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Seasonal Changes of Some Metabolites in *Hyoscyamus boveanus* (Dunal) Asch. & Schweinf – Saint Katherine, South Sinai, Egypt

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

This study was conducted to investigate the effects of seasonal fluctuations on some metabolites and to explore the correlation between soil and plant analysis in *Hyoscyamus boveanus* (*H.boveanus*) at the Wadi El-Sheikh Awad in Saint Katherine, South Sinai, Egypt. Significant differences ($p < 0.05$) were observed for physical and chemical properties of the soil associated with *H. boveanus* during 0-20 and 20-40 cm depths, which increased most of them during the first depth. All chemical composition contents of *H. boveanus* are influenced significantly ($p < 0.05$) by seasons studied, except Na^+, glycosides, total phenol, crude protein contents. A substantial increase in mineral composition (Na^+, K^+, P and Fe^{2+}), total alkaloids, glycosides, total phenol, proline, total carbohydrates and all photosynthetic pigments contents were recorded in *H. boveanus* during the summer season. While, the mineral composition (Ca^{2+}, Mg^{2+}, S, N and Cl^-), water content and crude protein contents appeared to be higher in the winter season. The relationships between soil and plant variables were delineated by performing the principal component analysis (PCA). The PC1 and PC2 displayed differences between the soil and plant variables, also, the variables Mg^{2+}, Cl^-, pH, EC, Ca^{2+} and K^+ in the soil associated with *H. boveanus* are variables with better chemical properties of the soil, which affect the plant distribution in Wadi El-Sheikh Awad during the two seasons. The PCA revealed high positive correlations among soil variables as well as among plant variables. Soil magnesium correlated highly and positively with

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the plant variables i.e., crude protein, water content, Chl b, Chl a+b, carotein and total pigment contents. The pH, EC, and Ca^{2+} in soil were positively correlated with all chemical composition contents of *H. boveanus*. Some metabolites in *H. boveanus* were significantly increased during the summer season compared to the other season, due to the activation of plant physiological stress tolerance mechanisms.

Keywords: Seasonal changes; metabolites; PCA; Hyoscyamus boveanus.

1. INTRODUCTION

The wild populations of plant species are exposed to severe danger, which could lead to their extinction due to climate change and human activities in their natural habitats. In Egypt, South Sinai is arid to the extremely arid region and characterized by ecological uniqueness due to its diversity in terrestrial forms, geological and climatic structures, which resulted in a diversity of plant species, which are mainly characterized by the dispersal and dominance of shrubs, sub-shrubs, and tree paucity [1, 2, 3]. Ayyad et al. [4] reported that the South Sinai region was more diverse compared to the entire Sinai peninsula. South Sinai contains 472 plant species including 19 Egyptian endemic species, 115 of medicinal interest, and approximately 170 species used in folk medicine [5].

The family Solanaceae is considered one of the largest and most important families of flowering plants. It contains about 102 genera and about 2460 species in the global flora [6]. In Egyptian flora, according to Hepper [7], the family Solanaceae comprises about 25 genera and about 91 wild and cultivated species, while, this family comprises about eight genera and 30-33 wild species according to Boulos [8]. Hyoscyamus genus belonging to the family Solanaceae have 26 species in the flora of the world [9, 10]. While Boulos [11] mentioned that there are fifteen species distributed in North Africa and Western Europe to Central Asia. Seven species of Hyoscyamus genus are found in Egypt [8].

Hyoscyamus boveanus (Dunal) Asch. & Schweinf (*H. boveanus*) is a flowering plant belonging to the genus Hyoscyamus. It is a plant species endemic to Egypt. It was found that the optimum height range for this species to grow is between 1300 and 1650 meters above sea level. *H. boveanus* is vulnerable to extinction due to natural factors such as area dryness, climate changes and fragmentation inherent in its habitats, as well as human activities such as overgrazing, cutting, killing, in addition to the dams and random human construction [12, 13]. All plant species of the genus Hyoscyamus which are abundant in deserts and on arid land are rich sources in tropane alkaloids, especially hyoscyamine and scopolamine contents [14, 15], thus these species have high medicinal importance [16], where are widely used as an analgesic, and an anti-spasmodic, mydriasis, and anti-cholinergic, as well as a sedative and painless in popular medicine [17].

Seasonal variations for the chemical composition or biochemical ingredients have been reported in various plant species by several researchers. Different seasons affect the different biochemical attributes of the plant species, as variation significantly was observed in the active ingredients of these plants, and this variation is due to differences in environmental variables such as rainfall, temperature, and other variables [18, 19]. The evaluation of the results concerning the seasonal variation supports a biosynthetic pathway in *Mentha pulegium* L. plants [20]. Seasonal variation on chemical composition in soil and plants of *Lycium showii* was also studied by Kamel and El-Absy [21] and concluded that significant differences were observed to represent genuine environmental variations on chemical composition during seasons, sites, locations, and their interactions.

Due to public concern about increasing soil productivity and crop input efficiency, there must understand a relationship between spatially diverse soil properties and fertility [22]. The principal component analysis (PCA) can be used to summarize the main sources of data variance between correlated variables, as the PCA is a multivariate statistical technique for dimensional reduction that uses correlated variables to recombine and define the orthogonal linear of variables [23]. PCA is one of the statistical tools that can be used to study the relationship between the chemical properties of the soil/plant or the relationship between them. Several studies investigating the relationship between soil properties and vegetation have been conducted using PCA [23, 24, 25, 26].

The major aim of this research was to Quantitative determination and study seasonal variations of some metabolites of *H. boveanus* plants growing under natural conditions in Saint Katherine, South Sinai, Egypt, as well as, to elucidate the relationship between soil chemical properties and plant chemical composition contents by principal component analysis.

2. MATERIALS AND METHODS

2.1 Study area and Plant Material

The field study was conducted in July 2019 and January 2020 in the Wadi El-Sheikh Awad in Saint Katherine, South Sinai, Egypt. The research area is located along an altitudinal gradient ranging from 1120 to 1140m above sea level, within longitude (E) 33° 39' 06.00" and latitude (N) 28° 39' 05.00" [27]. El-Sheikh Awad area is located outside the Ring Dyke at the northern end of Nabq Al Hawa, an area of mixed sandstone and metamorphic rocks with great exposure to slabs, shallow sand and gravel wadis and garden areas [28]. In Wadi El-Sheikh Awad (Table 1), the average air temperature was much higher in the summer than in the winter. While average relative humidity and rainfall increased with the winter than the summer.

H. boveanus is a stout and rare succulent perennial herb, 45 – 60 cm high, with an unpleasant smell. The full plant including the inflorescence spreads densely hairy. Stem erect, green, herbaceous, cylindrical, branched forming a rounded bush, solid. Leaves are petiolate, alternate, apex acute, margin entire. The flowers are crowded, bisexual, arranged in one-sided racemes, its color is white with purple blotches and stripes, filaments and anthers have cream color, it appears in late spring. Fruit is capsule deep brown, ellipsoid. Seeds flat at lateral faces, luster, yellowish-brown [14, 29, 30]. Reproduction by seeds occurs at the late of the summer.

2.2 Soil Analysis

The soil samples associated with *H. boveanus* were collected from three random points at the two successive depths 0-20 cm and 20-40 cm in the Wadi El-Sheikh Awad. Three replicates were taken from each sample and carried to the laboratory in closed tins to be used for soil analyses. Soil samples were air-dried before sieving, then sieved and used for mechanical analysis of soil particles as outlined by Jackson [31] and Rowell [32] for soil texture. Soil moisture content was estimated by the method described by Rowell [32]. For each soil sample, Electrical conductivity (EC) and pH value were carried out using soil-water paste, according to Jackson [33], EC was expressed as mmhos/cm. The mineral contents in soil i.e., chloride (Cl^-), calcium (Ca^{2+}), manganese (Mn^{2+}), sodium (Na^+) and potassium (K^+) were determined using a saturation paste [34].

2.3 Plant Analysis

From Wadi El-Sheikh Awad, three fresh samples of *H. boveanus* were randomly collected during the summer (July, 2019) and the winter (January, 2020) seasons, and sealed in plastic bags, and stored at 4°C under dark conditions. Drying of collected plant materials was done in the oven at 70°C to a constant weight after which dried samples were milled to fine powder and stored in brown bags at room temperature pending chemical analyses. The plant water content is the difference between fresh weight (Wf) and dry weight (Wd) on the basis of fresh weight, and it can be calculated from the equation; water content% = [(Wf-Wd)/Wf]x100 [35]. Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), sulfur (S), iron (Fe^{2+}), phosphorus (P) concentrations were determined by atomic absorption spectrophotometry (GBC Avanta E, Victoria, Australia) [36]. Whilst, the chlorides were estimated following the $AgNO_3$ method according

Table 1. Seasonal mean temperature, Relative humidity and Rainfall at Wadi El-Sheikh Awad in Saint Katherine, South Sinai, Egypt

Meteorological / Seasons	Air Temperature (°C)	Relative Humidity (%)	Rainfall (mm)
Winter	12.92	59.47	0.91
Summer	30.14	69.39	0.00

to Jackson and Thomas [37] after extraction from the ashed powdered samples. Total nitrogen (N) content was determined using the micro-Kjeldahl method [38]. The photosynthetic pigments parameters were quantified spectrophotometrically, and using the wavelengths of 663, 645 and 470 nm, the chlorophyll a, chlorophyll b and total carotenoids were calculated by equations of Lichtenthaler [39], respectively. The total alkaloids, glycosides and the total phenols contents were determined following the methods described by Harborne [40], Hikino et al. [41] and Singleton and Rossi [42], respectively. The crude protein % was determined by multiplying the total nitrogen by 6.25 according to Allen [43]. The proline and the total available carbohydrate contents were determined according to the methods of Bates et al. [44] and Chaplin and Kennedy [45], respectively.

2.4 Statistical Analysis

To perform statistical analyses in this study, the XLSTAT version 2020.5.1.1075 software for Windows was used. The water content % in soil was treated statistically by applying the two-way ANOVA test. The Student's t-test was used to confirm whether the difference between depths or seasonal variations was significant for soil and plant analysis. The values of $p \leq 0.05$ were considered to be statistically significant [46]. The data of soil analysis are expressed as the mean (±standard error) of three replicates. Mean values of soil and plant measurements are shown in the tables or figures and a sign (*) to inform statistical significance for the reported differences. The principal component analysis was done using a computer software program PAST version 2.17c.

3. RESULTS AND DISCUSSION

3.1 Soil Analysis

The physical properties (%) for the soil associated with *H. boveanus* from the two depths in Wadi El-Sheikh Awad are given in Table 2. According to Student's t-test, significant differences in all mechanical properties were observed between the 0-20 and 20-40 cm depths except clay %. The same tendency was observed by Kamel and El-Absy [21] as well as El-Absy and Kamel [47] who reported that most physical properties were significantly affected by depths in *Teucrium polium* and *Lycium showii* species during different habitat conditions, respectively. Compared to other mechanical properties (%), coarse sand, followed by fine sand was registered the highest percentage at the two depths in Wadi El-Sheikh Awad. Fine sand and silt plus clay showed significant differences in the simple alluvial fan vegetation as *H. boveanus* of southern Sinai [48]. Thus, the soil physical properties indicated that the soil associated with *H. boveanus* at the two depths in Wadi El-Sheikh Awad is characterized by sandy to fine gravelly in texture. Poor fertility conditions to sandy texture are associated with low inputs of organic matter in arid environments [49], as Wadi El-Sheikh Awad environment.

According to two-ways ANOVA, the water content % was significantly different ($P < 0.05$) between seasons and season x depths interaction, while, the difference between the two depths was not statistically significant (Fig. 1). Kamel and El-Absy [21] and El-Lamey [50] also noticed significant variations in the water content of *Retama raetam* and *Lycium showii* species between seasons at different depths under different environmental conditions, respectively. The values of water content % in the winter season were notably higher than in the summer season during the two depths. The seasonal effect can lead to beneficial changes in the water content of the soil, such as an increase in the availability of phosphorus content needed for plants nutrition [51]. The soil associated with *H. boveanus* was characterized by a high water content % in the surface layer (depth 0-20 cm). The seasonal variation was found in water content by Kamel and El-Absy [21] and Ahmed et al. [52] who reported maximum values in the winter season compared to the summer season, due to rainfall, which leads to normal plant growth. While, Larcher [53] attributed the reason to the increase in total ions accumulation due to increased soil salinity and soil moisture stress. Moustafa and Zayed [48] indicated that plant species as *H. boveanus* are richer in drier habitats, that is, in the absence of high moisture. Also they added the moisture gradient is complex and associated with many environmental factors, which are elevation, slope, climatic drought, soil texture and soil surface nature.

The chemical analysis comparison of the soil associated with *H. boveanus* between the two depths showed in Table 3. The Student's t-test showed that the chemical analysis of the soil associated with *H. boveanus* has significant differences ($P < 0.05$) between the 0-20 and 20-40 depths. This result was consistent with Kamel and El-Absy [21], El-Lamey [50] and El-Absy et al. [54].

Table 2. The values of mean and standard deviations (±SD) of physical properties at the soil associated with *H. boveanus* in the two depths during Wadi El-Sheikh Awad

Depths (cm)	Physical properties (%)							
	Very Coarse Sand	Coarse Sand	Medium Sand	Fine Sand	Very Fine Sand	Slit	Clay	Soil Texture Class
0-20	8.30±0.11	50.12±0.45	2.21±0.09	28.03±0.19	2.08±0.07	4.03±0.12	5.23±0.10	Sandy
20-40	6.72±0.08	46.11±0.31	4.52±0.06	30.40±0.15	3.21±0.03	3.90±0.10	5.14±0.08	Sandy
Probability	0.002*	0.004*	0.001*	0.003*	0.001	0.002*	NS	

According to Student's t-test, the asterisks (*) denote a significant difference (p<0.05) between the two depths.

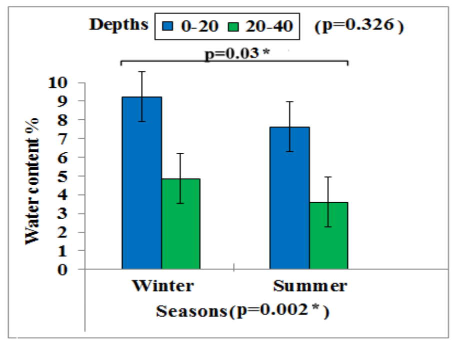

Fig. 1. Water content % at the soil associated with *H. boveanus* in the two depths and the two seasons during Wadi El-Sheikh Awad. According to two-way ANOVA test, the asterisks (*) denote a significant difference (p<0.05) between the two depths and the two seasons as well as their interactions

Table 3. The values of mean and standard deviations (±SD) of chemical properties at the soil associated with *H. boveanus* in the two depths during Wadi El-Sheikh Awad

Depths (cm)	Chemical Properties						
	pH	EC (dS/m)	Cl^- (meq/L)	Ca^{2+} (meq/L)	Mg^{2+} (meq/L)	Na^+ (meq/L)	K^+ (meq/L)
0-20	7.35±2.35	3.18±1.21	23.16 ±2.67	8.55±1.99	180.12±6.95	16.82 ±3.84	2.41±1.01
20-40	7.55 ±5.16	2.91±1.40	10.21 ±3.59	8.91±2.61	171.23±7.14	12.41±4.92	0.91±0.68
Probability	0.0001*	0.003*	0.0001*	0.002*	0.0001*	0.0001*	0.0001*

According to Student's t-test, the asterisks (*) denote a significant difference (p<0.05) between the two depths.

The values of electrical conductivity (EC), Cl^-, Mg^{2+}, $Na^+$and K^+ have increased significantly in 0-20 cm depth compared with their values in 20-40 cm depth. While, pH and Ca^{2+} values in 20-40 cm depth were significantly increased in comparison with the 0-20 cm depth. Of all chemical analysis in the soil associated with *H. boveanus* at the two depths in Wadi El-Sheikh Awad, Mg^{2+} content showed higher values relative to the other chemical analysis. Based on the pH values, the soil associated with *H. boveanus* in Wadi El-Sheikh Awad tended to be somewhat alkaline.

In general, our results agreed with Moustafa and Zayed [48] who mentioned that the soil associated with the *H. boveanus* plants was sandy to loamy sand, alkaline and not saline to slightly saline, also with a low content of basic nutrients and cation exchange capacity. Also, Labib et al. [55] reported that the soil properties indicated the weak organic nature of the study area in Saint Katherine. The results by Salama et al. [56] revealed significant differences for clay, calcium, magnesium, chlorides, electric conductivity, potassium and sulphates at the separated vegetation groups in South Sinai. Lower calcium, magnesium and potassium levels as well as total bases increased the acidic the soil [57]. Along the same lines, Zhao et al. [58] found that a higher concentration of calcium reduces the reaction of soil acids, in addition to affecting soil availability nutrient recycling and utilization. In agreement and disagreement with our results, they reported that soil pH and moisture content increased with increasing soil depth across the succession of vegetation cover, respectively. Often an inverse and adverse relationship was found between the high concentration of one cation in the soil and the availability of other cations for the plant to uptake [59]. As the availability of potassium depends on the availability of the relative quantities of potassium, calcium and magnesium in addition to the potassium content of the soil [60]. Hence, Laekemariam et al. [60] mentioned that the magnesium concentration is considered a potentially important factor in controlling potassium availability. The decrease in nutrient ion activities and the production of extreme ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+} and Cl^-/NO_3^- in the soil solution may be due to the high concentrations of Na^+ and Cl^- [61].

3.2 Plant Analysis

The effects of different seasons on photosynthetic pigments contents are presented in Fig. 2. All photosynthetic pigments contents of *H. boveanus* plant displayed statistically significant differences during the winter and summer seasons in Wadi El-Sheikh Awad, using Student’s t-test. These results are in line with earlier reports by Uvalle Sauceda et al. [62] and Devi et al. [63] who find significant differences in all photosynthetic pigments under different seasons. The concentrations of chlorophyll a (chl a), chlorophyll b (chl b), chl a+b, chl a/b, total carotenoids and total pigment were higher values in the summer season than in the winter season. Total carotenoids content was the highest of *H. boveanus* plant, followed by chl b and chl a contents during the two studied seasons. In contrast, Uvalle Sauceda et al. [62] mentioned that chlorophyll was higher than the carotenoids in all plants. There was a perceptible trend for seasonal variation in all photosynthetic pigments contents and were higher in the summer season than in the winter season [64], which might have been related to seasonal water deficits in summer and extremely low temperatures in winter [62]. The reasons for the decrease in the chlorophyll content during the winter season may be due to the chloroplasts degradation, the oxidative degradation of chlorophyll by the peroxidase enzymes degrading chlorophyll [65], low irradiance [66] and changing the pigment concentration [67]. The chlorophyll content of each leaf area reflects the adaptation of the different plant species to the environmental conditions of the area [68].

Based on Student’s t-test, statistically significant differences determined with respect to all concentrations of the mineral compositions (%) and water content (%) of *H. boveanus* in winter and summer seasons during Wadi El-Sheikh Awad, except Na^+ and N concentrations (Fig. 3). Significant differences trends among seasons in the minerals concentrations were in accordance with the results of Kamel and El-Absy [21], Glavac et al. [69] and Al-Qahtani et al. [70], but an opposite tendency was found by Williams and Chadwick [71]. During the two seasons, Ca^{2+}, Mg^{2+}, S, Cl^- and N concentrations and water content of *H. boveanus* plants increased in the winter season compared to the summer season. Whilst, the values of Na^+, K^+, P and Fe^{2+} concentrations in the summer season were higher than in the winter season of *H. boveanus* plants. These findings are in agreement with Kamel and El-Absy [21] and El-Lamey [50].

The water content % in plants is the most important physiological measure that affects the efficiency of photosynthesis, plant growth and biomass productivity, in addition to being an indicator of plant tolerance to drought and salinity, because water stress restricts transpiration, including stomata closure and water evaporation from the leaf surface [35]. Some chemical compositions such as K^+ and Na^+ revealed higher variation under salinity stress while some other chemical compositions like nitrogen showed higher variation under drought stress [72]. When stress intensity increases, the minerals uptake by the plant may decrease [73] and it was also found that some minerals in plants are reduced due to other minerals [21]. To adapt to diverse environmental stresses, the plants have evolved complex physiological and biochemical adaptations [74], and mineral concentrations in the tissues of the plant species have been positively correlated with their habitats [75]. The concentrations of Na^+ and K^+ and ion balance play important roles in plant salt tolerance [76].

In Fig. 4, the results of statistical analysis by Student's t-test exhibited that there was a significant influence on proline and total carbohydrates contents in *H. boveanus* plant between the two studied seasons. On the contrary, no statistically significant differences in crude protein % were noticed between the winter and summer seasons (Fig. 4). Similarly, results were reported by Gonzalez-Hernandez et al. [77] for crude protein, by Pouris et al. [78] for proline and by Al-Qahtani et al. [70] for total carbohydrates.

The contents of proline and total carbohydrates in *H. boveanus* plant in the summer season were higher than in the winter season, but crude protein % was lower in the summer season than in the winter season. Other studies have also the highest contents for total carbohydrates [70] and proline [79] in the summer season and for the crude protein in the winter season [50].

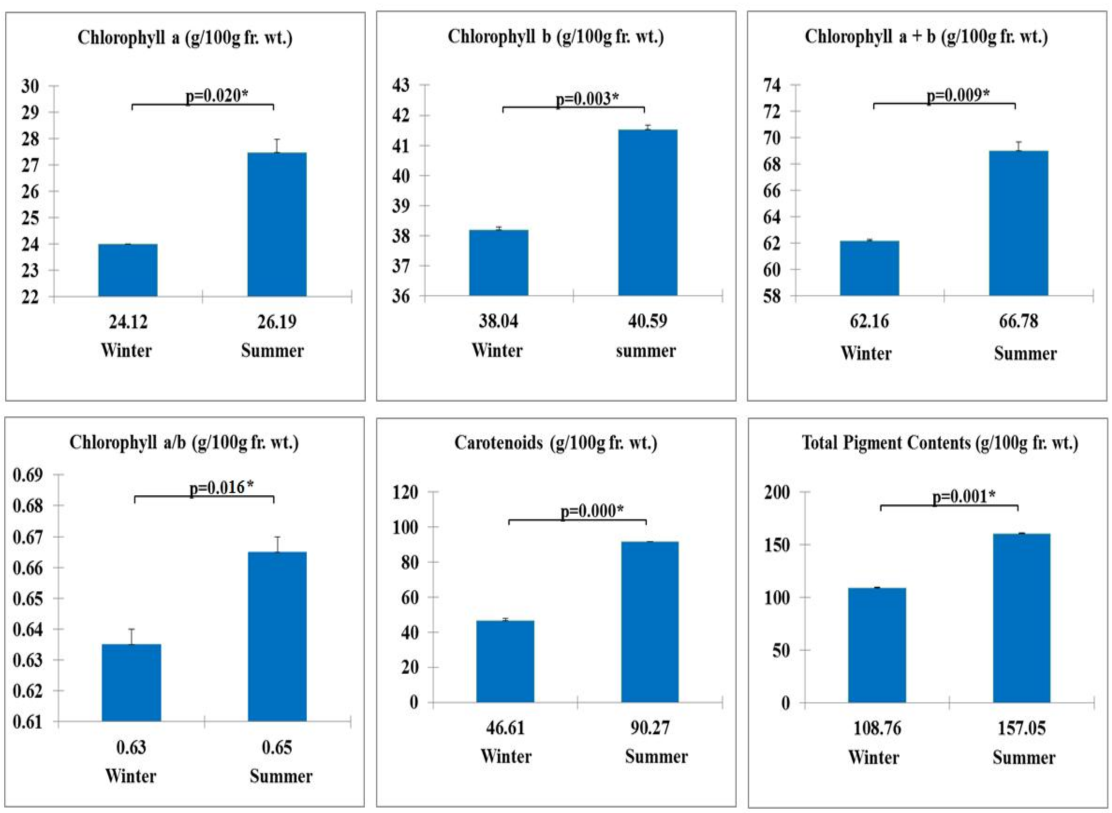

Fig. 2. Mean values of photosynthetic pigments contents in *H. boveanus* during different seasons. According to Student's t-test, the asterisks (*) denote a significant difference ($p<0.05$) between the winter and summer seasons

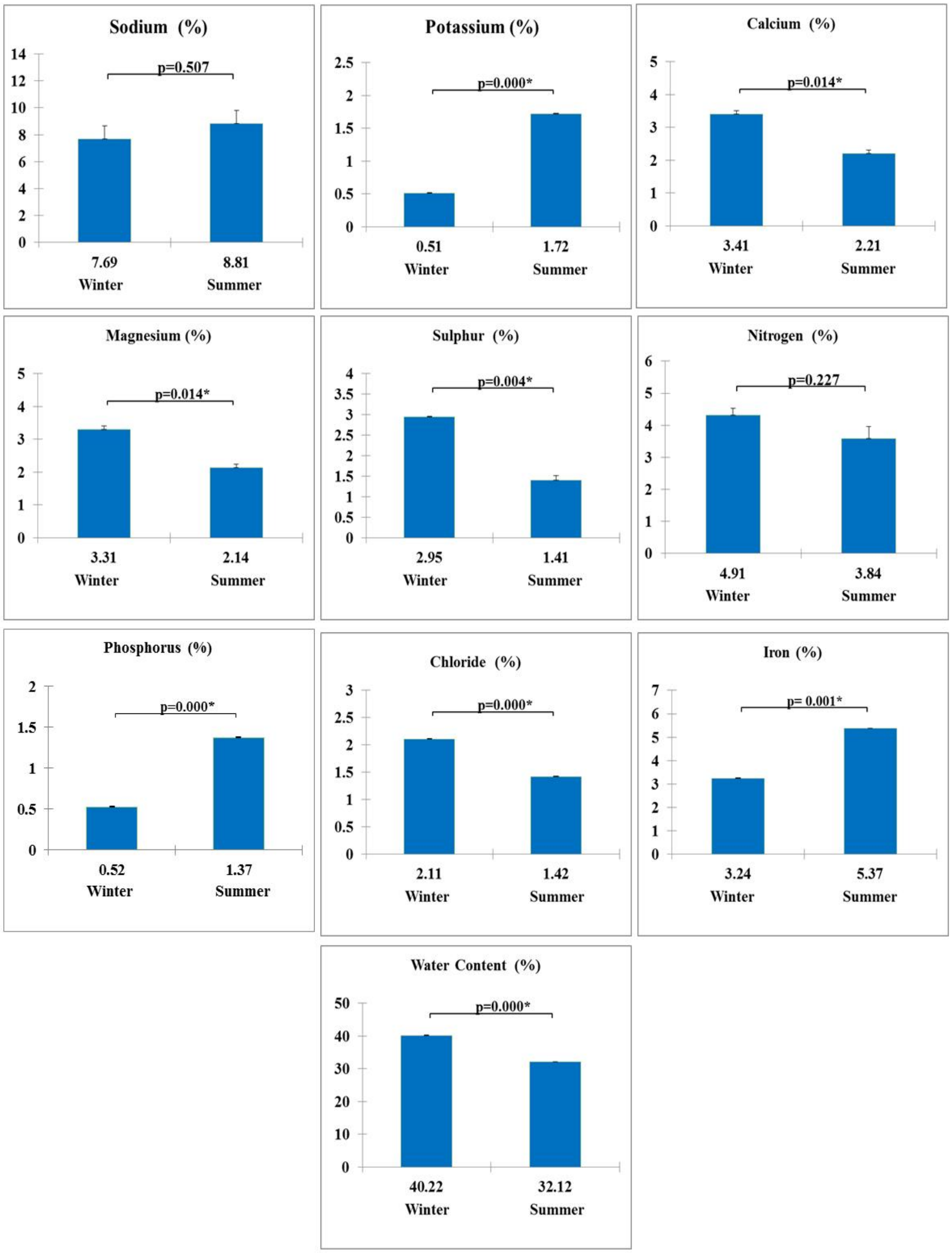

Fig. 3. Mean values of mineral composition and water content in *H. boveanus* during different seasons. According to Student's t-test, the asterisks (*) denote a significant difference ($p<0.05$) between the winter and summer seasons

Increased proline content can be used as an indicator of disturbed physiological conditions as drought and salinity stresses in most plant species [79, 80]. Proline accumulation in plants increased under drought and salinity conditions but was more under drought conditions [81, 82]. The increase in proline is due to the high total soluble salts in the soil [83].

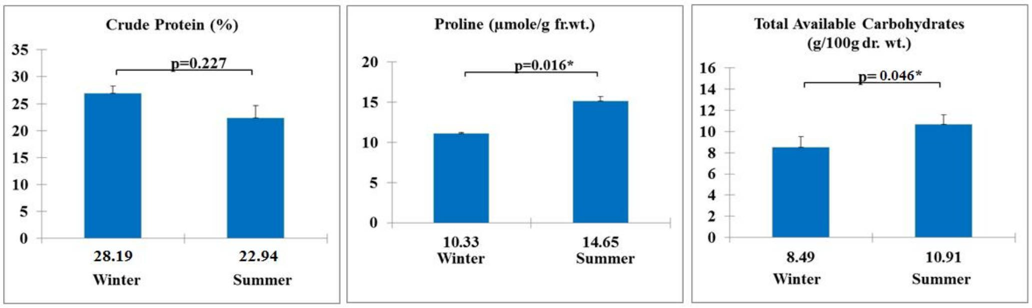

Fig. 4. Mean values of crude protein %, proline and total carbohydrates contents in *H. boveanus* during different seasons. According to Student's t-test, the asterisks (*) denote a significant difference ($p<0.05$) between the winter and summer seasons

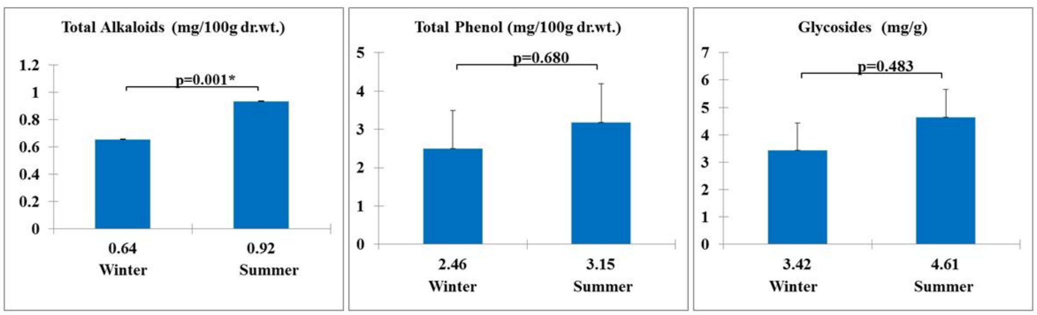

Fig. 5. Mean values of total alkaloids, glycosides and total phenol contents in *H. boveanus* during different seasons. According to Student's t-test, the asterisks (*) denote a significant difference ($p<0.05$) between the winter and summer seasons

Differences between the winter and summer seasons were statistically significant for the total alkaloids, but not statistical significance for the glycosides content and total phenol contents (Fig. 5). The studies carried out by Soni et al. [82], Salminen et al. [84], Elaloui et al. [85], Lin et al. [86] and Aoussar et al. [87] showed that secondary metabolites in some plant species are influenced significantly by seasonal variations. When comparing the two seasons, the total alkaloids, glycosides and total phenol contents in *H. boveanus* plant were higher in the summer season than in the winter season. El-Shazly et al. [14] detected 23 alkaloids in *H. boveanus*. Several studies noticed the effect of seasonal variation on secondary metabolites in different plants. Significantly higher total alkaloids and total phenolic compounds were higher in winter than summer [88], while the total alkaloids increased during winter than other seasons [89]. The difference in the secondary metabolites is due to the effect of the conditions of soil growth in addition to the humidity and temperature [90]. Cold weather decreases biosynthesis and consequently decreases some secondary metabolites compounds [91], while increasing with increasing light intensity [92]. On the basis of these results and the results of Soni et al. [82] and Deshmukh [93] secondary metabolites compounds increase with increasing water stress, especially the phenolic content through hydrolysis of glycosides.

3.3 Relationships between the Soil and Plant Variables

The PCA simplifies the complex data by transforming the number of correlated variables into a smaller number of variables in the data collection called principal components (PCs). Consequently, it has been used to understand the similarities and dissimilarities relationships between the soil and plant chemical variables during the winter and summer seasons of *H. boveanus*, which are graphically displayed in a biplot of PC1 and PC2 (Fig. 6). When comparing the winter and summer seasons, the PC1 and PC2 showed that the soil and plant chemical variables were distributed in different regions and formed different groups, and therefore these results indicate that there are differences between these variables during the winter and summer seasons. According to this biplot, the *H. boveanus* performance during the winter and summer seasons displayed a positive correlation among most soil and plant chemical variables, but, they differed in their degree and consistency in quantity. In this study, within PC1 and PC2, the soil and plant chemical variables that recorded the highest factor loading were selected as the most important contributor to the two PCs. In previous studies, the first two PCs indicated to those relationships between the variables, which were interpreted as a related response to some of the soil variables by Islabão et al. [24] as well as to some soil and plant variables by Juhos et al. [25], Ferraz et al. [26] and Gil et al. [94]. PCA indicated that changes in the relationship between variables under different stresses are reasonable [72].

The first PC1 had positively and highly correlated with soil Mg^{2+} as in the Fig. 6, and positively correlated with soil Cl^- (Fig. 6B). As for plant chemical variables, the PC1 showed positive and highly association with total pigment, Chl a+b (Fig. 6A) and water content (Fig B), and low positive association with carotenoids, Chl b (Fig. 6A) and crude protein (Fig. 6C). The PC2 had a high positive correlation with pH and Ca^{2+} (Fig. 6ABCD) and with EC (Fig. 6D), and moderate/low positive correlation with EC (Fig. 6ABC), Mg^{2+} (Fig. 6BCD) and K^+ (Fig. 6CD) in the soil of *H. boveanus*. Whilst, it had highly positively correlated with all photosynthetic pigments (Fig. 6A), with N, Cl^- (Fig. 6B), with crude protein (Fig. 6C) and with phenol (Fig. 6D), as well as low positive association with other variables in *H. boveanus*. On the other hand, the PC1 and PC2 under the winter and summer seasons were negatively correlated with the other soil and plant chemical variables. These results indicated that the PC1 had affected by soil magnesium and Chl b, Chl a+b, carotenoids and total pigment contents in plant, while, the PC2 had affected by most soil variables and all plant variables under study. The PC1 and PC2 in the Fig. 1 indicated that the chemical variables (Mg^{2+}, Cl^-, pH, EC, Ca^{2+} and K^+) of the soil associated with *H. boveanus* during the winter and summer seasons are variables with better soil chemical characteristics, which effect on the distribution of plants in the Wadi El-Sheikh Awad, South Sinai. This result was consistent with Omar [95] and Salama et al. [96]. The PC1 was highly positively correlated with EC and pH [25], with pH, K^+, Ca^{2+} and Mg^{2+} [26] and with N, P and K^+ [23], and highly negatively correlated with other soil variables. The PC2 showed a high positive correlation with soil pH [23, 25] and soil P [26], and displayed a negative correlation with other soil variables. Gil et al. [94] stated that the first two PCs has a correlation with some soil and plant variables studied. The PC1 correlation was low for crude protein content [97].

In soil variables, the high positive correlations were found among pH, EC and Ca^{2+}, among water content, Na^+ and K^+ as well as Na^+, water content and Cl^- under the winter and summer seasons (Fig. 6ABCD). This result was consistent with Islabão et al. [24], Ferraz et al. [26], who reported that the most properties of soil were significantly positively or negatively associated with each other. Regarding plant variables, high positive correlations were observed among all photosynthetic pigments (Fig. 6A), among all mineral contents (Fig. 6B), among crude protein, proline and total carbohydrates (Fig. 6C) as well as among alkaloids, phenol and glycosides (Fig. 6D) at the summer and winter seasons. A highly positive correlation between Na^+ and K^+, between proline and total protein, between proline and soluble carbohydrates, between chlorophyll a and carotenoids, between chlorophyll b and carotenoids as well as total chlorophyll and carotenoids were observed by Uvalle Sauceda et al. [62], Nejat and Sadeghi [72], Sims and Gamon [98], Murakeözy et al. [99], Kaspary et al. [100] and Sadeghi and Robati, [101]. Hendry and Price [102] confirm that the association between total chlorophyll and carotenoid concentrations plays an important role in protecting plants from stresses through photo-oxidation.

Soil magnesium had a highly positive correlation with plant variables i.e., crude protein (Fig 1C), WC (Fig 1B), Chl b, Chl a+b, carotenoids and total pigment contents (Fig. 6A). The pH, EC, and Ca^{2+} in soil were highly or moderately positively correlated with all photosynthetic pigments contents (Fig. 6A), with all the minerals contents (Fig. 6B), with crude protein, proline and carbohydrates (Fig. 6C) as well as with alkaloids, phenol and glycosides (Fig. 6D). A very low positive correlation was observed with crude protein, proline and carbohydrates (Fig. 6C) as well as with alkaloids, phenol and glycosides (Fig. 6D) at soil potassium. Non-correlations were noticed between other soil variables and studied plant variables. High correlation results between soil and plant variables indicate an effect of soil chemical variables on plant

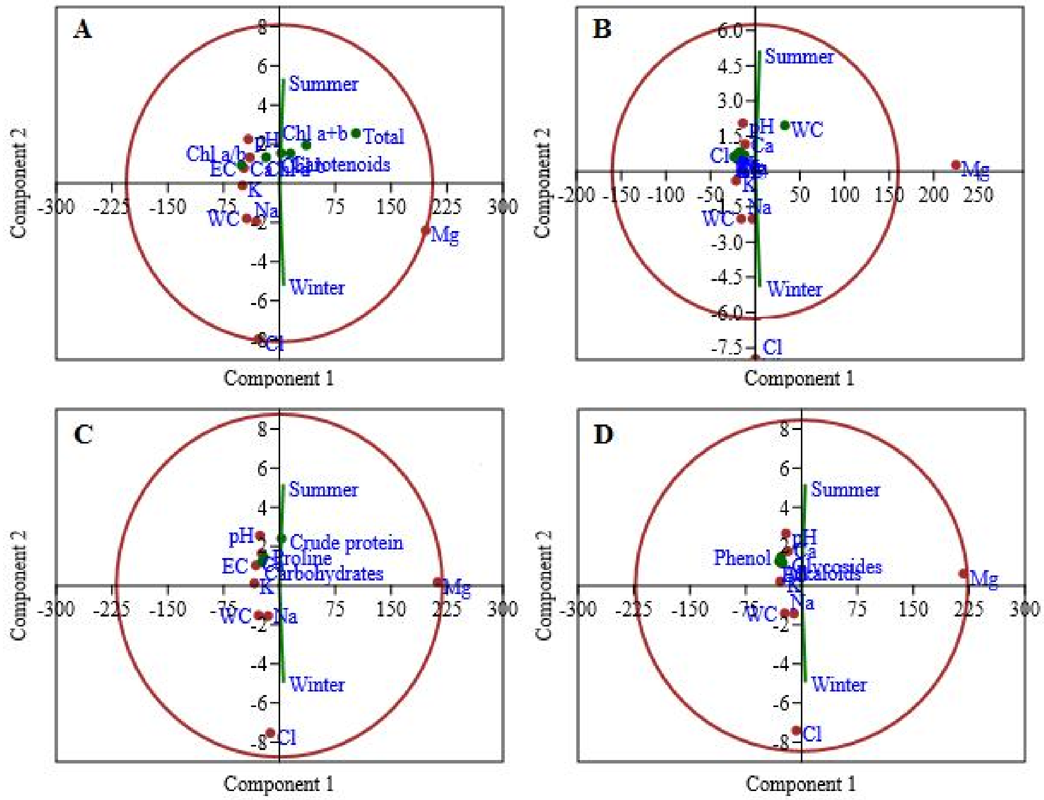

Fig. 6. Biplot diagram based on first two PCs axes of soil chemical variables (brown points) with (A) Photosynthetic pigments, (B) Mineral compositions and water content, (C) Crude protein %, proline and total carbohydrates, as well as with (D) total alkaloids, glycosides and total phenol contents in H. boveanus (green points) during different seasons. Symbols: EC: electrical conductivity; Cl-: chloride; Na+: sodium; K+: potassium; Ca2+: calcium; Mg2+: magnesium; S: sulfur; Fe2+: iron; P: phosphorus; N: total nitrogen; WC: water content; Total: total pigments

variables. Based on these correlations results, increasing these soil variables will increase plant variables. According to the PCA, the plant variables: Ca^{2+}, Mg^{2+}, proline, carbohydrate contents correlated significantly and positively with the most soil variables, but negatively with water content [94]. Carbohydrate contents show strong positive correlations with EC, but did not display any clear interactions with other soil variables studied [103]. While the plant species richness showed significant negative associations with EC, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cl^{-} [56]. The relationship between tropane alkaloids and nitrogen availability in *H. niger* was confirmed by Nassar et al. [104]. Gil et al. [94] reported that increased calcium and magnesium accumulation within plant cells could contribute to physiological salinity tolerance mechanisms. The harmful effect of sodium ions could be counteracted by increasing calcium and magnesium concentrations [105] and magnesium [106] to the extent that is non-toxic within cells, which gives plants a certain degree of tolerance. Gil et al. [94] cleared that the significant correlation between plant proline content and soil variables associated with environmental stress indicates the functional role of proline in the stress tolerance mechanisms of the plant species under study. Seasonal changes are the main factor effecting on the chemical composition of plants [107], as these reflect seasonal changes in physiological needs and efforts, rather than availability in plant content [108].

4. CONCLUSION

The results of the present study show that the soil and plant analysis of *H. boveanus* varied significantly during depths and seasons studied. Seasonal variation was observed for some plant chemical compositions, which increased significantly during the summer season than the winter season. The principal component analysis formed different groups from soil and plant variables based on the variation in the two seasons studied. According to the biplot diagram, clear and reasonable correlations were found among soil and plant variables, which may lead to the maintenance of cellular osmotic balance to protect the plant during different stress conditions.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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