40.07	clay	0.41 b
1(20-40)	Dry	4.01	17.23	24.33	19.40	10.25	12.75	12.03	Coarse M. sandy	0.26 c
	Wet	9.61	16.34	21.73	38.04	10.43	2.58	1.27	Medium sandy	0.51 a
2(0-20)	Dry	1.00	3.9	6.95	35.00	24.1	20.0	9.05	Medium sandy	0.18 d
	Wet	1.42	3.48	5.95	23.44	35.66	21.2	8.85	Fine sandy	0.46 b
2(20-40)	Dry		1.0	6.93	35.1	26.0	21.2	9.87	Medium sandy	0.23 c
	Wet	_	1.62	6.31	26.8	35.50	20.7	9.07	Fine sandy	0.50 a

Table 3: Some chemical analyses of soil depth profiles associated with the studied plant species during wet and dry seasons.

Plant species	Sites & depth	Season of sampling	E.C ds/m	pH	Organic carbon %	Total carbonate $CaCO_3$ %	Cations (meq/L)				Total Cations (meq/L)	Anions (meq/L)			Total Anions (meq/L)
							Na	K	Ca	Mg		HCO_3 -	Cl -	SO_4--	
L. spinosa	1(0-20)	Dry	0.86 c	8.05 a	0.087 b	3.99 d	7.44 b	0.925 a	28.6 a	24.2 a	61.2	0.702 a	0.091 a	1.85 a	2.64
		Wet	1.2 b	7.68 a	0.076 c	5.06 a	8.22 a	0.869 b	20.3 b	15.8 c	45.2	0.168 d	0.039 c	1.4 b	1.61
	1(20-40)	Dry	0.60 d	8.04 a	0.067 d	4.31 c	6.35 d	0.875 b	18.2 c	20.8 b	46.2	0.214 c	0.059b	0.95 c	1.22
		Wet	1.4 a	7.92 a	0.127 a	4.61 b	7.37 c	0.823 c	11.5 d	9.2 d	28.9	0.458 b	0.012 d	0.69 d	1.16
L. pyrotechnica	2(0-20)	Dry	1.5 b	7.98 a	0.117 a	4.16 b	8.35 b	0.925 a	20.3 a	18.1 a	47.7	0.320 c	0.029 a	7.05 a	7.40
		Wet	1.2 c	7.83 a	0.106 b	4.13 b	9.65 a	0.775 b	17.4 b	14.1 b	41.9	0.381 b	0.018 b	5.9 b	6.30
	2(20-40)	Dry	1.6 a	7.94 a	0.071 c	3.23 c	7.32 d	0.635 c	12.5 c	14.2 b	34.7	0.519 a	0.014 c	3.36 c	3.89
		Wet	0.66 d	8.14 a	0.106 b	4.91 a	8.15 c	0.403 d	8.4 d	10.4 c	27.4	0.137 d	0.012 d	2.78 d	2.93