MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Changes in metabolism, antioxidant compounds and enzymes in Portulaca oleracea L. in response to saline habitat

Alshaymaa A. Noureldeen; Amany M. Kazamel; Ghada A. El-Sherbiny; Samia A. Haroun and Omer A. El- Shahaby

* *Botany Department, Faculty of Science, Mansoura University*

Received:25/4/2021
Accepted:5/3/2021

Abstract: Acomparative study based on the measured conductivity (salinity) of the two studied habitats classified to mesophytic and saline habitats in the Nile Delta coast. Field survey was carried out during March and April 2015 where the *Portulaca oleracea* plants were collected from the two studied habitats.

In this study, physical and chemical soil characteristics of the two studied habitats showed marked variation. Salinity played a role in inducing *Portulaca oleracea* to produce more content of total carbohydrates and soluble sugars as well as proline, electrolyte leakage and antioxidant enzymes; peroxidase, polyphenol oxidase and catalase compared with mesophytic habitat. Regarding to elements content, nitrogen and potassium have a significant increase in mesophytic habitat, as compared with saline habitats. Meanwhile, phosphorus, sodium and calcium increase in saline habitats more than mesophytic habitat. Alkaloids content, total phenols, flavonoids, tannins and anthocyanin of *Portulaca oleracea* increased significantly in saline habitats as compared with mesophytic habitat.

keywords: *Portulaca oleracea,* salinity, metabolites, antioxidants

1.Introduction

Stress was described as physiological changes which occur when species are exposed to extraordinarily unfavorable conditions [1]. Plant stresses classified to a) Abiotic stresses which caused by un-living factors as variations in temperatures, drought and salt stress and b) Biotic stresses which caused by living organisms like insects, some other plants and some microorganisms [2] **.** Salts are considered very useful for the plants which can improve its growth and metabolism. However, they become toxic if found in excess concentration which impose salinity stress [3]**.** Salinity has many harmful effects on plants such as increase ionic and osmotic stresses around the roots which reduce the ability of the plant to water uptake, [4-3] and this reflects in reducing the rates of growth and metabolism. Another harmful effect of salinity on the plants formation of molecules called reactive oxygen species (ROS) which are molecules which are very dangerous for plants by damaging macromolecular and membrane as reported by [5-6].

Halophytes has a superior defense to resist the dangerous impact of salinity through more than one defense method as proline accumulation which increase plant defense to abiotic stress, by osmotic adjustment [7-8]**.** As reported by [9] stress factors e.g. temperature, salt, soil pH and aridity are essential factors for release of secondary metabolites with potent antioxidant activity. According to [10] plant secondary metabolites which as end products of primary metabolism but they don't involved in metabolic activity. One of the most important plant strategies for reduction ROS toxic components damaging effects is the utilization of many antioxidant defense which increase plant tolerance to different stress factors. Several antioxidants used by plants as catalase, APX, superoxide dismutase (SOD), glutathione reductase and non-enzyme molecules, such as glutathione, ascorbate, carotenoids and anthocyanins as mentioned by many researchers [3-5-11-12]

Protulaca oleracea (Common purslane) is edible herbs with nutritional value which contain both of healthy antioxidant compounds

and essential nutrients, as omega-3, omega-6 fatty acids, α-linolenic acid, ascorbic acid, glutathione, beta carotene and α-tocopherol [13]. Also [14] reported that *P. oleracea* is a rich source of omega-3 fatty acids, which is important in preventing heart attacks and strengthening the immune system. *P. oleracea* is known as 'vegetable for a long life' which can use as, traditional herbal medicine and an edible plant. It has been used for reducing swelling and pain in addition to it acts as antiviral, antibacterial, antidiabetic or increasing immunity properties [15]. Moreover *P. oleracea* is guessed that it reduces the occurrence of cardiovascular diseases and cancer [16]. Increase in temperatures, water deficit or high salinity on *P. oleracea* demonstrated by different authors [17-18]**.** Most of these studies have been executed in a single species, *P. oleracea,* [19-20] reached out to this and other taxa of the genus are able to resistant salt stress and/or drought when they compared with the major crops.

This study was conducted to evaluate the response of *P. oleracea* growing in two different habitats; mesophytic and halophytic by following up the changes in carbohydrates, proline, and electrolyte leakage, some elements as well as antioxidant enzymes.

2. Materials and methods

The study area situated in the north – eastern part of the Nile delta particularly El-Dakahlia and Damietta governorates. The habitats are classified according to the measured conductivity (salinity) for each one as follows: mesophytic, moderately saline and saline habitats.

1. Soil Analyses

Three soil samples (0-20 cm depth) of the two studied habitats; mesophytic and saline were collected from the rhizosphere around the species from each habitat. All samples were brought to laboratory after collection, air dried and sieved through a 2 mm sieve to get rid of debris and coarse gravel. These air dried samples were analyzed for determination of physical and chemical parameters.

a. Physical analysis of the soil samples

Soil samples texture determination reported by [21] method**.** Hilgard pan boxes were used for the estimation of water-holding capacity of each of the collected soil samples [22]. The total pore space (porosity) determination depends on measuring the volume of soil in both saturated and dry conditions and the pore–space volume is expressed as percentage of the original soil sample volume [22]**.**

b. Chemical analysis of the soil samples

Calcium carbonate was determined by titration against 1N NaOH and expressed as percentage [23]**. Organic carbon (OC)** oxidizable organic carbon (as indication of the total organic matter) was determined using Walkely and Black rapid titration method [24]**. Electrical conductivity (EC), pH and Total dissolved salts (TDS)** were estimated using a multi-meter CONSORT Model C535. **Chlorides (Cl^-)** content was estimated according to Mohar's method described by the American Public Health Association [25]**. Sulphates (SO_4^{-2})** content was estimated gravimetrically according to the assay described by [26] using 5% barium chloride solution. **Bicarbonates and carbonates** soil water-soluble bicarbonates and carbonates were estimated in soil extract by acidimetric titration method [27] using 0.1N HCl and phenolphthalein as indicator for CO_3^{-2} (pH more than 8.5) and methyl orange as indicator for HCO_3^- (pH less than 6). **Extractable cations** Sodium (Na^+) and potassium (K^+) were estimated in the tested samples using flame photometer (Model PHF 80 B Biologie spectrophotometer) according to [28]**.** Calcium (Ca^{++}) and magnesium (Mg^{++}) were determined by atomic absorption spectrometer (Perkin-Elmer; Model 2380 USA) according to [28]**. Total nitrogen determination** was determined in the soil extract using the micro-Kjeldahl [29]**. Phosphorus content** was estimated according to the method described by [24]**.** This method depends on the formation of a blue complex between phosphate and molybdic acid in presence of a reducing agent.

2. Plant analysis:

Portulaca oleracea plants were collected from different localities in plastic bags, transferred directly to the lab for determination of each the following:

a. Carbohydrates

the methods for extraction of the different

carbohydrate fractions tested were essentially those of [30-31]**.** Fructose was estimated using the resorcinol method of [32] as described by [33]**.** Sucrose content was determined using modification of the procedures of [31]**.** Total soluble sugars (TSS) content was determined using modification of the procedures of [30]**.** Polysaccharides estimated according to [34]**.** Total carbohydrates were calculated as the summation of the amount of total soluble sugars and polysaccharides of the same sample.

b. Antioxidant enzymes activity the enzyme extract of *Portulaca oleracea* prepared according to [35]**. Peroxidase (POX)** and **Polyphenol oxidase (PPO)** activities were assayed by the method of [33]. **Catalase** activity was assayed by the method of [36]that modified by [37]**.**

c. Quantitative determination of some secondary metabolites: Tannin content of *P. oleracea* from different studied habitats was determined by the method of [38]. While **flavonoid** content determined by the method accompanied by [39]**.** Meanwhile **Alkaloid** content was determined by the method of [40]. In addition, **total phenols** were determined by the modified Folin–Ciocalteau reagent method according to [38]**.**

d. Element's content: Total soluble Nitrogen was determined by the conventional semi-micropropagation of Kjeldahl method of [41] and described by [42]. **Phosphorus content** determined by the method depends on the formation of a blue complex between phosphate and molybdic acid in the presence of a reducing agent [43] as described by [44] and adopted by [42]**. Ca and Mg** ions were measured by atomic absorption spectrophotometry according to the methods described by [45]**. K and Na** determined by the flame emission technique [46]**.**

e. Electrolyte leakage and proline content electrolyte leakage of *P. oleracea* was determined to assess the membrane permeability according to [47]. Whereas proline was as essentially described by [48].

Statistical Analysis: Data were statistically analyzed and comparison among means was carried out using COHORT/ COSTAT program (798 Lighthouse Ave. PMB 329, Monterey, CA, 93940, USA). The least significant difference (LSD) test was chosen as a mean test with significance level at $P \leq 0.05$.

3.Results and discussion

Physical and chemical analysis of the soil samples

In the current study analysis of the soil from the two studied habitats showed that mesophytic habitat was categorized by its clayey texture, highest values of porosity, water holding capacity**,** OC, TN and TP (Tables 1-2) as compared with saline habitat. On the other hand, soil pH, TDS, $CaCO_3$, EC, Cl^-, SO_4, HCO_3, Na^+, K^+, Ca^{++} and Mg^{++} decreased in mesophytic soil as compared with saline soils. This may be attributed to the disturbed nature of the mesophytic habitat through application of excess organic and artificial fertilizer moisture availability by the webs of irrigation canals distributed in cultivated land of the Nile Delta. This was reported by [49] that compared the soil of vegetation types at the borders (saline coastal land) of the Nile Delta with those inside (cultivated) and found that silt, OC, P and N increased while the sand decreased from the borders towards the middle of the Nile Delta. Also, these results, in harmony with the present results, where soil of the saline habitat featured by sandy texture, high values of EC, TDS, $CaCO_3$, Cl^-, SO_4, HCO_3, Na^+, K^+, Ca^{++} and Mg^{++} and low values of OC, TN and TP. This coastal habitat subjected to less urbanization i.e. less plantations, less fertilizer application (less sources of OC, TN, TP). These results are in harmony with [50] who reported that saline soils are seen to possess high concentrations of potassium ions, chloride ions, and sulfate ions. Increased soil salinity is associated with increased concentrations of these ions.

Plant analysis

a. Carbohydrates content. The current results indicated that, content of total carbohydrate and soluble sugars of *P. oleracea* increased significantly at the saline habitat as compared with mesophytic habitat in which it recorded the minimum content of total carbohydrates and soluble sugars, as shown in table (3). These results are in harmony with [51-52] who reported that soluble sugars accumulated as the results of some environmental stresses, especially drought and salinity. In this respect,

the increase of *P. oleracea* soluble sucrose and carbohydrates in saline habitat was for osmotic adjustment as an adaptation mechanism.

b. Antioxidant enzymes activity. Peroxidase, polyphenol oxidase and catalase activity of *P. oleracea* have a significant increase in saline habitat as recorded in table (4). In this connection, [53]stated that plants use a number of enzymatic and non-enzymatic antioxidants to prevent oxidative damage and keep reactive oxygen species concentrations within a narrow functional range. Our results are in harmony with [54] who reported that tomato plant under high salt concentration showed higher antioxidant enzymes activity such as catalase, ascorbate peroxidase, glutathione reductase, and GST.

c. Secondary metabolites. Figure (1) showed that salinity catalyzed *P. oleracea* to produce the highest content of different secondary metabolites. Alkaloids content for *P. oleracea* have the maximum content in the plants which was collected from saline site. These results are in harmony with [55] who reported that steroidal alkaloid level increased by salinity stress in *Solanum nigrum* plant. Our research recorded that phenol for *P. oleracea* increased significantly in saline habitat as compared with mesophytic habitat as shown in figure (1). [56-57]reported that obtained correlation between antioxidant activity and total phenolic content recorded in plants of different origin. Also [58] reported that increase in leaf polyphenol content and antioxidant activity in leaves of the halophyte *Cakile maritima***.**

In this study *P. oleracea* flavonoids increase significantly in saline habitat as compared with mesophytic habitat as shown in figure (1). In this concerning, isoflavonoids which are flavonone derivatives play important role in plant defense and development as reported by [59]. *P. oleracea* that was implanted in saline habitat recorded the maximum content of tannins, comparing with that growing in the mesophytic one. These results are in agreement with those of [60] who reported more respiration, increase soluble phenolic and tannin contents in the two different phylogenetically poplar species has been observed under salt stress.

Also *P. oleracea* collected from saline habitat showed the largest content of anthocyanin as compared with the plant which was collected from mesophytic habitat. In this respect [61] recorded that a high polyphenol content, mainly "anthocyanins" reduced the oxidative damages which result to the "H_2O_2" generation**. Also** [62]**)** reported that salinity increased anthocyanins content in plants [63].

d) Elements content.

The current study showed that nitrogen and potassium showed the maximum content in case of *P. oleracea* which was collected from mesophytic site as compared with the plants which was collected from saline site. In contrast phosphorus, sodium and calcium showed the maximum content at saline habitat as shown in table (5). On the other hand, magnesium showed a non-significant difference between the two studied sites. In support, [64] reported that intracellular K^+ and Na^+ homeostasis has a vital role in maintaining membrane potential and a proper osmotic and in regulation many cytosolic enzymes. Our results are in harmony with [65] who reported that the decrease of K^+ concentration under stress is owing to accumulation of Na^+ ion

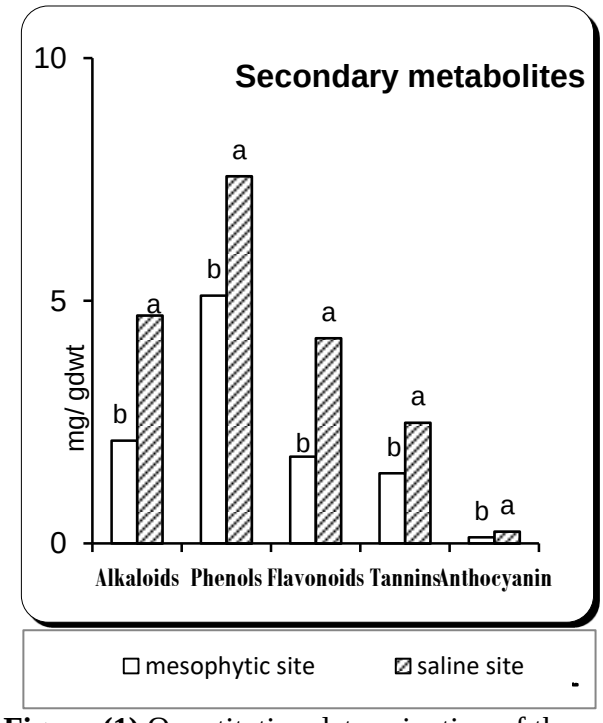

Figure (1) Quantitative determination of the secondary metabolites of *P. oleracea* shoots at mesophytic and saline habitat

Table (1): Soil physical characteristics of studied habitats**.**

Habitat	Soil texture (%)			Porosity(%)	W.H.C.(%)
	Sand	Silt	Clay		
Mesophytic	13.37[b]	19.20[a]	65.81[a]	72.00[a]	42.00[a]
Saline	75.70[a]	14.90[b]	8.06[b]	30.00[b]	19.00[b]
L.S.D	1.86	1.86	1.86	1.75	2.02

-WHC: water holding capacity

-Different letters indicate statistically significant differences and identical letters are not significant

Table (2) Soil chemical characteristics of studied habitats.

Habitat	TDS (mg/)	$CaCO_3$ (%)	Organic carbon (%)	pH	EC (dSm^{-1})	Cl^- (%)	SO_4^{--}(%)	CO_3 (%)	HCO_3 (%)	Cations (mg/100 g dry soil)				TP (mg/ 100g dry soil)	TN (mg/ 100g dry soil)
										Na^+	K^+	Ca^{++}	Mg^{++}		
M	402[b]	1.65[b]	1.40[a]	7.90[b]	0.73[b]	0.06[b]	0.18[b]	0.00	0.10[b]	32.90[b]	8.10[b]	20.10[b]	2.69[b]	13.8[a]	29.00[a]
S	827[a]	2.40[a]	0.45[b]	8.48[a]	2.30[a]	0.29[a]	0.34[a]	0.00	0.16[a]	90.90[a]	29.20[a]	37.80[a]	8.31[a]	8.00 [b]	12.70[b]
L.S.D	20.57	0.43	0.043	0.16	0.12	0.04	0.08	0	0.02	3.33	1.19	1.39	0.75	2.21	2.70

-TDS: total dissolved salts, EC: electrical conductivity, TN: total nitrogen, TP and total phosphors.

-Different letters indicate statistically significant differences and identical letters are not significant

- (M) and (S) are the mesophytic and saline site, respectively

Table (3) Carbohydrate contents (mg/g dwt.) of *P. oleracea* shoots at mesophytic and saline habitats

Habitat	Fructose	Sucrose	Total soluble sugar	Polysaccharides	Total carbohydrate
Mesophytic	4.52[b]	49.30[b]	83.00[b]	104.00[b]	187.00[b]
Saline	7.16[a]	63.70[a]	124.00[a]	198.00[a]	322.00[a]
L.S.D	0.79	2.78	5.11	8.86	8.07

-Different letters indicate statistically significant differences and identical letters are not significant.

Table (4) Enzymes activity of *P. oleracea* shoots at mesophytic and saline habitats

Habitat	Peroxidase (ug/g fresh weight/ min)	Polyphenol oxidase (ug/g fresh weight/ min)	Catalase (µ moles H_2O_2 consumed/min/mg protein)
Mesophytic	42.20[b]	2.34[b]	100.00[b]
Saline	52.63[a]	3.38[a]	162.00[a]
L.S.D	1.79	0.69	8.70

-Different letters indicate statistically significant differences and identical letters are not significant

Table (5) Elements content of *P. oleracea* shoots at mesophytic and saline habitats

Habitat	N%	P%	K%	N%	C%	M%
Mesophytc	29.90[a]	7.90[b]	5.32[a]	0.23[b]	0.16[b]	1.5[a]
Saline	28.60[b]	9.80[a]	4.77[b]	0.69[a]	0.46[a]	1.3[a]
L.S.D	1.01	0.84	0.21	0.03	0.08	0.69

Different letters indicate statistically significant differences and identical letters are not significant

since Na^+ hinder uptake of K^+ ion. From other side of view, increase of *P. oleracea* calcium at saline site consider an adaptation method to tolerate salinity this result is in agree with [66] who reported that Ca^{2+} is a non-toxic inorganic nutrient and has a function of detoxification under saline medium.

e) Electrolyte Leakage and Proline content

Electrolyte leakage from plasma membranes is reported as one of the most important selection criterion for identification of salt-tolerant plants [67]. This is in similar with the current results in which electrolyte leakage of *P. oleracea* showed observed increment in case of saline site as shown in table (6). Table (6) also showed that *P. oleracea* proline has a significant increase at saline site. These results are in harmony with [68] find out that stress increase the proline content in *P. oleracea* leaves. Also, [69] reported that proline has important role in maintaining the cell turgor and also affects the various proteins solubility.

Table (6): Electrolyte leakage (% ion leakage) and proline content (ug/g) of *P. oleracea* shoots at mesophytic and saline habitats

Habitat	Electrolyte leakage	Proline
mesophytic	**38.56^b**	**4.09^b**
saline	**48.36^a**	**6.75^a**
L.S.D	**1.24**	**0.47**

-Different letters indicate statistically significant differences and identical letters are not significant

In conclusion: This study confirmed that soil as a major growth medium for plants plays an extremely important role in the plant life through its physical, chemical and biological processes which provide the favorable air-moisture regime for plant growth. This is reflected directly, in this study on the various determined parameters of P. oleracea that growing at two different habitats; mesophytic. Although the saline habitat have negative effects on P. oleracea, this habitat enhances synthesis of antioxidant compounds and enzymes

References

1 Gaspar, T., Franck, T. and Bisbis, B. (2002): Concepts in plant stress physiology. Application to plant tissue cultures. Plant Growth Regul, **37**: 263-285.

2 Kranner, I., Minibayeva, F. V., Beckett, R. P. and Seal, C.E. (2010): What is stress? Concepts, definitions and applications in seed science. New Phytol., **188**: 655–673.

3. Sazzad, K. (2007): Exploring plant tolerance to biotic and abiotic stresses. Swedish University of Agricultural Sciences, Uppsala.

4. Flowers, T.J., Troke, P.F. and Yeo, A.R. (1977): The mechanism of salt tolerance in halophytes. Annu Rev Plant Physiol., **28**:89–121.

5. Blokhina, O., Virolainen, E. and Fagerstedt, K. V. (2003): Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot., **91**:179–194.

6. Farnese, F.S., Menezes-Silva, P.E., Gusman, G.S. and Oliveira, J.A. (2016): When bad guys become good ones: the key role of reactive oxygen species and nitric oxide in the plant responses to abiotic stress. Front Plant Sci., 7:471.

7. Rains, D.W. (1989): Plant tissue and protoplast culture: applications to stress physiology and biochemistry. In: Jones HG, Flowers TJ ،Jones MB, eds. Plants under Stress: Biochemistry, Physiology and Ecology and Their Application to Plant Improvement; Seminar Series, **39**: 181-196.

8. Kavi- Kishore, P.B., Sangam, S., Amrutha, R.N, Laxmi, P.S., Naidu, K.R., Rao, K.R.S.S., Rao, S., Reddy, K.J., Theriappan, P. and Sreenivasulu, N. (2005): Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr Sci., **88**: 424-438.

9. Selvam, R., Jurkevich, A., Kang, S.W., Mikhailova, M.V., Cornett, L.E., Kuenzel, W.J. (2013): Distribution of the vasotocin subtype four receptor (VT4R) in the anterior pituitary gland of the chicken ، Gallus gallus, and its possible role in the

avian stress response. *J Neuroendocrinol*, **25**:56–66.
10. Salah, A.I. (2015): Chapter 8: Plant secondary metabolites of halophytes and salt tolerant plants. In :El Shaer HM, Squires VR (eds) Halophytic and salt-tolerant feedstuffs, impacts on nutrition ، physiology and reproduction of livestock, 1st edn. CRC Press, Boca Raton, pp 127–142.
11. Mittler, R. (2002): Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., **7**:405–410.
12. Gould, K.S., McKelvie, J. and Markham, K.R. (2002): Do anthocyanins function as antioxidants in leaves Imaging of H2O2in red and green leaves after mechanical injury. Plant Cell Environ, **25**:1261-1269.
13. Liu, L., Howe, P., Zhoum, Y.F., Hocart, C. and Zhang, R. (2002): Fatty acid profiles of leaves of nine edible wild plants: an Australian study. *J Food Lipids*, **9**: 65-71.
14. Simopoulos, A. P. (2004): Omega-3 Fatty Acids and Antioxidants in Edible Wild Plants. Biol Res **37**: 263-277.
15. Zheng, Z.H., Dong, Z.H. and Yu, J. (1997): Modern study of traditional Chinese medicine, Xue Yuan Press, Beijing University of Traditional Chinese Medicine, Beijing.
16. Simpoulos, A.P. (1991): Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr*, **54**: 438-463.
17. Grieve, C.M. and Suarez, D.L. (1997): Purslane (P. oleracea L.): a halophytic crop for drainage water reuse systems. Plant and Soil 1997; **192(2**): 277-283.
18. Teixeira, M. and Carvalho, I.S.(2009): Effects of salt stress on purslane (P. oleracea) nutrition. Ann App Bio, **154(1):** 77-86.
19. Alam, M.A., Juraimi, A.S., Rafii, M.Y., Abdul Hamid, A. and Aslani, F.(2014): Screening of purslane (*P. oleraceaL*.) accessions for high salt tolerance. *Sci World J*; 9.
20. Ren, S., Weeda, S., Akande, O., Guo, Y., Rutto, L. and Mebrahtu, T. (2011): Drought tolerance and AFLP-based genetic diversity in purslane (P. oleracea L.). *J Biotech Res*, **3**: 51-61.
21. Richards, L.A. (1954): Diagnosis and improvement of saline and alkali soil, United States department of Agriculture, hand book, 60-160.
22. Klute , A. (1986): Water relation: Laboratory methods. In Methods of Soil Analysis, part 1. 2 nd ed. A. Klute (ed) Agronomy Monograph, No.9.ASA, Madison W(1): 653-661.
23. Jackson, M.L. (1962): Soil chemical analysis. Constable Co. Ltd. London, p. 296.
24. Margesin, R. and Schinner, F. (2005).Manual for soil analysis monitoring and assessing soil bioremediation, with 31 figures, Springer-Verlag Berlin Heidelberg, Germany, pp 366.
25. APHA (American Public Health Association) (1998): Standard methods for the examination of American Public Health Association, American Water Work Association , Water Pollution Control Federation, Washington, D.C.
26. Jackson, M.L. (1967). In soil chemical analysis. Prentica Hall Inc Inglewood Cliffs, London, pp 498.
27. Baruah, T.C. and Barthakur, H.P. (1997): A Text Book of Soil Analysis, Vika poblishing House Pvt Ltd, New Delhi, pp 334.
28. Allen, S. E., Grimshaw, H. M., Parkinson, J. A., Quarmby, C. and Roberts, J. D. (1974). Chemical Analysis of Ecological Materials. Blackwell Scientific Publications. Osney, Oxford, London.
29. Allen, E. B., Chambers, J.C., Connor, K.F., Allen, M.F. and Brown, R.W. (1987): Natural reestablishment of mycorrhizae in disturbed alpine ecosystems . Arctic and Alpine Res, 11-20.
30. Yemm, E.W. and Willis, A. J. (1954): The estimation of carbohydrates by anthrone. *Biochemist Journal*, **57:** 508-514.
31. Handel, E. V. (1968): Direct micro determination of sucrose. Anal. Biochemist, **22**: 280-283.
32. Roe, J.H. (1934): *J. Biol. Chem*, 107: 15.

33. Devi, P. (2002): Principals and Methods in plant molecular biology, biochemistry and Genetics. Agro biology, India, pp .41.
34. Sadasivam, S. and Manickam A. (1996): Biochemical method. Second Ed. New Age Int. Pvt. Ltd. Pub and T.N. Agricultural Univ., Coimbagore, 108-11035.
35. Agarwal, S. and Shaheen, R. (2007): Stimulation of antioxidant system and lipid peroxidation by abiotic stresses in leaves of Mormoordica charantia. Braz. Plant Physiol, **19**: 149-161.
36. Sinha, A.K. (1972): Colorimetric assay of catalase. Anal Biochem, **47**: 389-394.
37. Gopalakrishnan, V. K., and T. Starlin (2013) "enzymatic and non-enzymatic antioxidant properties of Tylophora pauciflora wight and arn. â€an in vitro study". Asian *J. of Pharmace. and Clin*. Res., Vol, **8**: 68-71.
38. Sadasivam, S. and Manickam, A. (2008): Biochemical Methods, 3rd Edition, New Age International Publishers, New Delhi, India.
39. Boham, B. A. and Kocipai-Abyazan, R. (1974): Flavonoids and Condensed Tannins from Leaves of Hawaiian Vaccinium vaticulatum and V. calycinium. Pacific Sci, **48**: 458-463.
40. Harborne, J. B. (1973): Phytochemical Methods, London. Chapman and Hall, Ltd. p. 49-188.
41. Rees, M. W. and Williams, E. F. (1943): The total nitrogen content of egg albumin and other proteins. *Journal Biochemist*, **37**: 354-359.
42. Haroun, S. A. (1985): Studies on adaptation of plants to water stress. Ph.D. Thesis, Botany Department, Faculty of Science - Mansoura University, Egypt.
43. Kuttner, T. and Lichtenstein, L. (1932): Estimation of organically-bound phosphorus. A system of analysis of phosphorus compounds in blood. *J. of Biological Chem* , **95**: 661-670.
44. Humphries, E.C. (1956): Mineral components and ash analysis. In: Modern method of plant analysis .Vol. **1,** (edited by K. Paech and M.V. Tracey). Berlin: Springer Verlag, 468–502.
45. Chapman, H.D. and Pratt, P.F. (1978): Method of Analysis for Soil, Plant and Water, California University, Division Agrc. Sci., Priced Publication, pp 50-169.
46. Ranganna, S. (1977): Manual analysis of fruit and vegetable products. Tata MC craw-Hill publishing company limited New York.
47. Lutts, S., Kinet, J.M. and Bouharmont, J. (1996): NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance. Ann. Bot, **78**:389-398.
48. Snell, F.D. and Snell, C.T. (1954): Colorimetric method of analysis volume IV.D. Van Nostrand Co. Inc., New York. pp: 786.
49. Shaltout, K.H. and El-Sheikh, M.A. (2003): Vegetation of the urban habitats in the Nile Delta region, Egypt Urban Ecosystems, **6:** 205–221.
50. Hashem, A., Abd_Allah, E.F., Alqarawi, A.A., Al-Huqail, A.A. and Egamberdieva, D. (2014). Alleviation of abiotic salt stress in Ochradenus baccatus (Del.) by Trichoderma hamatum (Bonord.) Bainier. *J. Plant Int*, **9**: 857–868.
51. Murakeozy, E.P., Nagy, Z., Duhaze, C., Bouchereau, A. and Tuba, Z. (2003): Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hung. *J. of plant physiol*, **160**: 395-401.
52. Sotripopoulos, T.E, (2007): Effect of NaCl and CaCl2 on grown and contents of minerals, chlorophyll, proline and sugar in the apple rootstock M4 cultured in vitro. Biol. Plant, **51(1)**: 177-180.
53. Ozgur, R., Uzilday, B., Sekmen, A.H. and Turkan, I. (2013): Reactive oxygen species regulation and antioxidant defence in halophytes. Functional Plant Biology, **40**: 832–847.
54. Rodriguez-Rosales, M.P., Kerkeb, L., Bueno, P. and Donaire, J.P. (1999): Changes induced by NaCl in lipid content and composition, lipoxygenase, plasma membrane H+ATPase and antioxidant enzyme activities of tomato (Lycopersicon esculantumMill.) calli. Plant Sci, **143**: 143–150.

55. Sachan, N, Rogers, D.T., Yun, K.Y., Littleton, J.M.and Falcone, D.L. (2010): Reactive oxygen species regulate alkaloid metabolism in undifferentiated N. tabacum cells. Plant Cell Rep, 29:437.
56. Piluzza, G. and Bullitta, S. (2011): Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethno veterinary use in the Mediterranean area. Pharm Biol, **49(3**):240–247.
57. Sadeghi, Z., Valizadeh, J., Azyzian Shermeh, O. and Akaberi, M. (2015): Antioxidant activity and total phenolic content of Boerhavia elegans (choisy) grown in Baluchestan, Iran. Avicenna *J Phytomedicine,* **1:**1-9.
58. Ksouri, R., Megdiche, W., Debez, A., Falleh, H., Grignon, C. and Abdelly, C. (2007): Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte Cakile maritima. Plant Physiol Biochem, **45 (3–4**):244–249.
59. Sreevidya, V.S., Srinivasa, R.C., Rao, C., Sullia, S.B., Ladha, J.K., Reddy, P.M. (2006): Metabolic engineering of rice with soyabean iso flavone synthase for promoting nodulation gene expression in rhizobia. *J Exp Bot*, **57**:1957-1969.
60. Janz, D., Behnke, K., Schnitzler, J.P., Kanawati, B., Schmitt-Kopplin, P. and Polle, A. (2010): Pathway analysis of the transcriptome and metabolome of salt sensitive and tolerant poplar species reveals evolutionary adaption of stress tolerance mechanisms. BMC Plant Biol, 10:150.
61. Hajlaoui, H., Denden, M., El Ayeb, N. (2009): Differential responses of two maize (Zea mays L.) varieties to salt stress: changes on polyphenols composition of foliage and oxidative damages. Ind Crop Prod, **30(1**):144–151.
62. Slama, I., M'Rabet, R., Ksouri, R., Talbi, O., Debez, A. and Abdelly, C. (2017): Effects of salt treatment on growth, lipid membrane peroxidation, polyphenol content, and antioxidant activities in leaves of Sesuvium portulacastrum L. Arid Land Res Manag, **31**:1–14.
63. Parida, A.K. and Das, A.B. (2005): Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf, **60(3):**324–349.
64. Munns, R. and Tester, M. (2008): Mechanisms of salinity tolerance. Ann.Rev. of plant boil, **59**: 651-681.
65. Gomes, M.A.C., Suzuki, M.S. Cunha, M. and Tullii, C.F. (2011): Effect of salt stress on nutrient concentration, photosynthetic pigment, proline and foliar morphology of Salvinia auriculata Aubl. Acta Limnologica Brasiliensia, **23(2**): 164-176.
66. Izzo, R., Incerti, A. and Bertolla, C. (2008): Seawater irrigation: Effects on growth and nutrient uptake of sun flower plants. Biosaline Agriculture and High Salinity Tolerance, 61-69.
67. Ashraf, M. and Ali, Q. (2008): Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (Brassica napus L.). Environmental and Experimental Botany, **63:** 266–273.
68. Mulry, K.R., Hanson, B.A. and Dudle, D.A. (2015): Alternative strategies in response to saline stress in two varieties of P. oleracea (purslane) PloS ONE, **10 (9**).
69. Abraham, E., Rigo, G., Szekely, G., Nagy, R., Koncz, C. and Szabados, L. (2003): Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in Arabidopsis. Plant Mol. Biol, **51**: 363-3