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Seasonal behavior of chlorophyll in some shrubs grown under mesic and xeric habitat conditions

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ABSTRACT

At two distinctive habitats, the chlorophyll contents (Chls.), its thermo-stability (CSI), and some metabolic substances in four shrubs were studied. The obtained results revealed that, Chl. contents, Chl. *a/b* ratio and CSI were variables among the investigated species in response to seasonality, habitat, species and their interactions. Generally, low Chl. contents were accompanied by an increase in CSI. Statistically, single factors and their interactions had significant effects on the investigated constituents of plants with some exceptions. Seasonality greatly affected Chl. *b*, Chl. *a/b* ratio and total chlorophylls. The species factor had a major role in the Chl. *a* and CSI. The same was true in the case of the rest constituents in plant tissues, whereas the habitat factor had a dominant role in Mg^{+2}. The results showed that the *Calotropis procera* was the highest plant chlorophyll stability to heat and the lowest content of chlorophyll, and vice versa was reported in the case of *Olea europaea* plant. The correlations between CSI and free amino acids were significantly positive. Also, Chl. contents were positively correlated with soluble sugars. Within species, the specific correlations between different chlorophyll parameters and other components in plant tissues were discussed.

INTRODUCTION

Chlorophyll was the basic compound of productivity and development of “natural ecosystems”, through food chains and “energy pyramids” characteristics of the system. Accordingly, its response to environmental aridity was quite basic for the life of living organisms. Mostly, anthropogenic activities were recently participating in global climatic changes. The climatic changes greatly caused disturbance in the biological processes of plants, particularly in the photosynthetic system, ultimately on the productivity and yields of different plants. Under the climatic changes such as high temperature, atmospheric aridity and high light intensities the Chl. efficiency decreases and cause constant suppression of CO_2 assimilation rate [1]. Therefore, the chlorophyll content and its thermo-stability were greatly used parameters as an indicator of photosynthetic efficiency in most plants insert under drastic natural conditions.

The usefulness of leaf chlorophyll as an economic alternative for screening large numbers of plants for water use efficiency, together with other traits, to predict yield under moderate and severe water deficit conditions [2]. The arid climate affects the synthesis of essential plant pigments, such as chlorophyll *a* and chlorophyll *b*. Therefore, the abundance of solar radiation and water scarcity in arid environments selects for plants with reduced Chl. content in the photosynthetic reaction [3]; [4]. Consequently, the adaptation of plants in their natural habitats to enduring the drastic conditions contributed to decreased Chl. *a/b* ratio balanced by increased chlorophyll thermo-stability [5]. Hence, the strategies of plants against Chl. destruction depend on the alternation of Chl. stability to heat between Chl. *a* and Chl. *b*. Furthermore, the increased chlorophyll stability index and/or Chl. accumulation was indicative of a high efficiency of the photosynthetic system under extreme conditions [6]. Also, the adaptation of plants to heat stress induced the accumulation of metabolites as compatible solutes in chloroplasts which were related enhance thermo-stability [7]. Consequently, plant selection for drought tolerance should be made using drought tolerance indices based on chlorophyll, yields under both conditions for widely adapted genotypes [8]. However, the chlorophyll content was very sensitive to the climatic change all over the year and the chlorophyll stability to heat was mainly dependent on the species genotype and habitat conditions, especially in hot desert areas.

Accordingly, the present work, aimed to studying the effects of seasonality, species, habitat type and their interactions on the chlorophyll content and its stability to heat stress in four shrubs. The study also was extended to evaluate the relationships between different chlorophyll parameters with free amino acids and soluble sugars, as well as the Mg^{+2} content in the investigated species. Statistical analysis of the data was carried out to evaluate the effects of the experimental factors on the different measured parameters of these plants.

The studied area:-

The Nile Valley in the Assiut area is bounded from the east and the west by the Eocene Limestone plateau and is located between latitudes 26° 50′ and 27° 40′ N and longitudes 30° 40′ and 31° 32′ E. The study area (Botany & Microbiology Farm, as a mesic habitat) is a part of the Nile Valley and lies within the stable shelf of Egypt where the major surface features are a reflection of basement lines and the main fault systems are NW, NE and N–S. It is composed mainly of coarse sand and sandy loam mixed with limestone, marble and gravel [9]. According to Assiut Meteorological Station, the average values of temperature ranged between 15.4-33°C, irradiance 20.8-29.6 MJ/M^2/day, relative humidity 58-25% and wind speed 3.7- 5.1 m/s during the cold and hot season, respectively.

Wadi El-Assiuty (as a xeric habitat) is one of the most notable features of the Egyptian Eastern Desert. The climate in Wadi El-Assiuty is extremely arid. Commonly, it is characterized by the scantiness of rainfall for many years and it amounts to less than 15 mm/year between November and March. The mean temperature ranged between 15° and 35 °C in both winter and summer, respectively. The sand and gravel of the Pleistocene age cover the surface of the old alluvial plains bordering the valley which are subject to the new reclamation activities [10].

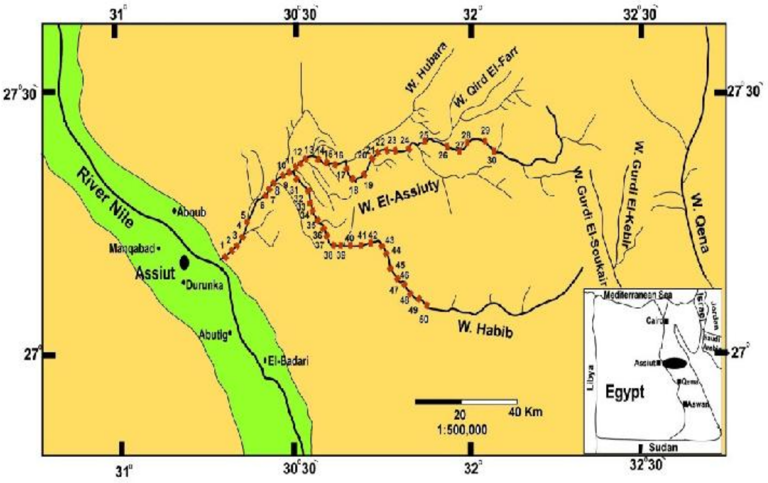

Figure (1): Location map of the studied areas.

MATERIALS AND METHODS

This study focused on native and cultivated species inhabited soils of Wadi El-Assiuty Reserve, which represents a xeric habitat, in the Egyptian eastern desert and Botany & Microbiology Farm – Faculty of Science – Assiut University, which represented a mesic habitat in the western side of Nile valley region. The plants were sampled four times: in August (summer), in November (autumn), in February (winter), and in May (spring) to cover seasonal changes in tested parameters as a result of climatic conditions.

Collection of plant samples:-

Studied species were four shrubs namely: *Olea europaea* L., *Calotropis procera* (Ait.) Ait., *Dodonaea viscosa* Jacq and *Psidium guajava* L.. Samples of two native species representing the xeric type [*Calotropis procera* (F: Asclepiadaceae) and *Dodonaea viscosa* (F: Sapindaceae)] and two cultivated species representing the mesic habitat [*Olea europaea* (F: Oleaceae) and *Psidium guajava* (F: Myrtaceae)] were collected from the inhabited area once encountered. The studied native species were identified according to Täckholm [11], Boulos [12] and El-Hadidi and Fayed [13]. Also, the cultivated species were identified according to Soliman and Amer [14].

Determination of dry matter:-

The collected samples (branches bearing leaves) were transferred immediately through tightly closed plastic containers from their habitats to the laboratory. Samples of leaves were washed with distilled water and thoroughly dried on filter paper. Three samples were chosen at random from each species, oven-dried at 70 °C for 24 hours, and reweighted to calculate dry matter as following:

$$\text{Dry matter (\%)} = \frac{\text{Dry weight of plant}}{\text{Fresh weight of plant}} \times 100$$

Relative water content (Relative Turgidity) measurement:-

Relative water content (RWC) using the method of Weatherly and Barrs [15] was adopted to correct mainly for continued water uptake by leaf tissue after attaining full turgidity due to growth. Leaf cuts (one to two centimeters in length) were immediately floated in distilled water, at a low temperature (5 °C) and dark or under low light intensity. Saturation of tissue cuts was attained in 24 to 36 hours. Leaf cuts were then rapidly and thoroughly blotted dry, weighed immediately (turgid weight), oven dried at 70 °C for 24 hours, and reweighed (dry weight), for fresh weight determination. The RWC of leaves was expressed as a percentage using the equation:

$$\text{RWC (\%)} = \frac{\text{Fresh wt. of leaf cuts} - \text{oven dry wt. of leaf cuts}}{\text{Saturation wt. of leaf cuts} - \text{oven dry wt. of leaf cuts}} \times 100$$

Determination of chlorophyll content:-

Chlorophylls (*a* and *b*) were extracted from of fresh, healthy, unblemished leaf material (0.1 g in10 ml 85% acetone). The extract was centrifuged at 7000 r.p.m. for a few minutes to get rid of coarse material. Chlorophyll *a* and chlorophyll *b* were measured spectrophotometrically at 664 and 648 nm, respectively. The chlorophyll (*a* and *b*), total chlorophyll content (Chl. *a* + Chl. *b*) and chlorophyll *a/b* ratio were calculated according to Lichtenthaler [16].

Chlorophyll stability index (CSI):-

Another 0.1 g of leaf tissue was placed in 20 ml of distilled water and heated in a water bath at $56 \pm 1^{\circ}$C for 30 minutes. The sample was extracted in the same way [16]. According to Murty and Majumder [17], the CSI was calculated as the ratio of chlorophyll content in heated leaf ($56 \pm 1^{\circ}$C) to that in fresh leaf, (expressed as %) as following:-

$$\text{CSI } a = \frac{\text{Content of chlorophyll(a) in heated sample}}{\text{Content of chlorophyll(a) in fresh sample}} \text{ X } 100$$

$$\text{CSI } b = \frac{\text{Content of chlorophyll(b) in heated sample}}{\text{Content of chlorophyll(b) in fresh sample}} \text{ X } 100$$

Differences between fresh and heated *a/b* ratios were calculated in order to evaluate the destruction of Chl. *a* or Chl. *b* according to Farghali [3] as the following equation:-

Specific thermo-stability of Chl. *a* or *b* = $\text{Fresh } a/b - \text{heated } a/b =$ -ve or +ve value

Analysis of plant extracts:-

A known weight of fresh leaf sample was quickly blended with 10 cm³ of ice-cold distilled water, and the supernatant was deep frozen until analysis of metabolites and Mg^{+2} ions.

Determination of water soluble metabolites and magnesium (Mg^{+2}):-

1. Soluble sugars were determined according to Dubois *et al*. [18].
2. Total free amino acids were determined according to procedures described by Lee and Takahashi [19].
3. Magnesium was determined by using an Atomic Absorption Spectrophotometer according to Stewart [20].

Statistical evaluation of experimental data:-

The effects of single factors (Season, habitat and species) and their interactions on the contents of chlorophyll, Mg^{+2}, and metabolites parameters in different species were evaluated statistically by the factorial analysis (two-way method) of variance (F test). The relative roles of every single factor and their interactions in the total response were determined by using the coefficient of determination (Sharing %) to indicate the degree of control of each factor on the tested parameter [21]; [22]. A simple linear correlation coefficient (r) between all parameters for each plant species separately and the correlation between chlorophyll parameters and Mg^{+2}, soluble sugars and free amino acids were calculated by using SPSS program [23]**.**

RESULTS

1- Relative water content (RWC):-

The seasonal changes of RWC (%) in different shrub species at both mesic and xeric habitats were shown in figure (2). It was quite clear that the highest RWC (87.79 and 90.47%) was detected in *P. guajava* at xeric and mesic habitats respectively. The RWC in *O. europaea* was the lowest (34.52% and 34.88%), among the investigated species, particularly in the autumn and summer seasons at xeric habitat. In general, the RWC in all plants was higher at mesic habitat than that at xeric habitat. This means that, the availability of soil water conservation leads to high RWC in species grown under mesic habitats particularly in the autumn season. In both *C. procera* and *D. viscosa* the RWC was moderate and ranged between 56.35 and 74.36%. Statistically, the single factors of season, habitat, species and their interactions had highly significant effect on the RWC of different species. While the species factor had a dominant effect on RWC (Sharing 55.8%), the interaction (Season × habitat) had a secondary role (Sharing 10.45%, table 1).

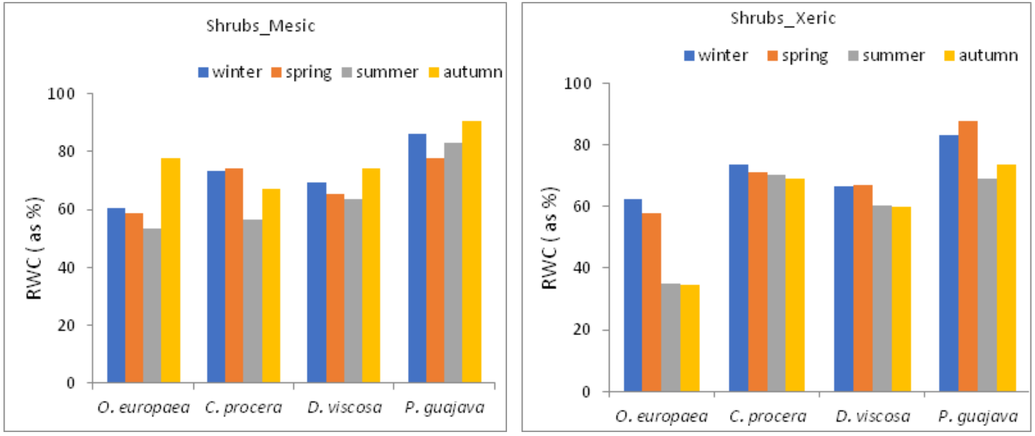

Figure (2): The average values of seasonal percentage (%) of the relative water content (RWC) in investigated shrub plants at mesic and xeric habitats.

2- The dry matter (as %):-

Figure (3) showed the dry matter of testing plants as a percentage of the fresh weight. Apparently, the dry matter of *C. procera* was the lowest (13.45 and 17.94%) among the experimental shrubs at both mesic and xeric habitats, respectively. While the building up materials in *O. europaea* had the highest values (49.94% and 68.31%) in the winter and summer seasons at mesic and xeric habitats, respectively. In both *D. viscosa* and *P. guajava* the dry matter % produced moderate values at different seasons at both habitats. Statistical analysis (F values) indicated that, all single factors and their interactions had highly significant effect on the dry matter of various species. It was noticed that the species factor had the predominant role in dry matter accumulation (Sharing 82.96%, table1).

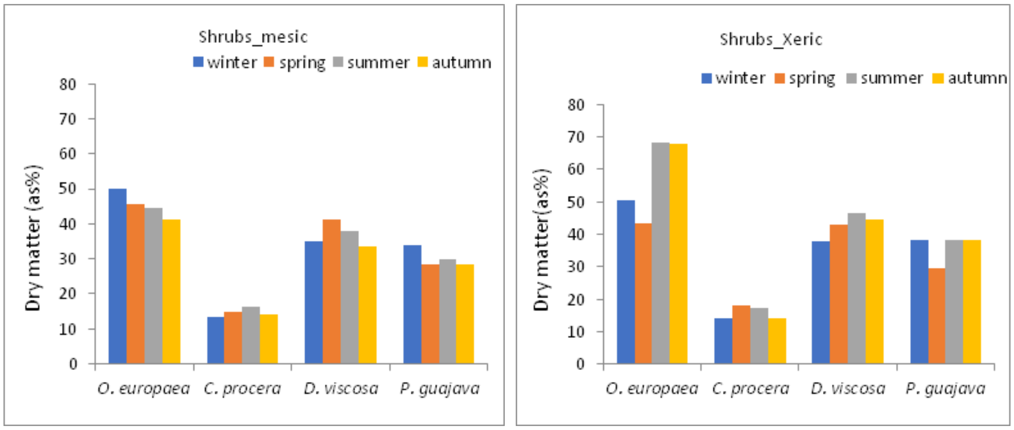

Figure (3): The average values of seasonal percentage (%) of the dry matter of investigated shrub plants at mesic and xeric habitats.

Table (1): ANOVA test showed the effects of the season (S.), habitat (H.), species (Sp.) and their interactions on RWC (%) and dry matter (%) of investigated shrub palnts at mesic and xeric habitats.

Parameters	**df**	**RWC**		**Dry matter**	
Source of variance		F	Sharing %	F	Sharing %
S.	3	52.56**	9.95	19.46**	1.25
H.	1	88.14**	5.56	229.01**	4.9
Sp.	3	294.77**	**55.8**	1291.78**	**82.96**
S. × H.	3	55.19**	**10.45**	45.39**	2.91
S. × Sp.	9	4.08**	2.32	15.62**	3.01
H. × Sp.	3	31.56**	5.97	28.39**	1.82
S. × H. × Sp.	9	17.51**	9.94	16.32**	3.14

**Significant at 0.01 level   * Significant at 0.05 level

3- Chlorophyll parameters:-

3.1- Chlorophyll contents (Chl.):-

The chlorophyll *a*, chlorophyll *b* and the total chl. contents were illustrated in figure (4). Apparently, Chl. *a* content was high in plants under xeric and mesic habitats during the winter and spring seasons. At xeric habitat, the Chl. *a* content in shrubs ranged between 0.72 mg g^{-1} to 1.92 mg g^{-1} leaf f. wt., While the Chl. *a* content in shrubs grown under mesic habitat conditions ranged between 0.94 mg g^{-1} and 1.99 mg g^{-1} leaf f. wt. Commonly, the highest Chl. *a* content existed in *D. viscosa*, particularly from the spring to autumn at mesic habitat. The lowest Chl. *a* content was reported in *C. procera* followed by *O. europaea* under xeric habitat conditions. Chl. *b* content in all tested species was lower than Chl. *a* content at different seasons at xeric and mesic habitats. At both habitats, the maximum Chl. *b* content (1.57 mg g^{-1} leaf f. wt.) was showed in *O. europaea*. The lowest Chl. *b* values were detected in *C. procera* (0.3 mg g^{-1} leaf f. wt.). Also, the highest Chl. *b* content in all investigated shrubs was observed at spring season, whereas the low Chl. *b* content was found during the winter and autumn seasons. Apparently, at the xeric habitat, the summation of chlorophyll content in all plants ranges between 1.15 mg g^{-1} leaf f. wt. in *C. procera* and *O. europaea* in the summer & autumn respectively and 3.41 mg g^{-1} leaf f. wt. in *O. europaea* during the spring season. Generally, the high total Chls. was observed in plants under mesic habitat, particularly in *O. europaea* followed by *D. viscosa*.

The F values (Table 2) indicated that, all single factors and their interactions had highly significant effect on the Chl. *a*, Chl. *b* and total chlorophyll contents. The seasonality factor played the major role on the Chl. *b* (Sharing 40.61%), whereas plant species factor had the same role on the Chl. *a* and total chlorophyll content (Sharing 22.97 and 24.44%, respectively), and played a secondary effect on the Chl. *b* (Sharing 26.28% in all plants).

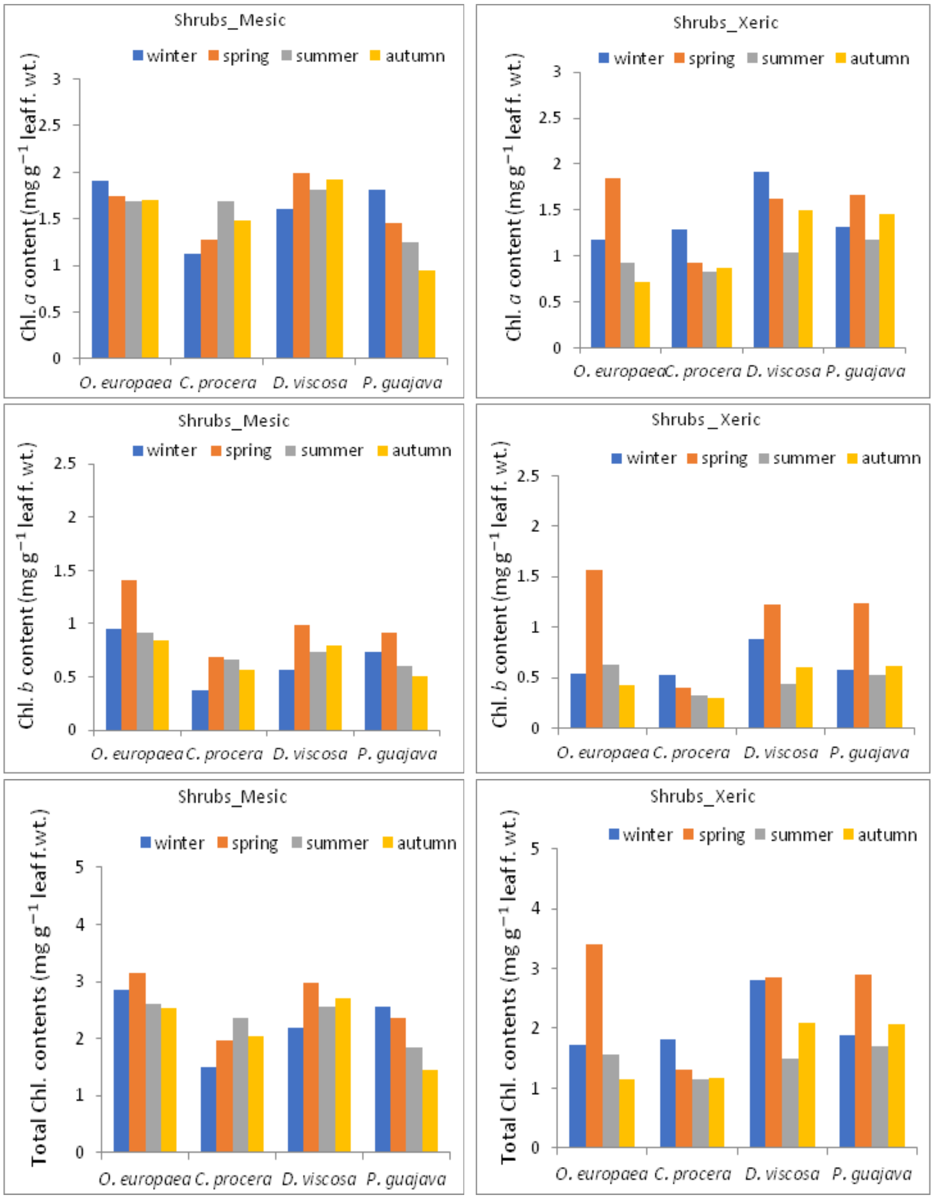

Figure (4): The average values of Chl. *a*, Chl. *b* and total chlorophyll contents (mg g^{-1} leaf f. wt.) of investigated shrub plants in different seasons at mesic and xeric habitats.

3.2- The Chl. *a/b* ratio:-

The Chl. *a/b* ratio in shrubs investigated in different seasons at both xeric & mesic habitats was shown in figure (5). Cleary, at both habitats, the Chl. *a/b* ratio in different species was reached high values during the cold season (winter), while the low values were existed under mild temperature season (spring). At mesic habitat a maximum ratio was achieved in *C. procera* (2.98) followed by *D. viscosa* (2.9) during the winter. Under xeric habitat, also *C. procera* attained the highest Chl. *a/b* ratio (2.88) in the autumn, whereas the ratio in the rest species was high at the same season. Only, the *C. procera* gained high Chl. *a/b* ratio at all seasons at both habitats. It seemed that, the lowest Chl *a/b* ratio was detected in *O. europaea* (1.18 and 1.26) during the spring season at both xeric and mesic habitats respectively. ANOVA data (Table 2) indicated that all single factors and their interactions had highly significant effect on Chl. *a/b* ratio except for the (Season × species) interaction. Single factors season and species had the dominant and sub dominant role on the Chl. *a/b* ratio (Sharing 42.8 and 41.09%, respectively).

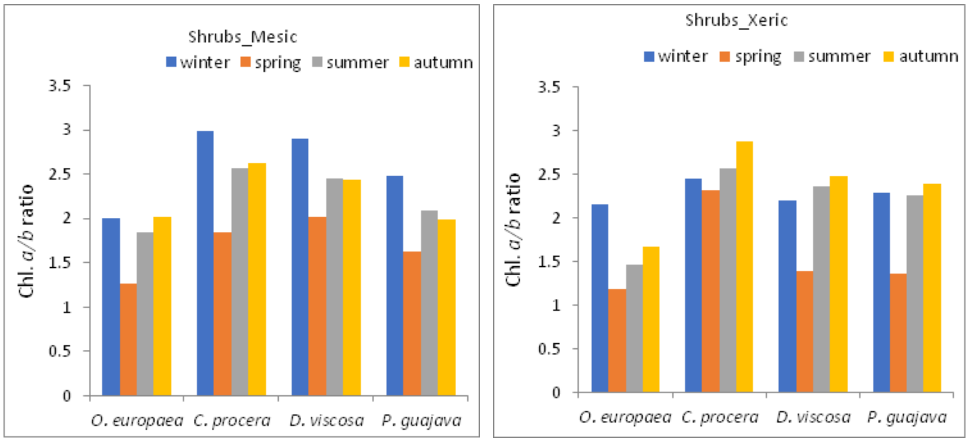

Figure (5): The average values of Chl. *a/b* ratio of investigated shrub plants in different seasons at mesic and xeric habitats.

Table(2): ANOVA test showed the effects of the season (S), habitat (H.), species (Sp.) and their interactions on Chl. *a*, Chl. *b* , total Chl. contents and Chl. *a/b* ratio of investigated shrub plants at mesic and xeric habitats.

Parameters	**df**	**Chl. *a* content**		**Chl. *b* content**		**Total Chl. contents**		**Chl. *a/b* ratio**	
Source of variance		F	Sharing%	F	Sharing%	F	Sharing%	F	Sharing%
S.	3	17.42**	9.93	83.12**	**40.61**	42.66**	21.89	90.08**	**42.8**
H.	1	101.58**	19.3	13.69**	2.23	64.48**	11.03	8.41**	1.33
Sp.	3	40.30**	**22.97**	53.81**	**26.28**	47.64**	**24.44**	86.48**	**41.09**
S. × H.	3	12.69**	7.23	10.75**	5.25	13.87**	7.12	5.06**	2.41
S. × Sp.	9	5.87**	10.03	9.16**	13.43	8.17**	12.58	1.11	1.58
H. × Sp.	3	17.21**	9.81	8.42**	4.12	14.81**	7.6	5.96**	2.83
S. × H. × Sp.	9	12.12**	20.73	5.51**	8.08	9.97**	15.35	5.59**	7.96

**Significant at 0.01 level   * Significant at 0.05 level

Differences between *a/b* ratios:-

Chl. *a* and Chl. *b* were greatly dependent on the Chl. thermo-stability of the studied species (Table 3). The differences between fresh and heated Chl. *a/b* ratios markedly increased in heated leaves (negative value) of *C. procera* during the spring and summer seasons and *O. europaea* during the spring at xeric habitat conditions due to destruction of Chl. *b* molecules. Adversely, the decreased ratio in heated leaves (positive value) in the rest species at different seasons at both habitats was attributed to the destruction of Chl. *a*. However, a similar Chl. *a/b* ratios between fresh and heated leaves of *C. procera* and *P. guajava* in the spring and autumn seasons under mesic habitat emphasized the high thermo-stability of Chl. *a* and Chl. *b* to heat. Also, at xeric habitat, the same results were obtained in *D. viscosa* during the winter and autumn seasons as well as in *C. procera* during the summer season.

Table (3): Differences between fresh and heated Chl. *a/b* ratios of investigated shrub plants at mesic and xeric habitats.

Season	**Winter**		**Spring**		**Summer**		**Autumn**	
Species	mesic	xeric	mesic	xeric	mesic	xeric	mesic	xeric
O. europaea	0.50	0.80	0.31	**-0.56**	0.13	0.23	0.74	0.29
C. procera	0.61	0.36	**0.06**	**-0.25**	0.11	**-0.07**	0.24	0.33
D. viscosa	0.77	**0.01**	0.37	0.26	0.22	0.28	0.59	**0.05**
P. guajava	0.14	0.55	0.49	0.11	0.42	0.47	**0.02**	0.74

3.3- Chlorophyll stability index (CSI):-

The thermo-stability of Chl. *a*, Chl. *b* and total chlorophylls in the experimental shrubs were shown in figure (6). At xeric habitat, the CSI *a* produced highest value during the spring season in *C. procera* (97.67%). While a minimum CSI *a* value (45.21%) was observed in *O. europaea* at summer. At mesic habitat, the summer season had a boosting effect on the CSI *a* and tended to be highest (86.81%) in *C. procera*, while the lowest CSI *a* (23.67%) was detected in *O. europaea* in the autumn. Commonly, the CSI *a* of species under xeric habitat conditions exerted a higher value than that at mesic habitat. Only, *P. guajava* had exerted the highest value in winter (75.2%).

The stability index of Chl. *b* was higher than that of Chl. *a* in all studied shrubs. Consequently, at xeric habitat, the CSI *b* values ranged between 52.55% (*O. europaea* during the summer) and 96.81-96.98% (In both *C. procera* at autumn and *D.viscosa* at spring, respectively). At mesic habitat, the lowest CSI *b* was noticed in *O. europaea* (36.7%) during the autumn, whereas the highest CSI *b* value (90.83 and 91.2%) in *C. procera* during the summer and winter seasons, respectively.

The thermo-stability of total chlorophylls (*a* + *b*) exerted high values in *C. procera* particularly under xeric habitat conditions in the spring season. The decreased total CSI was reported in *O. europaea* during the autumn season at mesic habitat. Also, at mesic habitat, the total CSI in all plants was increased under hot season. At xeric habitat the same increasing effect was true at spring season, and at autumn in (*C. procera*). In general, the total chlorophyll stability in studied shrubs was higher at xeric habitat than those at mesic habitat.

The F values (Table 4) indicated that, season, species factors and their interactions had a significant effects on CSI *a* (Except for season x species). The species factor, bi-(Season × habitat) and tri-factorial interactions had a significant effect on CSI *b*. While, the season, habitat, species factors and (Season × habitat) interactions had significant effect on total CSI of investigated species. The species factor played the dominant role on CSI *a*, CSI *b* and total CSI (Sharing 37.7, 42.53 and 45.39%, respectively). Whereas, (Season × habitat) had the secondary role on CSI *a* (Sharing 14.45%), the tri-factorial interaction had the secondary role on CSI *b* and total CSI (Sharing 25.59 and 14.52% respectively).

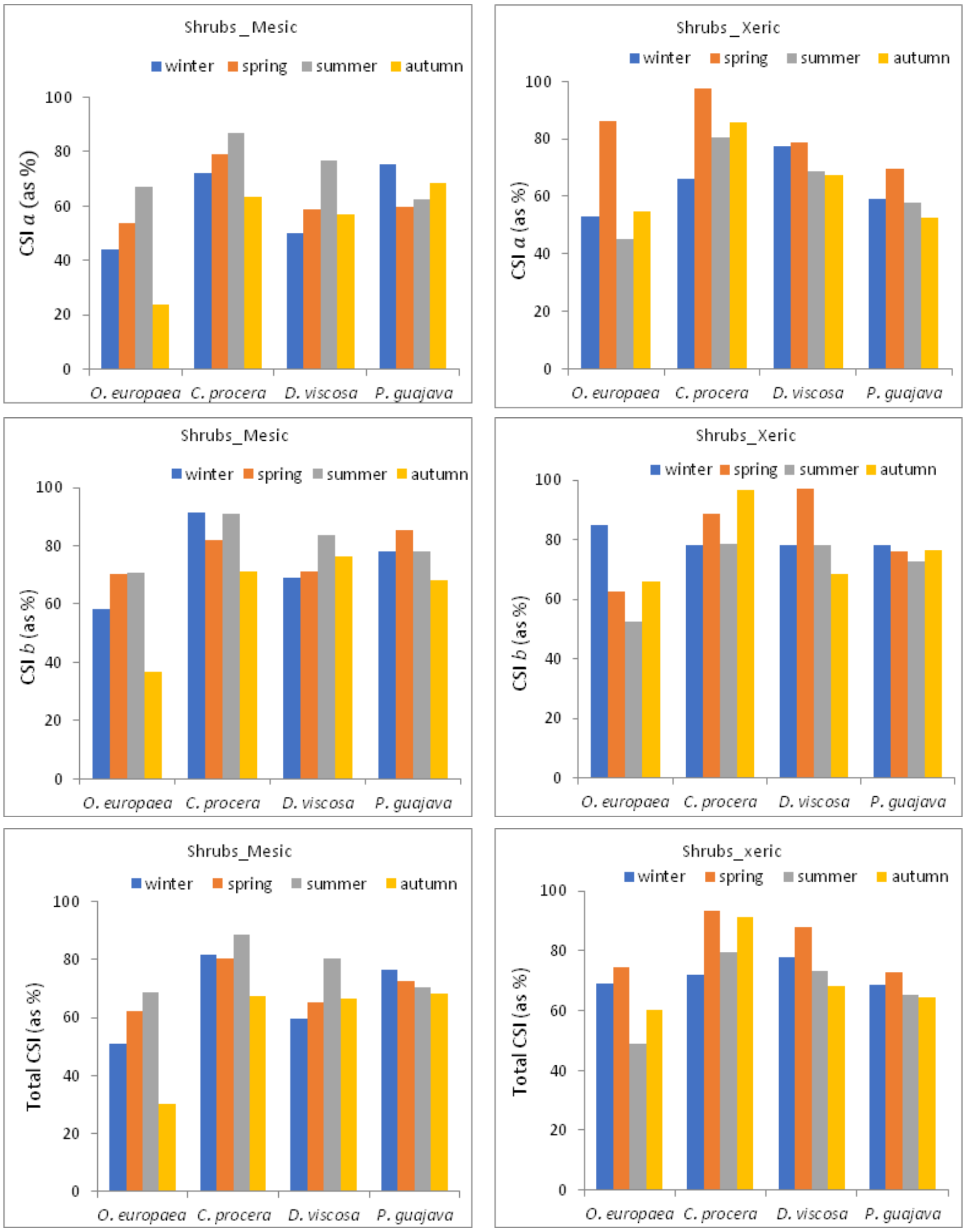

Figure (6): The average values of CSI *a*, CSI *b* and total CSI (%) of investigated shrub plants in different seasons at mesic and xeric habitats.

Table (4): ANOVA test showed the effects of the season (S.), habitat (H.), species (Sp.) and their interactions on CSI *a*, CSI *b* and total CSI of investigated shrubs at mesic and xeric habitats.

Parameters	**df**	**CSI *a***		**CSI *b***		**Total CSI**	
Source of variance		F	Sharing%	F	Sharing%	F	Sharing%
S.	3	6.44**	12.9	2.3	7.71	4.32**	10.96
H.	1	6.88*	4.59	1.6	1.79	4.37*	3.69
Sp.	3	18.81**	**37.7**	12.66**	**42.53**	17.89**	**45.39**
S. × H.	3	7.21**	**14.45**	3.77*	12.68	5.44**	13.8
S. × Sp.	9	1.78	10.69	0.75	7.59	0.86	6.57
H. × Sp.	3	3.52*	7.05	0.63	2.12	2	5.08
S. × H. × Sp.	9	2.10*	12.6	2.54*	**25.59**	1.91	**14.52**

**Significant at 0.01 level * Significant at 0.05 level

4- Metabolic compounds:-

4.1- Total soluble sugars (SS):-

The total soluble sugar contents in the different species were illustrated in figure (7). Among the investigated species, the *O. europaea* had the highest SS content (34.84 mg g^{-1} leaf f. wt.) followed by *D. viscosa*. The lowest SS content was detected in *C. procera* (2.14 and 2.5 mg g^{-1} leaf f.wt.) in the winter and summer seasons at xeric habitat, respectively and (3.02 mg g^{-1} leaf f. wt.) during summer at mesic habitat. Commonly, the highest SS content was observed during the mild seasons, whereas the hot season caused decrease in SS content at mesic habitat.

The single factors and their interactions (Table5) had highly significant effect on the SS contents in various species. The predominant role on SS content was exerted by the species factor (Sharing 76.81%) and the interaction (Season × species) had a secondary role (Sharing 9.09%).

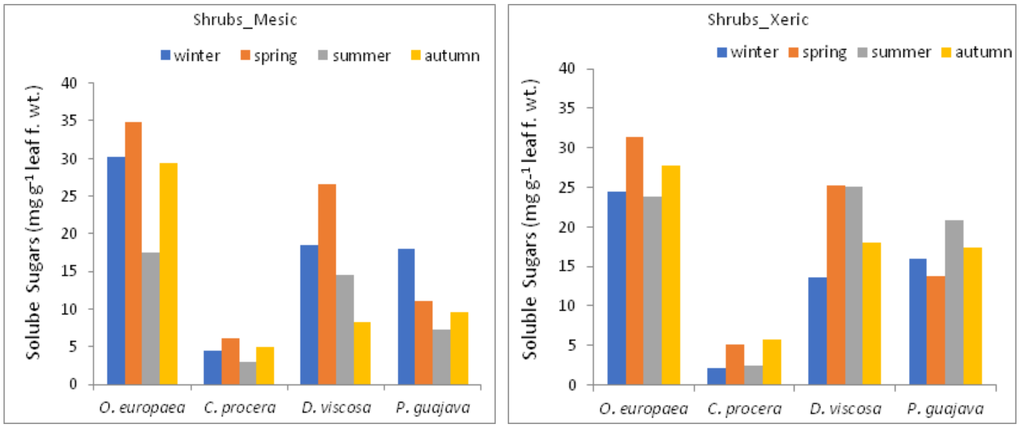

Figure (7): The average values of soluble sugar contents (mg g^{-1} leaf f. wt.) of investigated shrub plants in different seasons at mesic and xeric habitats.

4.2- Free amino acids (FAA):-

The free amino acids in different investigated species were illustrated in figure (8). In the spring season, the free amino acids in different species attained the highest values at mesic habitat. Only, *D. viscosa* had the highest value of FAA at the same season at xeric habitat. The maximum FAA contents (3.36 and 3.39 mg g^{-1} leaf f. wt.) were gained by both *D. viscosa* and *C. procera* at xeric and mesic habitats, respectively. However the *O. europaea* had lowest values (0.04-1.12 mg g^{-1} leaf f. wt.) during the autumn and winter seasons under xeric and mesic habitat conditions, respectively.

F test (Table5) pointed to highly significant effect of season, habitat, species factors and their interactions on the FAA contents in plants. The FAA contents depended mainly on the species factor and the seasonality had the subdominant role (Sharing 45.43% and 17.68%, respectively).

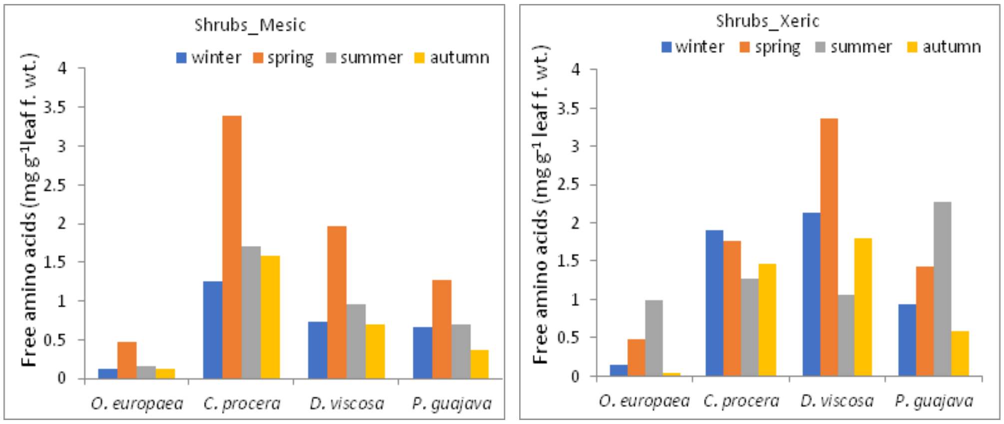

Figure (8): The average values of free amino acid contents (mg g^{-1} leaf f. wt.) of investigated shrub plants in different seasons at mesic and xeric habitats.

5- Magnesium (Mg^{+2}) content:-

The contents of Mg^{+2} in different experimental species were shown in (Figure 9). Mg^{+2} contents in different species were high at xeric habitat, particularly in *D. viscosa* (4.94 mg g^{-1} leaf f. wt.) followed by *P. guajava* (4.57 mg g^{-1} leaf f. wt.) during summer season. At mesic habitat, the high Mg^{+2} contents were observed in *D. viscosa* (3.42 mg g^{-1} leaf f. wt.) followed by *C. procera* (1.85 mg g^{-1} leaf f. wt.) as well as *P. guajava* (1.45 mg g^{-1} leaf f. wt.) in winter season. Commonly, the lowest values of Mg^{+2} contents were reported in *O. europaea* (0.13 mg g^{-1} leaf f. wt.) and *P. guajava* (0.17 mg g^{-1} leaf f. wt.) during the spring season at mesic and xeric habitats respectively. The effect of single factors (S., H. and Sp.) and their interactions was highly significant on the Mg^{+2} contents of all tested species (Table5). The trifactorial interaction (S. × H. × Sp.) had dominant role (Sharing 29.15%) in the Mg^{+2} contents and the habitat factor role (Sharing 17.28%) was subsidiary.

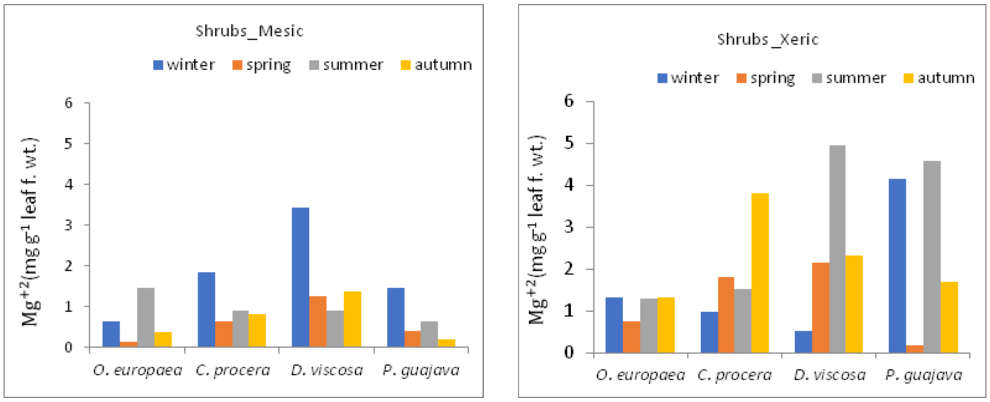

Figure (9): The average values of Mg^{+2} contents (mg g^{-1} leaf f. wt.) of investigated shrub plants in different seasons at mesic and xeric habitats.

Table (5): ANOVA test showed the effects of the season (S.), habitat (H.), species (Sp.) and their interactions on soluble sugars, free amino acids and Mg^{+2} contents of investigated shrub plants at mesic and xeric habitats.

Parameters	**df**	**Soluble sugars**		**Free amino acids**		**Mg^{+2}**	
Source of variance		F	Sharing%	F	Sharing%	F	Sharing%
S.	3	28.56**	3.97	344.98**	**17.68**	136.92**	10.68
H.	1	19.88*	0.92	237.18**	4.05	665.06**	**17.28**
Sp.	3	552.97**	**76.81**	886.41**	**45.43**	146.61**	11.43
S. × H.	3	37.48**	5.21	37.39**	1.92	145.18**	11.32
S. × Sp.	9	21.83**	**9.09**	73.59**	11.32	65.60**	15.34
H. × Sp.	3	15.83**	2.2	176.26**	9.03	61.48**	4.79
S. × H × Sp.	9	4.33**	1.8	68.76**	10.57	124.64**	**29.15**

**Significant at 0.01 level * Significant at 0.05 level

Correlation coefficient (r.) between total chlorophyll contnets and its total thermo-stability (CSI) with different constituents:-

1- RWC and dry matter:-

The total Chl. contents had significant negative correlation with total CSI under habitat factor and its interaction with species (Table 6). The RWC was positively correlated with the total chlorophyll content under the habitat factor. In contrast, the total Chl. contents had a significant negative correlation with the dry matter in shrubs under the effect of habitat type and its interaction with season. Also, the RWC had highly significant negative correlation with total CSI under the habitat factor. While highly significant positive correlation was observed between the total CSI and the dry matter under the habitat effect. While the total CSI had a significant negative correlation with the dry matter under the effect of different investigated factors with some exceptions.

Table (6): Correlation coefficient (r) between total Chl. contents and total CSI with RWC and dry matter in shrubs studied at mesic and xeric habitats.

Parameters		**Total Chl. contents**			**Total CSI**	
Source of variance	df	Total CSI	RWC	Dry matter	RWC	Dry matter
S.	3	0.764	0.542	-0.846	-0.064	-0.314
H.	1	**-1.000****	**1.000****	**-1.000****	**-1.000****	**1.000****
Sp.	3	-0.736	-0.427	0.918	0.516	**-0.941***
S. × H.	7	0.367	0.647	**-0.834***	-0.264	0.068
S. × Sp.	15	-0.145	-0.139	0.467	0.399	**-0.807****
H. × Sp.	7	**-0.742***	-0.123	0.53	0.328	**-0.750***
S. × H. × Sp.	31	-0.171	0.052	0.197	0.175	**-0.574****

**Significant at 0.01 level *Significant at0.05 level

2- RWC and dry matter in different specific species:-

The total Chl. contents had significant positive correlation with the RWC in *O. europaea* and *C. procera* at mesic habitat only, but its total thermo-stability had no significance in various species (Table 7). Adversely, the dry matter content was independent of the total Chls. in all investigated species, whereas the total CSI was negatively correlated with the dry matter in both *O. europaea* (At mesic & xeric habitats) and *P. guajava* under mesic habitat conditions.

Table (7): Correlation coefficient (r) between total Chl. contents and total CSI with RWC (%) and dry matter (%) in different shrubs studied at mesic and xeric habitats.

	Total Chl. contents				**Total CSI**			
Parameters	RWC		Dry matter		RWC		Dry matter	
Species	mesic	xeric	mesic	xeric	mesic	Xeric	mesic	xeric
O. europaea	**0.969***	0.726	-0.038	-0.857	0.066	0.403	**-0.975***	**-0.990****
C. procera	**0.960***	-0.784	-0.092	-0.651	-0.41	0.842	-0.698	0.929
D. viscosa	0.451	0.935	0.853	-0.949	-0.253	-0.289	-0.724	0.326
P. guajava	-0.941	0.526	0.121	0.467	-0.28	-0.263	**-0.998****	-0.328

**Significant at 0.01 level *Significant at 0.05 level

3- Different metabolites and Mg^{+2}:-

It was noticed that, the correlation between total Chl. contents with different metabolites was significantly negative under the habitat factor (Table 8). Adversely, the relationships between total Chl. contents and SS were significantly positive under the season factor, bi-(Season × species) and tri-factorial (Season × habitat × species) interactions. On the other hand, the total CSI was positively correlated with the free amino acids under the different single factors and their interactions (Except for season × habitat interaction). Also, the relationships between total CSI with soluble sugars were significantly positive as the habitat change and negative in response to change in species factor, (Season × species), (Habitat × species) and tri-factorial interactions.

The relationships between total Chl. and Mg^{+2} contents were significantly negative under the habitat, bi-factorial (Season × habitat) and tri-factorial interactions. Only, the total CSI was positively correlated with Mg^{+2} ions under the habitat factor (Table 8).

Table(8): Correlation coefficient (r) between total Chl. contents and total CSI with different metabolites (SS and FAA) and Mg^{+2} in shrubs studied at mesic and xeric habitats.

Parameters		**Total Chl. contents**			**Total CSI**		
Source of variance	df	Soluble sugars	Free Amino acids	Mg^{+2}	Soluble sugars	Free amino acids	Mg^{+2}
S.	3	**0.983***	0.887	-0.811	0.675	**0.923***	-0.391
H.	1	**-1.000****	**-1.000****	**-1.000****	**1.000****	**1.000****	**1.000****
Sp.	3	0.89	-0.507	0.053	**-0.952***	**0.955***	0.603
S. × H.	7	0.029	0.283	**-0.854****	0.111	0.511	-0.057
S. × Sp.	15	**0.663****	-0.055	-0.278	**-0.663****	**0.761****	0.254
H. × Sp.	7	0.672	-0.378	-0.201	**-0.859****	**0.842****	0.395
S. × H. × Sp.	31	**0.446***	-0.011	**-0.419***	**-0.570****	**0.524****	0.158

**Significant at 0.01 level *Significant at 0.05 level

4- Different metabolites and Mg^{+2} in different specific species:-

There was significant positive & negative correlation between the total Chl. contents and soluble sugars in *O. europaea* at xeric & mesic habitats, respectively. At xeric habitat the most significant correlations between total Chl. contents and free amino acids in *C. procera* and *P. guajava* were positive, but also there was a negative significant correlation in *O. europaea* at the same habitat. Also, under xeric habitat conditions, there was a significant positive correlation between total CSI and soluble sugars in *P. guajava*. At mesic habitat, the same positive correlation was found between the total CSI (CSI *a* + CSI *b*) and free amino acids in *C. procera* (Table 9 & 10).

It was quite clear that, the total Chl. contents were positively correlated with Mg^{+2} in *D. viscosa* under mesic habitat conditions. Meanwhile, the total CSI was negatively correlated with Mg^{+2} in *D. viscosa* at mesic habitat (Table 9 & 10).

Table (9): Correlation coefficient (r) between total Chl. contents and different metabolites (SS and FAA) & Mg^{+2} in shrubs studied at mesic and xeric habitats.

Total Chl. contents						
Parameters **Species**	Soluble sugars		Free amino acids		Mg^{+2}	
	mesic	xeric	mesic	xeric	mesic	xeric
O. europaea	**-0.952***	**0.992****	-0.597	**-0.986***	0.712	-0.926
C. procera	-0.19	-0.514	-0.502	**1.000****	0.041	-0.723
D. viscosa	0.249	0.874	0.193	-0.871	**0.992****	0.091
P. guajava	0.743	0.791	-0.525	**0.998****	-0.476	0.302

**Significant at 0.01 level   * Significant at 0.05 level

Table (10): Correlation coefficient (r) between total CSI and different metabolites (SS and FAA) & Mg^{+2} in shrubs studied at mesic and xeric habitats.

Total CSI						
Parameters	Soluble sugars		Free amino acids		Mg^{+2}	
Species	mesic	xeric	mesic	xeric	mesic	xeric
O. europaea	-0.124	0.865	-0.679	-0.844	0.559	-0.707
C. procera	0.871	-0.644	**0.984***	-0.296	-0.788	0.888
D. viscosa	-0.039	-0.917	0.018	0.14	**-0.997****	-0.846
P. guajava	0.622	**0.987***	-0.817	0.722	0.907	0.903

**Significant at 0.01 level   * Significant at0.05 level

DISCUSSION

In the presented data, the RWC (%) in all plants was higher at mesic habitat compared to xeric habitat which contributed to soil water availability. Among the investigated species, the high RWC was detected in *P. guajava* at both habitats, whereas the RWC in *O. europaea* was the lowest, particularly in the autumn and summer seasons at xeric habitat. In contrast, the building up materials in *O. europaea* had highest values all over the year (Except in spring) at xeric habitats. While, at both habitats, the dry matter of *C. procera* was the lowest among the experimental shrubs. Cleary, the dry matter production and partitioning was greatly affected by the environmental factors, ultimately the growth and development of plant organs [24]. The statistical analysis (F values) indicated that, all single factors and their interactions had highly significant effect on the RWC and dry matter production of various species. Species factor had a predominant effect on RWC and dry matter content of investigated species. Consequently, the water status in plants had essential effects on the photosynthetic apparatus. Therefore, the total chlorophyll reduction in atmospheric aridity was thought to be related to the decrease in relative water content [25].

The chlorophyll content and CSI were affected by the water status in plants which was corresponded to chlorophyll reduction and high thermo-stability. In most plants, the Chl. *a* content was higher in plants growing at mesic than that under xeric habitat conditions during the winter and spring seasons. Commonly, the highest Chl. *a* content existed in *D. viscosa*, particularly from spring to autumn season at mesic habitat followed by *O. europaea*. The lowest Chl. *a* and Chl. *b* contents were showed in *C. procera.* On the other hand, the Chl. *b* content in all tested species was lower than Chl. *a* content at different seasons at both habitats. Maximum Chl. *b* content was shown in *O. europaea* at both habitats. In general, the highest Chl. *b* content in all investigated shrubs was observed at spring season, whereas the low Chl. *b* content existed at winter and autumn seasons. Accordingly, the highest total Chls. were observed in plants under mesic habitat, particularly in *O. europaea* and followed by *D. viscosa*. The F values indicated that, all single factors and their interactions had highly significant effect on the Chl. *a*, Chl. *b* and total chlorophyll contents. Seasonal factor played a major role on the Chl. *b*, whereas the species factor had the same role on the Chl. *a* and total chlorophylls. This means that, the acclimation potential of photosynthesis to temperature variation greatly varies with the plant species and ecotypes [26].

According to Gogoi and Basumatary [27], the Chl. *a/b* ratio plays an essential role to higher plants to adapt to high irradiance areas and make optimal use of ambient light intensities and quantities. Apparently, the Chl. *a/b* ratio in different species was reached to high values during cold season (winter), while the low values were existed during/within mild temperature season (spring). Regardless of habitats, *C. procera* had the highest Chl. *a/b* ratio at all seasons followed by *D. viscosa* during the winter. The lowest Chl *a/b* ratio was detected in *O. europaea* during the spring season. ANOVA values indicated that, the single factors and their interactions had highly significant effect on Chl. *a/b* ratio (Except for season × species interaction). Single factors (season and species) had the dominant and sub dominant role on the Chl. *a/b* ratio. The increased Chl.

a/b ratio indicated the synthesis of photosystem cores takes metabolic preference over the synthesis of light harvesting complex II [28]. The decreased chlorophyll ratio in plants under drastic conditions was existed to be balanced by increased chlorophyll thermo-stability [6].

The increased thermo-stability in plants implied the high adaptability of photosynthetic apparatus against severe desert conditions. At xeric habitat, the CSI *a* and CSI *b* produced high values during the spring season (Which was tented to a maximum in *C. procera*). While a minimum CSI *a* value was observed in *O. europaea* at summer. At mesic habitat, summer season had a boosting effect on the CSI *a* and tended to be highest in *C. procera*, while the lowest CSI *a* and CSI *b* values were detected in *O. europaea* at autumn. In spring season, the high CSI *b* in both *D. viscosa* and *P. guajava* was observed at xeric and mesic habitats respectively. This means that, the adaptation of plants to enduring drought and elevated temperatures was contributed to increased chlorophyll thermostability. Furthermore, the stability index of Chl. *b* was higher than that of Chl. *a* in all studied shrubs. Consequently, the thermo-stability of total chlorophyll (*a* + *b*) exerted highest values in *C. procera* particularly under xeric habitat conditions in the spring season. The decreased total CSI was showed in *O. europaea* during the autumn season at mesic habitat. Also, at mesic habitat, the total CSI in all plants was increased during hot season. In general, the total chlorophyll stability in studied shrubs was higher at xeric habitat compared to those at mesic habitat. Therefore, the increase in CSI and /or chlorophyll contents was referred to the high efficiency of photosynthetic apparatus in plants survives extreme conditions [5]. F values indicated that, the single factors and interactions (with some exceptions) had a significant effects on the CSI *a*, CSI *b* and total CSI of investigated species. The species factor played the main role in all parameters of CSI.

Chl. *a* and Chl. *b* efficiency were greatly depended on the Chl. thermo-stability of the studied plant species. At xeric habitat the differences between fresh and heated Chl. *a/b* ratios markedly had a negative value in the case of *C. procera* during the spring and summer seasons also in *O. europaea* during spring due to the destruction of Chl. *b* molecules. Adversely, the decreased Chl. *a/b* ratio in heated leaves produced a positive value in the rest species in different seasons at both habitats was attributed to the destruction of Chl. *a* [3]. However, a similar Chl. *a/b* ratios between fresh and heated leaves of *C. procera* and *P. guajava* in the spring and autumn seasons under mesic habitat clarified the high thermo-stability of Chl. *a* and Chl. *b* to heat. At xeric habitat, the same results were found in *D. viscosa* during the winter and autumn seasons as well as in *C. procera* during the summer season. Conspicuously, the plant strategies against chlorophyll destruction depended on the alternation of heat tolerance between both chlorophyll fractions.

The osmotically adjusted plants were mainly dependent on the solutes accumulation. Therefore, the seasonal accumulation of sugars would influence the plant growth and reproduction in the following seasons [29]. Among the investigated species, the *O. europaea* had the highest soluble sugars content (SS) followed by *D. viscosa*. The lowest SS content was detected in *C. procera* during the summer season at both xeric and mesic habitats. Commonly, the highest SS content was observed during mild season, whereas the hot season caused decreasing in SS content at mesic habitat. Likewise, the free amino acids (FAA) in different species obtained high values in spring season at mesic habitat. Only *D. viscosa* had the highest value of FAA at the same season at xeric habitat. The maximum FAA content was gained by both *D. viscosa* and *C. procera* at xeric and mesic habitat, respectively. This indicated that, the plants might tolerate severe conditions by accumulating free amino acids [30]. It was found that *O. europaea* had the lowest value during the autumn and winter seasons under xeric and mesic habitat conditions, respectively. F test pointed to a highly significant effect of single factors of (S., H. and Sp.) and their interactions on the SS and FAA contents in studied plants .The SS and FAA contents were mainly depended on the species factor, but the seasonality had the subdominant role.

Mg^{+2} ions were an essential for the chlorophyll molecule formation. During the summer season, the Mg^{+2} contents in different species were high at xeric habitat, particularly in *D. viscosa* followed by *P. guajava*. At mesic habitat, the highest Mg^{+2} contents were observed in *D. viscosa* followed by *C. procera* as well as *P. guajava* in winter season. Commonly, the lowest values of Mg^{+2} contents were shown in *O. europaea* followed by *P. guajava* during the spring season at mesic and xeric habitats respectively. The effect of single factors and their interaction was highly significant on the Mg^{+2} contents of tested species. The sharing percentage indicated that, the tri-factorial interaction (S. × H. × Sp.) had a dominant role on the Mg^{+2} contents and the habitat factor role was subsidiary. Therefore, the interaction between the environmental factors played the major role on the Mg^{+2} contents in plants [3]; [5].

The correlations between chlorophyll parameters with different constituents in species were variables in response to single factors and/or their interactions. The seasonality exerted positive correlation between SS and chlorophyll contents. The same correlation was found between the CSI and free amino acids. This implied the crucial role of the metabolic osmolytes on the Chl. contents and CSI in plants subjected to climatic changes. Also, positive correlation between the chlorophyll stability to heat was reported with different constituents, and was negative between the Chl. content due to translocation of sugars to different plant organs [31]; [32]. In general, the positive correlation between CSI and free amino acids reflected its role on chlorophyll molecule protection in chloroplast; ultimately enhancing the chlorophyll thermo-stability against heat stress and atmospheric aridity. Hence, the warming could result in an increase in photosynthesis capacity and contributed to an increase in chlorophyll and nitrogen concentration [33]. The positive correlation between the chlorophyll content and soluble sugars was probably referred to as protection and increased chlorophyll molecules efficiency.

Many specific correlations had existed between both Chl. content and its stability in the investigated species. Apparently, the chlorophyll molecule construction was mainly depended on the Mg^{+2} availability in the leaf tissues. Therefore, at mesic habitat the positive correlation between the chlorophyll content and Mg^{+2} in *D. viscosa* plants means a construction process of chlorophylls. In *C. procera* (true xerophyte) , a significant positive correlation was found between Chl. parameters with osmolytes free amino acids and soluble sugars which was corresponding to an increase in the relative turgidity of tissues [5]. This correlation was true in the case of both *O. europaea* and *P. jaugava*, which was indicated the essential role of both nitrogen and carbon metabolites in the water conservation in plants exposed to drastic stresses. Conversely, In some circumstances such as in the case of *O. europaea*, the free amino acids and soluble sugars had a negative correlation with chlorophyll content might be due to anabolic or catabolic processes which were participated in the osmotic adjustment and increased chlorophyll thermo-stability of plants grown under drastic habitat conditions [34].

CONCLUSION

Many facts could be concluded from the present work, these were: (1) The seasonal variations and ecotypes played a major role on the different chlorophyll parameters of investigated plants. (2) High CSI was exerted by *C. procera* particularly under xeric habitat conditions in spring season and vice versa in the case of *O. europaea* at mesic habitat. (3) The free amino acids played a crucial role in the CSI of plants under different single factors and their interactions. (4) Decreased chlorophyll content was compensated by an increase in CSI of plants. (5) The differences between fresh and heated chlorophyll ratios can be used as a criterion for chlorophyll thermo-stability magnitude. Therefore, the resistance of chlorophylls must be taken into consideration in any cropping projects for cultivation by economic shrubs in arid and semi-arid areas.

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