Eco-physiological Studies on *Hyoscyamus muticus* L. inhabiting El-Kharga Oasis in the Egyptian Western Desert

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Abstract

Hyoscyamus muticus L. (Egyptian Henbane) plant (Fam.: Solanaceae) is characterized by high economic importance due to its content of bioactive alkaloids and hypnotic medical benefits. The aim of the current study was to identify physiological and metabolic processes *H. muticus* L. uses as way of adapting to survive in arid and drought environments. El-Kharga is the largest Oasis in the Egyptian Western Desert. Four sites during the wet (winter) season were chosen for collecting plant and soil samples. The collected data indicated that the high summer temperatures and scattered vegetation had a detrimental effect on the soil's organic matter (OM%), which correlates with species' richness. As a result of accumulating more amino acids and soluble proteins during the summer rather than the winter, when existing environmental conditions may be better for such plants. The studied plant is often adapted for drought conditions that are prevalent in the habitats. Drought stress lowers the chlorophyll content in leaves and the activity of photosynthetic enzymes, which lowers the efficiency of photosynthesis. Results indicated that *H. muticus* L. is regarded as a very economical plant, and there is increasing interest in its cultivation in severely arid areas that are subject to drought stress.

Keywords: *Hyoscyamus*; Drought resistance; Plant-environment relations; Chlorophyll; Carbon and Nitrogen metabolism.

Introduction

Hyoscyamus muticus L. is a shrub growing in the sandy soils of Egypt [1]. The presence of bioactive alkaloids such as tropane alkaloids (hyoscyamine and hyoscine), which have sedative, hypnotic, anticholinergic, mydriatic, and other medical benefits, makes them consider to have high economic significance [2, 3, 4]. *Hyoscyamus muticus* L. is a member of the xerophytic plant community and is distinguished by its scarce water source.

One of the driest regions on earth is the Egyptian Western Desert, which makes about two thirds of Egypt's area. El-Kharga Oasis is the biggest and most urbanized oasis in the Western Desert and also serves as the seat of the New Valley Governorate. El-Kharga Oasis is one of highly arid regions with scarce vegetation. In these types of environments, vegetation plays a very minor role and soil development is mainly a geo-mechanical process. The soil types that sustain *Hyoscyamus muticus* L. include sandy clay, loam, gravelly sand, and sandy limestone. Thus, sandy soil with a significant fine sand fraction and low medium carbonate content is the most suitable for *Hyoscyamus muticus* L., which exhibits a wide range of amplitude in the desert.

The majority of the literature on the botanical traits of *H. muticus* deals with the *Hyoscyamus niger* L., or European henbane, then that with *Hyoscyamus muticus* L., or Egyptian henbane. Thus, it is imperative that any new botanical information regarding Egyptian Henbane be provided. Desert ecosystems are among the toughest on Earth because they combine severe temperatures and drought [5]. In order to survive in harsh conditions and maximize their benefits from nutrients and other resources most desert plants have evolved special morphological, physiological, reproductive, and anatomical traits. Adaptation refers to these plant-exhibited methods to the adverse conditions of their habitat.

According to [6], drought avoidance refers to a plant's potential to maintain a high plant water capacity during periods of water scarcity through adaptive traits including optimizing water intake through spenders and minimizing water loss through savers.

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Plants can typically withstand drought conditions owing to their tolerance and avoidance mechanisms, but the frequency and intensity of drought episodes can put additional stress on the plants [7]. Plants in xeric habitats have evolved to be as turgid as possible while absorbing the most possible water. The most important method for maintaining plant water reserves is the ability of plants to store large amounts of inorganic solutes within their tissues [8]. To keep plant cells stable, osmolytes such proline, soluble proteins, soluble carbohydrates, and betaine accumulate in the cells [9, 10].

According to previous investigations, *H. muticus* L. is metabolically active throughout the year. This behavior indicates the plant's resistance to water stress. The main objective of the current study was to determine which physiological functions and metabolic processes *H. muticus* L. uses as adaptation strategies to survive in arid and drought conditions. Numerous ecophysiological investigations have clarified the value of the plant's functions and indicated features that are adaptive in particular conditions [11].

Materials and Methods

Study area

The largest oases in the Western Desert are El-Kharga Oasis (southern Oasis), which is located in the arid region of the Great Sahara, which lacks any rain. Assiut City is located 224 km to the west, whereas Dakhla Oasis is located 104 km west. The desert lands are located between latitudes 24° 30´ and 25° 40´N and longitudes 30° and 31°E. With a total surface area of around 3000 km^2, the depression is long and narrow in shape. It extends for approximately 185 km from north to south and between 15 and 30 km from east to west. Only 1% of the depression is covered by cultivated land. Nearly 18 meters below sea level is where the El-Kharga Oasis is lowest. Barchan-type mobile sand dunes are extremely common. Their movement (10-20 m $year^{-1}$) across the floor swamps, cultivated land, wells, roads, and buildings is remarkable. Mean annual relative humidity is lower in summer (26-32%) than in winter (53-60%), whereas rainfall is irregular, averaging virtually zero (mm year-1). Winter temperatures are moderate, ranging from 6.0 to 4.8 °C at its lowest point to 22.1-21.5 °C at its highest point. In contrast, summer temperatures are quite high, reaching as high as 39.2-39.5 °C at its highest point and from 23.4-23.1 °C at its lowest point. It is not rare to record severe maxima of roughly 50 °C. Meteorological data over the past five years (2020–2024) indicates that the mean temperature varied from 5 °C in January to 43 °C in June and August. Meanwhile, there was a noticeable variation in the relative humidity (RH) based on latitude, longitude, and the season. December saw a range of 66%, while June and April recorded 7%.

Soil analysis

Soil sample taken were plants found in significant quantity. During the wet season (winter), samples were taken from four locations. In plastic bags, three duplicates were removed from each stand and brought to the lab. The same locations were used to gather plant samples of *Hyoscyamus muticus* L. during the summer and winter. After weighing the fresh soil sample and drying it for 24 hours at 105 °C in an oven, the dry weight of the sample was calculated to ascertain its water content [12]. The sample's water content was calculated as a percentage of the dry weight of the sample. Preparation of soil extracts 1:5 (soil: water) were made in line with the US Salinity Laboratory staff's protocol [13]. In order to prepare soil extracts (1:5) for this proposal, 20 gm of soil was shaken for 60 minutes with 100 ml of distilled water. A clear filtrate was then obtained by filtering the mixture. The dichromate oxidation method, as described by [14], was used to assess the amount of organic matter in the soil samples. While sulphate was measured turbid metrically as $BaSO_4$ using methods outlined by [15], chloride was determined volumetrically in accordance with [16]. Flame photometry was used to quantify potassium and sodium, as stated by [17]. Volumetric measurements of calcium and magnesium using the Versene method were estimate according to [18]. Phosphorus has been measured calorimetrically as phosphomolybdate, according to [16]. As stated by [16], a conductivity meter (model 4310, JEN WAY) was used to measure the soil filtrate's total soluble salts (TSS) and electric conductivity (EC). The samples' soil reactivity that were collected was measured using an electric pH meter (model AD-8000). **Jackson's (1967)** methodology was used to calculate the soil's total carbonates [16].

Plant analysis

Hyoscyamus muticus L. A perennial herb or shrub that grows 30 to 60 centimeters high, it has a sturdy, green, and fleshy stem, thick leaves that are thickly branched from the neck, and sessile leaves that are alternating, succulent, and have long petioled edges on the lower side and angled or toothed-lobed margins on the upper side. Flower is dense in the inflorescence, which resembles a one-sided spike or raceme. According to [19], 10 ml of 80% aqueous ethanol was used to extract chlorophyll from a specific weight of fresh, healthy leaves in test tubes. After weighing an accurate amount of fresh, healthy leaves in 10 milliliters of distilled water, the leaves were placed in a water bath at 56 ± 1 °C for 30minutes to determine the chlorophyll stability. For either chlorophyll an or b, the chlorophyll stability index (CSI) was given as a percentage comparing the amount of chlorophyll in the heated sample with the fresh sample. Ultimately, mg g^{-1} fresh weight (FW) was calculated for each of these pigment components. The chlorophyll a/b ratio was also calculated. Fresh leaves were dried for 24 hours at 70 °C in an oven before being ground into a fine powder for plant extraction [20]. The examination of soluble osmotically active metabolites included the determination of soluble carbohydrates (carbon metabolites), soluble proteins (nitrogen metabolites), and total free amino acids. The methods outlined by [21, 22, 23]were utilized to determine these metabolites. As was previously noted in the soil extract, it was determined which distinct anions and cations were present in plant extracts. The ions and soluble osmotically active metabolites were measured in mg g^{-1} dry weight (DW).

Statistical analysis

To assess the impact of individual components and their interactions on the parameters under evaluation, statistical inferences were required, such as determining the coefficient of determination (η2) and finding the analysis of variance (F value). The SPSS software was used to statistically analyze the data. To evaluate each component's and interaction's proportional contribution to the entire response, the determination coefficient (η2) has been developed.

Results

Soil analysis

Among the many components of soil particles, the mechanical soil analysis showed that clay soil is the major component. Four sites had sand proportions ranging from 66.9% to 78.9%.

Total soluble salt concentration and soil water content

The soil sample's water content (Figure 1) varied from 0.20% to 5.05%. Site 2 had the lowest water content (0.20%), whereas site 1 had the highest water content (5.05%). In the majority of the research sites where *Hyoscyamus muticus* L. was found to be present, the soil extracts total soluble salts (TSS%) varied from 0.15% to 2.09% (Figure 1). At location 1, the highest TSS value (2.09%) was found. At location 4, the lowest value (0.15%) was recorded.

Organic matter, pH value, electric conductivity, and total carbonate in soil

As shown in table (1) the organic matter content (%) of soil samples, the maximum value was (1.14%) was at site 1, whereas the minimum value (0.52%) occurred at site 2. It is observed in table 1 that the soil solution is slightly alkaline pH varied between 8.07 at site 2 and 8.68 at site 1. Table 1 indicates that values of electric conductivity (EC) in soil solution are slightly elevated. The highest value of EC (6.53 mS cm^{-1})

was recorded at site 1. Generally, total carbonate contents ranged between 5.5% and 6.22% in table 1 (at sites 2 and 4, respectively).

Table 1. Includes the pH values, total carbonate (CO_3^-%), organic matter content (OM%) and electric conductivity (EC mS $cm^{-1}$of soil samples of different four sites inhabited by *Hyoscyamus muticus* L. plants in El-Kharga Oasis during the winter season.

Sites	pH	EC mS cm^{-1}	OM %	CO_3^-
S1	8.68	6.53	1.14	5.98
S2	8.07	0.94	0.52	5.5
S3	8.20	2.41	0.78	6.21
S4	8.37	0.46	0.62	6.22

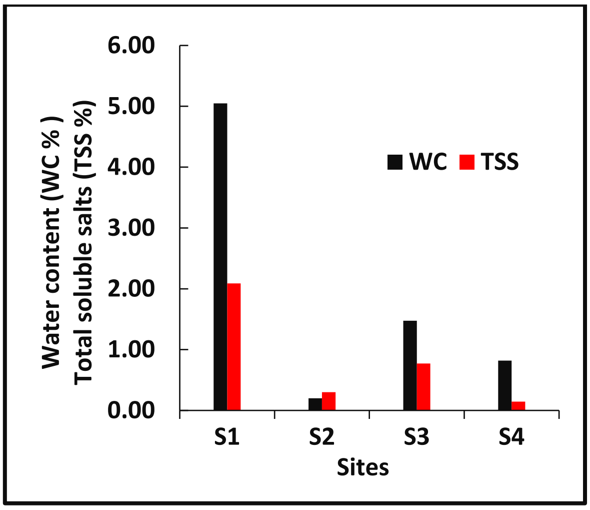

Figure 1. The amount of total soluble salts (TSS%) and water content (WC%) in soil samples collected from the El-Kharga Oasis study sites where *Hyoscyamus muticus* L. inhabited.

The concentration of major ions in the soil

Table 2 shows the concentrations of the main soluble anions and cations in samples of soil from sites where *Hyoscyamus muticus* L. plants were detected. Among the other sites, site 1 had the highest concentration of cations. The range of sodium contents in the soil was 1.75 mg/g to 15.66 mg/g. The soil samples' calcium and magnesium concentrations from 0.05 to 1.65 mg g^{-1} soil, but the potassium content ranged from 0.16 to 2.49 mg g^{-1} soil. The range of chloride values observed in soil samples was 1.69 mg g^{-1} to 4.60 mg g^{-1}. Site 3 had the highest concentration of chlorides detected. The sulphates and phosphate contents of the studied sites appeared in small quantities.

Plant analysis

Water content

As shown in (Figure 2), the water content of winter leaves ranged between 90.10% and 92.31%. In summer, it ranged between 87.84% and 93.50%. According to statistical analysis, a highly significant impact was caused by regionality and the interaction of two components. while seasonality had a significant effect (Table 4). The regionality factor had the dominant effect ($\eta2 = 0.59$), followed by interaction ($\eta2 = 0.35$), while the seasonality factor was the minor one ($\eta2 = 0.06$).

Table 2. The concentrations of the main soluble ions and cations in the examined areas where *Hyoscyamus muticus* L. inhabits El-Kharga Oasis. The soluble ions [Cl^- (mg g^{-1} soil), SO_4^{-2}, PO_4^{-3} (µg g^{-1} soil)], cations [Na^+, K^+, Ca^{+2}, Mg^{+2} (mg g^{-1} soil)].

Sites	Cations				Anions		
	Ca^{+2}	Mg^{+2}	Na^+	K^+	Cl^-	SO_4^{-2}	PO_4^{-3}
S1	1.65	1.43	15.66	2.49	1.69	6.05	0.72
S2	0.58	0.05	2.52	0.16	1.96	2.09	0.84
S3	1.40	0.18	7.51	0.61	4.60	4.97	0.48
S4	0.43	0.44	1.75	0.17	2.08	1.53	0.52

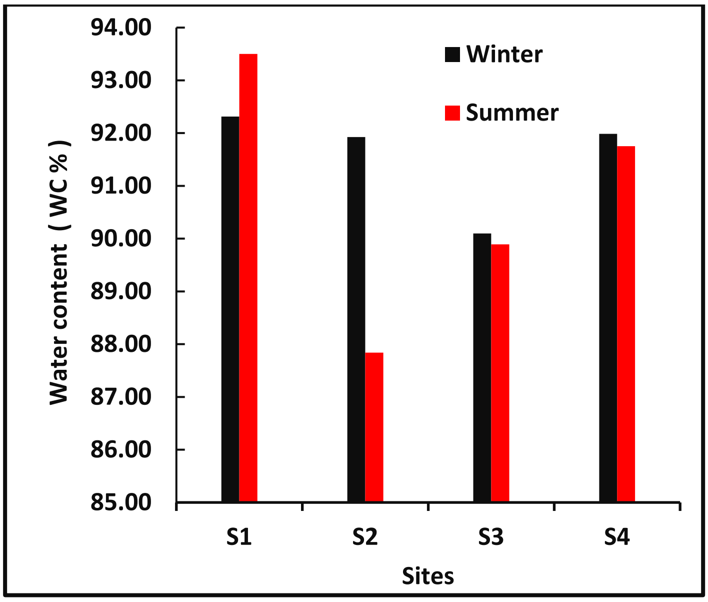

Figure 2. Water content (%) in the leaves of *Hyoscyamus muticus* L. plants at different studied sites in El-Kharga Oasis during the winter and summer seasons.

Plants pigment and chlorophyll stability index

In winter, sites 3 and 4 reported concentrations of chlorophyll a that varied from 0.13 mg g^{-1} FW – 0.19 mg g^{-1} FW; in summer, site 2 and 4 had concentrations of 0.08 mg g-1 FW and 0.24 mg g^{-1} FW, respectively (Figure 3 and Table 3). At all locations, the summertime concentration of chlorophyll b was greater than the wintertime concentration. In Summer data at site 4 revealed the highest concentration of chlorophyll b, measuring 0.13 mg g^{-1} FW (Figure 3 and Table 3).
Carotenoid content was slightly low, ranging 0.05 mg g^{-1} FW – 0.08 mg g^{-1} FW in both seasons (Table 3). With the exception of site 2, Summer values for total chlorophyll a+b were higher than winter (Table 3). At all sites, the ratio of chlorophyll a to b was greater in the winter than in the summer. In the winter, the ratio fluctuated between 3.81 and 5.51, while it varied from 1.03 to 1.91 in summer (Figure 3 and Table 3). Statistical analysis is shown in table 4; in chlorophyll a, while regionality and the interaction between the two components were extremely significant, the seasonality factor's effect was not statistically significant. The seasonality component had a moderate influence ($\eta2 = 0.0004$), whereas the regionality factor had the dominating one ($\eta2 = 0.57$), followed by the interaction ($\eta2 = 0.43$). Two elements and their interaction significantly impacted chlorophyll b. The main influence was seasonality ($\eta2 = 0.85$). Regionality dominated for carotenoids ($\eta2 = 0.87$). The impacts of individual components as well as their combinations were quite significant in chlorophyll a+b. Regionality had the most significant impact on the chlorophyll a+b ($\eta2 = 0.42$), followed by interaction ($\eta2 = 0.35$), while the seasonality role was

subdominant (ɳ2 = 0.23). In chlorophyll a/b ratio, Single-factor effects as well as interaction of these effects were significant. The seasonality was main factor (ɳ2 = 0.89).

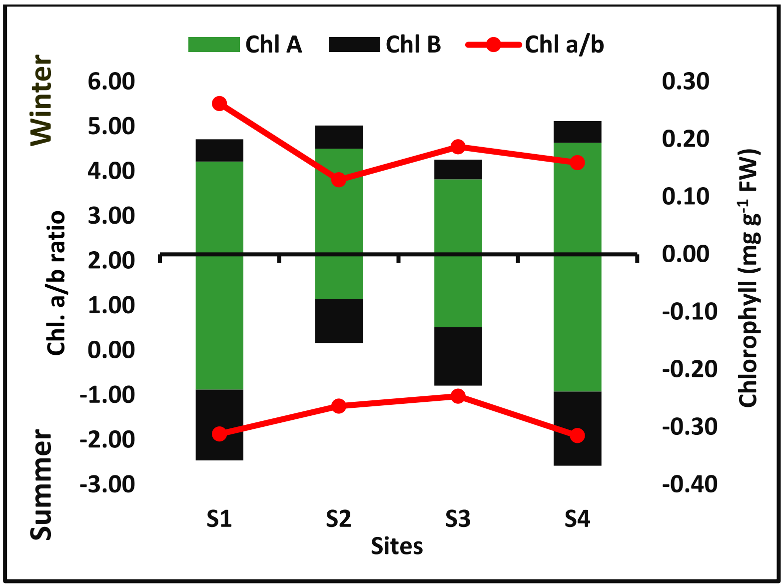

Figure 3. Winter and summer concentration of Chlorophyll (Chl. a and Chl. b) (mg g^{-1} FW) and Chl. a/b ratio in *Hyoscyamus muticus* L. at different studied sites in El-Kharga Oasis.

With the exception of site 1, which exhibited a reversed trend, the summer stability index (CSI) of chlorophyll a was higher than the winter CSI. In winter, the chlorophyll-a stability index varied from 29.20% to 56.84%, whereas in summer, it varied from 56.81% to 92.94%. Table 3 shows that the range of chlorophyll stability b was 53.42% and 96.48% in the summer and 53.43% and 88.99% in the winter. According to the statistical analysis shown in table (4) seasonality had the main influence (ɳ2 = 0.60) in affecting CSI, followed by interaction (ɳ2 = 0.35), while regionality played a minor role (ɳ2 = 0.05). For CSI b, regionality was most significant factor (ɳ2 = 0.59), followed by interaction (ɳ2 = 0.40), while the seasonality had a negligible influence (ɳ2 = 0.01).

Table 3. concentrations of Chlorophyll a, Chlorophyll b, Carotenoids, and Chlorophyll a+b (mg g^{-1} FW), Chlorophyll a/b ratio, and chlorophyll stability index (CSI%) in *Hyoscyamus muticus* L. at sites in El-Kharga Oasis during the winter and summer seasons.

St	Chl. A		Chl. b		Carotenoids		Chl. a+b		Chl. a/b		CSI a		CSI b	
	W	S	W	S	W	S	W	S	W	S	W	S	W	S
1	0.16	0.23	0.04	0.12	0.05	0.05	0.20	0.36	4.19	1.91	56.84	56.81	72.54	54.42
2	0.18	0.08	0.04	0.08	0.07	0.08	0.22	0.15	4.55	1.03	29.20	92.94	88.99	96.48
3	0.13	0.13	0.03	0.10	0.05	0.05	0.16	0.23	3.81	1.25	48.87	69.99	85.71	60.37
4	0.19	0.24	0.04	0.13	0.06	0.07	0.23	0.37	5.51	1.87	29.73	68.34	53.43	75.91

The ionic content of plant tissue

The main ions contents in leaves of *H. muticus* L. collected from different sites are given in figure 4 and 5, respectively. At each location, the winter concentrations of sodium in *H. muticus* L. leaves were greater than those in the summer. The winter concentrations at site 3 (42.83 mg g^{-1} DW) and the summer concentrations at site 1 (29.02 mg g^{-1} DW) were the greatest. Results of the statistical analysis (Table 5) showed that both the individual components' and their interactions' impacts were significant. The main effect was seasonality (ɳ2 = 0.44). *H. muticus* L. leaves had potassium values that varied from 9.80 mg g^{-1} DW to 20.87 mg g^{-1} DW in the winter to 7.87 mg g^{-1} DW to 20.67 mg g^{-1} DW in the summer. Site 4 recorded the highest concentration (20.87 mg g^{-1} DW) in the winter, while site 3 recorded the lowest value (7.87 mg g^{-1} DW) in the summer. Table 5 shows that both the single-factor effects and their interactions were significant. The seasonality influence was minimal (η2 = 0.05), but the interaction had the dominant effect (η2 = 0.57). At every site but site 4, there was a higher concentration of calcium in the leaves of *H. muticus* L. plants during the winter than during the summer. The winter plant samples taken from location 1 had the greatest calcium contents (6 mg g^{-1} DW). The results of the statistical analysis (Table 5) showed that while the interaction was not significant, the impacts of the seasonality and regionality components were. Regionality had the greatest effect (η2 = 0.60). Magnesium Concentration of *H. muticus* were higher in winter than in summer in all sites and ranged between 2.11 mg g^{-1} DW at site 2 and 4.88 mg g^{-1} DW at site 1 in winter and between 1.95 mg g^{-1} DW at site 2 and 3.66 mg g^{-1} DW at site 1 in summer. Table 5 of the statistical analysis revealed that the effects of the seasonality and regionality factors have significant impact, while interaction was non-significant. Regionality had the great effect (η2 = 0.72).

Table 4. Statistical analysis of *Hyoscyamus muticus* L. water content, chlorophylls, and organic components, showing analysis of variance (F-value) and determination coefficient ($η^2$).

Parameters	S.O.V	F	$η^2$
Water content	Seasonality	7.3*	0.06
	Regionality	22.887**	0.59
	Interaction	13.482**	0.35
Chlorophyll a	Seasonality	0.525ns	0.0004
	Regionality	224.951**	0.57
	Interaction	169.215**	0.43
Chlorophyll b	Seasonality	399.106**	0.85
	Regionality	11.249**	0.06
	Interaction	12.467**	0.09
Carotenoids	Seasonality	6.07*	0.06
	Regionality	31.347**	0.87
	Interaction	2.703ns	0.08
Chl. a/b ratio	Seasonality	818.643**	0.89
	Regionality	22.692**	0.07
	Interaction	10.542**	0.03
Chl. a+b	Seasonality	106.286**	0.23
	Regionality	100.378**	0.42
	Interaction	83.165**	0.35
CSI_a	Seasonality	280.359**	0.60
	Regionality	8.363**	0.05
	Interaction	53.733**	0.35
CSI b	Seasonality	4.628*	0.01
	Regionality	74.599**	0.59
	Interaction	50.527**	0.40
Soluble sugars	Seasonality	633.438**	0.65
	Regionality	35.887**	0.11
	Interaction	80.604**	0.24
Soluble proteins	Seasonality	70.521**	0.29
	Regionality	12.697**	0.14
	Interaction	41.839**	0.57
Total Amino acids	Seasonality	803.138**	0.56
	Regionality	104.657**	0.22
	Interaction	103.432**	0.22

****Significant at 0.01 confidence level.**
***Significant at 0.05 confidence level.**

Table 5. Statistical analysis of the inorganic components (anions and cations) in the leaves of *Hyoscyamus muticus* L. showing analysis of variance (F-value) and determination coefficient (η^2).

Parameters	S.O.V	F	η^2
Na^+	Seasonality	166.89**	0.44
	Regionality	29.224**	0.23
	Interaction	41.749**	0.33
K^+	Seasonality	18.232**	0.05
	Regionality	47.587**	0.38
	Interaction	70.785**	0.57
Ca^{+2}	Seasonality	4.745*	0.29
	Regionality	3.32*	0.60
	Interaction	0.588ns	0.11
Mg^{+2}	Seasonality	23.539**	0.22
	Regionality	25.5**	0.72
	Interaction	1.944ns	0.06
Cl^-	Seasonality	4.459ns	0.03
	Regionality	37.671**	0.88
	Interaction	3.488*	0.08
SO_4^{-2}	Seasonality	8.13*	0.04
	Regionality	32.717**	0.54
	Interaction	25.147**	0.42
PO_4^{-3}	Seasonality	3.404ns	0.01
	Regionality	32.818**	0.38
	Interaction	52.437**	0.61

****Significant at 0.01 confidence level. *Significant at 0.05 confidence level.**

At every site, the summer had greater chloride contents in the leaves of *H. muticus* L. plants than winter. According to statistical analysis (Table 5) the main effect was regionality ($\eta2$ = 0.88), subsequently followed by seasonality ($\eta2$ = 0.03) and their interaction ($\eta2$ = 0.08). In comparison to chlorides, phosphates and sulphates have lower values in the summer and winter. Sulphates varied from 10.80 µg g^{-1} DW and 25.33 µg g^{-1} DW in winter and from 5.50 and 19.06 µg g^{-1} DW in summer. During winter, site 3 had the highest value of sulphates (25.33 µg g-1 DW) in plants. The result of statistical analysis (Table 5) demonstrated the high significance of the effects of regionality and interaction. Role of seasonality was minor (η^2 = 0.04), while the interaction role was subdominant (η^2 = 0.42), and regionality had the largest influence (η^2 = 0.54). Phosphate concentrations ranged between 83.97 µg g^{-1} DW and 102.71 mg g^{-1} DW in winter, while in summer, they ranged between 73.19 µg g^{-1} DW and 125.42 µg g^{-1} DW. According to statistical analysis (Table 5), regionality and interaction exhibited significant effects. The greatest effect (η^2 = 0.61) was by interaction, with seasonality role (η^2 = 0.01) and regionality role (η^2 = 0.38) rather prominent.

Metabolic components

In comparison to the other metabolites examined, the TAA level was the greatest, and the SP concentration was the lowest metabolic component of the cell. At every site under investigation, the concentrations of soluble sugars recorded during the winter were greater than those during the summer, as figure 6 illustrates. In the winter, site 4 had the highest value for soluble sugars in the samples of plant, with 0.33 mg g^{-1} DW. At site 3, the lowest value over the summer was 0.02 mg g^{-1} DW. The results of the statistical analysis showed that both the individual components' and their interactions' effects were significant (Table 4). The main affect was seasonality ($\eta2$ = 0.65), following by interaction ($\eta2$ = 0.24). Figure 6 displayed the soluble proteins concentrations. Soluble protein concentrations in the winter were higher than in the summer, with the exception of site 2. In winter, the values ranged between 0.08 mg g^{-1} DW and 0.10 mg g^{-1} DW, while ranged between 0.04 mg g^{-1} DW and 0.11 mg g^{-1} DW in summer. Table 4 of statistical analysis showed that both the individual components' and their interactions' impacts were significant. seasonality ($\eta2$ = 0.29) was the second most prominent effect, after the interaction ($\eta2$ = 0.57). Total free amino acid content was higher in the summer than in the winter at all sites, as figure 6 illustrates, when compared to the other metabolites. The values varied from 0.17 mg g^{-1} DW and 0.48 mg g^{-1} DW in winter, 0.36 mg g^{-1} DW, and 1.94 mg g^{-1} DW in summer. The highest values of amino acid content were recorded at site 2 during the summer. The individual components and their interactions had significant effects, according to statistical analysis (Table 4). The main factor was seasonality (η2 = 0.56), followed by regionality and interaction (η2 = 0.22).

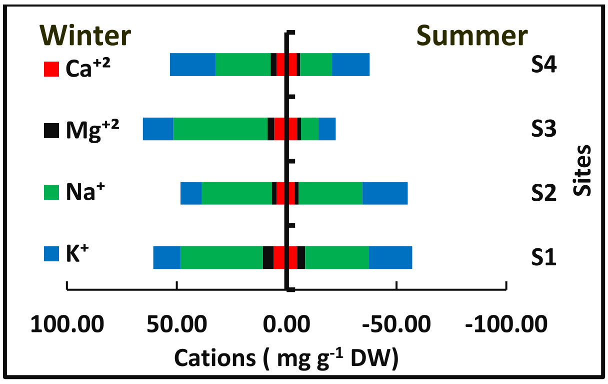

Figure 4. Concentrations of main soluble cations (K^+, Na^+, $Ca^{+2}$and Mg^{+2}) in mg g^{-1} DW of leaves of *Hyoscyamus muticus* L. plants at studied sites in El-Kharga Oasis during the winter and summer seasons.

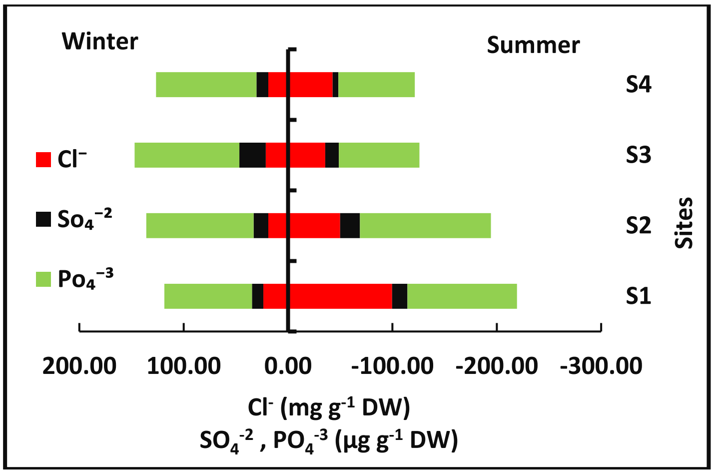

Figure 5. Concentrations of soluble anions Cl^- in mg g^{-1} DW and (PO_4^{-3} and SO_4^{-2}) in µg g^{-1} DW in leaves of *Hyoscyamus muticus* L. plants at different studied sites in El-Kharga Oasis during the winter and summer seasons.

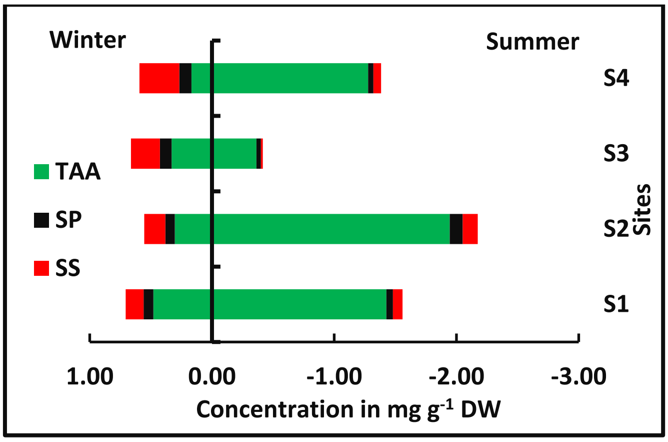

Figure 6. Total free amino acids (TAA), soluble proteins (SP), and Soluble sugars (SS) concentrations in leaves of *Hyoscyamus muticus* L. expressed as mg g^{-1} DW at studied sites in El-Kharga Oasis during the winter and summer seasons.

Discussion

Recent data aim to study the effects of environmental factors on the eco-physiological responses of *Hyoscyamus muticus* L. in El-Kharga Oasis. Therefore, four sites were chosen to be inhabited by plant under investigation in order to clarify the ecological features of these settings and the means by which this species endures such extreme aridity. The western desert is one of the driest regions on earth, which accounts for the low soil water content. Its remote location from the oceans and lack of high heights, which could draw orographic rain, contribute to its extreme aridity. Even though it rarely rain, a rise in the groundwater table could increase the possibility of perennials being served.

In desert ecosystems, soil texture has a significant influence in influencing the spatial distribution of soil moisture [**24, 25**]. The mechanical soil analysis revealed that clay soil predominates among other components of soil particles. The obtained results demonstrated that the high summer temperatures and dispersed vegetation had a detrimental impact on the organic matter percentage (OM%) of the soil, which is connected with species richness [**26**]. Soil carbonate was weakly to moderately calcareous, and the estimated pH values in the soil solution tended to be slightly alkaline. In dry regions where soluble sodium salts (such Na_2CO_3) may accumulate, a pH of alkaline is typically reached. These findings were in line with the general traits of dry region soils and their correlation with vegetation, climate, and other factors as reported by multiple authors [**27**]. This is typical of the general characteristics of soil in arid regions, according to earlier research; the association between this soil feature and vegetation, indicating low organic matter (OM) levels, an alkaline soil surface reaction (pH), and low biological activity [**28**]. The total of soluble salts was high. The extreme aridity and low moisture content in the studied area might be reasons for that. The rate of evaporation and precipitation had an impact on the total amount of soluble salts in the soil. Salt can be dissolved by allowing water to seep into the unsaturated zone due to high rates of evaporation [**29**]. As a result, the area's soil solutions investigated had comparatively high electric conductivity. This illustrates how abundant certain ecosystems are in soluble salts. Consequently, in semi-arid regions, the evaporation process causes groundwater to become more ionic enriched, thereby raising salinity. Na^{+2} and Cl^{-} dominated the predicted soluble salts in the soil. The high concentration of salt may have resulted from a rapid rate of evaporation that concentrated the soil solution. The current statistics are consistent with the results [**30, 31, 32**]. Phosphates were generally the least predicted anion in the soil across all sites.

Plants have evolved two adaptive mechanisms to deal with environmental stress. In order to prevent water loss, plant cells typically readjust their osmotic potential. This can be accomplished by absorbing inorganic ions from the environment and by synthesizing compatible solutes from scratch, such as soluble proteins, amino acids, and soluble sugars that function as osmolytes [**33, 34**]. When compared to the synthesis of suitable solutes, Ion flux variations rapidly cause the osmotic readjustment [**35, 36**].

The recent data showed that demonstrated that a higher reduction occurred in the water content of *Hyoscyamus muticus* L. plants in the summer than in the winter as a result of high summer temperatures and limited water supply. When stomata can close due to high temperatures, plant transpiration continues through the cuticle [**37**]. The plant under study had significantly more chlorophyll a and chlorophyll b in the summer than in the winter, according to the data. According to [**38**], plants in the desert have adapted to arid conditions by accumulating larger quantities of chlorophyll. Furthermore, the Chl a/b ratio is greater than 1 in all sites. The range of the Chl a/b ratio, according to [**39**], was 1.5 to 3. Stress from drought considerably reduced chlorophyll a and b contents as well as the chlorophyll a/b ratio, which had an adverse effect on photosynthetic efficiency [**40**] Chl a/b ratio in the leaves may have decreased because of either degradation of Chl an or an increase in

Chl b in comparison to Chl a. It has recently been shown that Chl b is transformed to Chl an in higher plants as a result of the Chl a/b inter-conversion cycle, allowing plants to adjust to fluctuating light levels [**41**]. Within chloroplasts, carotenoids are one of the most prevalent classes of lipid-soluble antioxidants [**42**]. There is increasing evidence that plants' responses to dehydration stress depend on carotenoids [**43**]. The ability of plant pigments to withstand extremely high temperatures is shown by the chlorophyll stability index. It is therefore an effective indication for arid plants. In general, summer values of the chlorophyll a and b stability index were substantially greater than winter estimates. According to the current data, chlorophyll b exhibited greater stability compared to chlorophyll a during the summer months in adaptation to rising temperatures and a progressive reduction in soil moisture; these results are consistent with those reported by [**44**].

The simplest way to manage external biotic and/or abiotic challenges brought through an accumulation of inorganic solutes is by osmoregulation [**45**]. In stressed plants, osmotic gradient re-adjustment can be effectively achieved by the absorption, exclusion, or removal of inorganic osmoregulatory ions such as K^+, Na^+, Ca^{+2}, Mg^{+2}, and Cl^- [(26)]. Soluble proteins, soluble carbohydrates, and free amino acids are examples of organically compatible solutes that can be accumulated using the second technique. According to [**46**], osmotic compensation through a high accumulation of osmolytes is dependent upon the kind of plant, the degree of stress, and the stage of plant growth at which the stress is administered. According to [**47**], a higher chlorophyll stability index could compensate for a decreased chlorophyll content. This is supported by the higher chlorophyll stability index in the plants under study when exposed to high temperatures. Additionally, the author revealed a strong relationship between CSI and chlorophyll level in connection to Mg, K, Fe, water content and soluble proteins. According to [**48, 49, 50**], desert plants have adapted to their dry surroundings, they contain higher levels of carotenoids and chlorophyll.

In order to withstand the external conditions brought on by drought, plants typically increase their osmotic pressure. Additionally, Salt is concentrated in the soil solution due to evaporation being accelerated by the high temperatures found in desert. Moreover, increased transpiration rates will cause the cells to become more turgid and will add to the tendency of absorbing more salts into the shoots. The simple and quick reaction of inorganic solute accumulation can also lead to risks that could wipe out the plant. According to [**51**], in order to maintain charge balance and prevent toxicity, within numerous organs, plants need to absorb, eliminate, and translocate the solutes. The molar sum of accumulated cations was often greater than that of accumulated anions, regardless of the season. Plants lose some of their biological activity throughout the summer. As a result, they gather the essential concentrations of organic solutes to keep the growing branches' cells turgid. However, biological activity will resume in the winter and the accumulated organic solutes will be brought to use. Therefore, instead of accumulating organic solutes which are necessary for biological processes, the plants will prefer to collect inorganic solutes. It appears that two of the most important aspects of tolerance are the regulation of Ca^{+2} and Cl^- uptake from soils and the partitioning of these ions inside plants. The examined species therefore relied more on K^+ and Ca^{+2}. These findings corroborated those presented by [**52**]. The research results of this investigation show that the investigated plant had significant calcium and magnesium accumulation. According to [**52**], under drought stress, succulent species accumulated significant levels of Ca^{+2} and Mg^{+2}. In desert environments, plants tend to accumulate more sulfates in order to maintain their succulence and the production of amino acids containing a thiol group (-SH). *Hyoscyamus muticus* acquired notable amounts of Ca^{+2} and K^+, which were crucial to this physiological response. In line with [**30**], who observed that *Ochradenus baccatus* collected considerable quantities of Ca^{+2} and Mg^{+2} during drought stress, more Ca^{+2} ions were accumulated than Mg^{+2} in all plants. To prevent Na^+ toxicity, plants may eliminate or accumulate Na^+ in the vacuole. Osmotic pressure is raised because Na^+ and Cl^- are frequently held in the vacuoles, according to [53]. The most significant osmoregulatory anion is thought to be Cl^-. Because of its crucial roles in stomatal movement, plant growth and reproduction, photosynthesis, stress resistance, enzyme activation, nitrogen uptake, osmoregulation, and protein synthesis, accumulated K^+ in *Hyoscyamus muticus* L. may be used in drought tolerance mechanisms during the summer [54]. It is also preferred in the cytoplasm. Phosphates contain phosphorus in the soil. Plant growth and productivity are correlated with phosphorus uptake [55]. Small traces of phosphates were found in the plants under study. This can be the result of the soil's low phosphate content or the fast assimilation of phosphates into plant metabolism. Complementary solutes provided by the plants themselves are known as organic solutes. Biological processes require organic solutes. Plants keep them under stress so they can withstand environmental stresses. The examined plants often accumulate more soluble proteins and amino acids in the summer than they do in the winter, when environmental conditions could be suitable for these plants. This adaptation helps the plants cope with drought conditions in their environments. These findings corroborated the research conducted in Wadi Qena by [30], on *Ochradenus baccatus*. Generally, under conditions of stress, soluble sugars act via hydrogen bonding to interact with proteins and cell membranes to avoid protein denaturation. In order to preserve the hydrophilic contacts in the cell membrane and the structure and function of proteins, the free hydroxyl groups of soluble carbohydrates balance those of water. Because of this, the research plant's soluble sugar levels plummeted during the summer. These findings corroborated the research by [56]. In response to environmental stress, plants can either make more molecules that bind water or stop amino acids from being incorporated into proteins. The amount of soluble protein increased significantly in the winter. The surface exposed to binding water rises with protein content, and drought resistance is associated with bound water [33]. Nitrogen metabolites may help certain xerophytic organisms osmotically respond to stress [57]. According to [58], amino acids function similarly through three different mechanisms: serving as suitable osmolytes, controlling pH, or serving as a reservoir for carbon or nitrogen. Total free amino acid concentration was higher than that of soluble protein and sugar. Several other researchers [59, 60, 61] who used different economic plants also had similar outcomes. One could consider this rise in amino acid content to be a plant's adaptation strategy to salinization. According to recent findings, *H. muticus* L. is regarded as a very economical plant, and cultivation of it in extremely arid areas under drought stress is increasingly prominent. It is among the factors that has a major impact on photosynthesis and plant productivity [62, 63]. Increased drought stress lowers the amount of chlorophyll in leaves and the activities of photosynthetic enzymes, which lowers the efficiency of photosynthesis [64, 65]. The decrease in photosynthetic activity might also be attributed to a reduction in stomatal mobility [66, 67].

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