

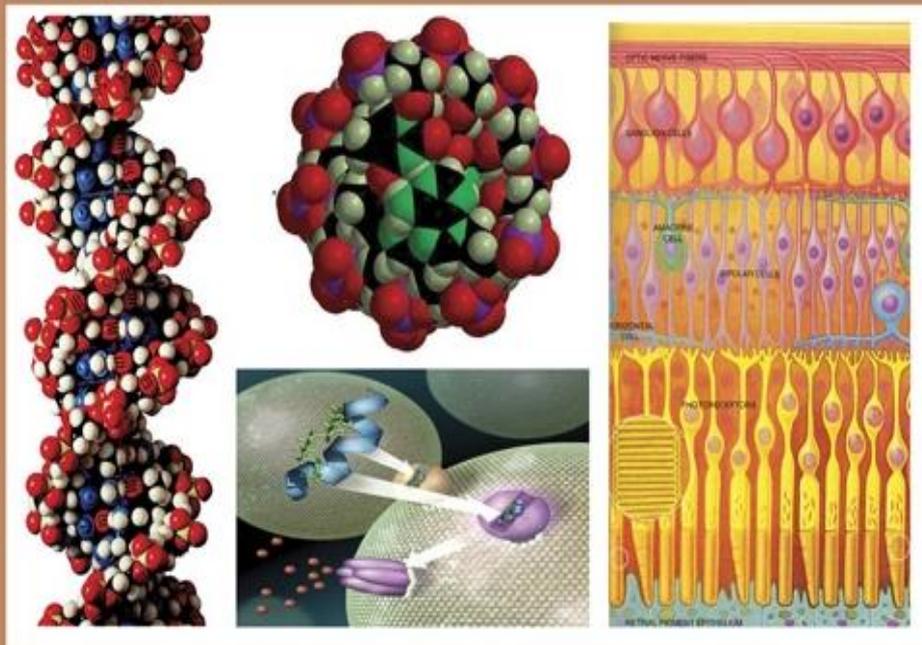


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EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULAR BIOLOGY



ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)



Ecophysiological Studies on the Oxidative Stress Responses of *Phlomis aurea*, *Ballota undulata* and *Nepeta septemcrenata* Endemic Plants in Saint Katherine Mountain, Egypt

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ARTICLE INFO

Article History

Received:30/5/2022

Accepted:12/7/2022

Available:16/7/2022

Keywords:

P. aurea, *B. undulata*,
N. Septem crenata,
Environmental stress,
Physico-chemical
parameters,
Antioxidant enzyme,
catalase (CAT),
superoxide dismutase
(SOD) and peroxidase
activity (POD).

ABSTRACT

Responses to antioxidants are reported in this research. Some indigenous plants have catalase (CAT), superoxide dismutase (SOD), and peroxidase activity (POD) as scavengers of reactive oxygen species (ROS) for mitigating environmental circumstances. In May 2019, physicochemical parameters such as EC, TDS, TSS, pH, HCO₃, CO₃⁻, Cl⁻, and SO₄ were measured. Five locations in Egypt's Saint Katherine Mountain were chosen. During the sampling process, A statistical analysis of Physico-chemical components discovered considerable variations in numerous chemical and physical soil variables among diverse wadis. Changes in HCO₃ were found to be non-significant, but substantial and highly-significant variations in other chemical and physical property measures were discovered among the five wadis examined. The current study found that in a stressed ecosystem, the plants chosen overcome the stress by altering their stress enzyme activities, implying evidence of adaptive mechanisms to thrive in a stressful environment and the suitability of three plant species—*Phlomis aurea*, *Ballota undulata*, and *Nepeta Septemcrenata* for environmental matrices (as indicators), particularly under altered climatic conditions.

INTRODUCTION

The mountains are known for their diverse biodiversity. As compared to lowland plants, most plant species at high elevations are isolated and have a limited number of niche habitats (Williams *et al.*, 2009). As a result, mountain plant populations exhibit a larger percentage of endemism than lowland plant populations (Alhathloul 2019, Habib *et al.*, 2020) Because of their narrow altitudinal distribution ranges, endemic species are more commonly endangered at high elevations (Grabherr *et al.*, 1994, Moustafa-Farag *et al.*, 2019). Most plants grow only near their climatic boundaries (El-Keblawy & Khedr 2017, Elkelish *et al.*, 2019) and climate restrictions largely influence high-altitude habitats.

The area over 2800 meters above sea level (a. s. l.) in the Saint Katherine protectorate (SKP), Sinai Peninsula, is remarkable not only for its magnificent sceneries but also for its richness of medicinal plants of national and international significance (Moustafa and Klopatek 1995., Abdel-Azeem *et al.*, 2019). As a result of various climatic conditions, elevations, and geography, SKP has a diverse range of ecosystems (Ayyad *et al.*, 2000).

SKP is home to 44 percent of Egypt's indigenous plant species, making it one of the most floristically varied places in the Middle East (Tackholm 1974).

Sinai has over 1285 species, with about 800 of them found in its southern portion (El-Esawi *et al.*, 2018). Several indigenous species in Egyptian flora have been found in previous investigations (Shaltout *et al.*, 2019). In Egypt, there are about 60 endemic plant species, with 31 of them on the Sinai Peninsula (which accounts for 51.6 percent of Egyptian endemism) (Boulos 1995). Twenty-four of these are unique to South Sinai and are recognized for their therapeutic characteristics as well as their usage in traditional medicine and treatments (El-Demerdash 2020).

Because of SKP's high elevation, severe and complicated climatic conditions limit plant to spread and variety (Shaltout *et al.*, 2015). Reduced O₂ and CO₂, strong winds, high sun irradiation, shallow rocky soils, cold temperatures, and poor water and nutrient content are some of the harsh circumstances (Roupioz *et al.*, 2016). Even though these stress conditions may slow plant development, many species have survived and evolved distinct adaptation mechanisms in response to them (Wonsick and Pinker 2014).

Several determinants affect the number of secondary metabolites in plants, including age, season, and nutrition status (Batiha *et al.* 2020., Rashad *et al.*2020). Similarly, environmental factors such as altitude, high/low temperature, drought, and light conditions cause significant quantitative

MATERIALS AND METHODS

Study Area:

Saint Katherine Mountain, Egypt's highest mountain, was selected for this research (2641 meters above sea level) It is situated in the heart of the southern Sinai triangle. Shaq Musa and Wadi Garagneia are two big deep canyons, and three mountains of black volcanic granite stand out starkly against the surrounding cliffs. The Katherine pluton is part of a Precambrian basement

and qualitative variations in secondary compound production among plants and population groups (Julkunen-Tiitto *et al.*1996). These factors include altitude, high/low temperature, drought, and light conditions (Gong *et al.*2018., Gea-Izquierdo *et al.*2012). Increased reactive oxygen species (ROS) production is caused by a combination of high-altitude environmental stress, which increases the risk of oxidative damage (Polle *et al* 1999., Soliman *et al.*2020).

Antioxidative enzymes such as ascorbate peroxidase, catalase, superoxide dismutase, and glutathione reductase may scavenge various ROS such as superoxide, hydroxyl radicals, and singlet oxygen (Conklin 2001 and Zamin *et al.* 2020). High mountain plant species have previously been shown to be adapted to high irradiance and cooling stress (Streb *et al.*2003). The synchronized action of enzymatic and non-enzymatic antioxidants in plants regulates the level of reactive oxygen species (ROS). Catalase (CAT), superoxide dismutase (SOD), and peroxidase activity (POD) are examples of enzymatic antioxidants that are important ROS scavengers (Chai and Wong 2012).

The antioxidant defense capability of plant samples from diverse habitats was explored in this study, as well as the eco-physiological reactions of some indigenous species as a result of their exposure to stressors existing at various habitats on Saint Katherine Mountain under varying conditions.

complex that contains acid plutonic and volcanic rocks in the southern Sinai Peninsula.

Plant Sample Gathering:

Plant samples were collected from five wadies (Wadi Gebal, Wadi Gragina, Wadi El-Arbae'en, and Wadi Abu-Tuweita) and Musa's Gorge in May 2019, for three plant species *Phlomis aurea*, *Ballota undulata*, and *Nepeta Septem crenata*.

Soils: Physico-Chemical Properties of

Soils:

A stainless-steel auger was used to obtain soil samples from 20 cm deep at each elevation. The samples were gathered at random from three locations and then pooled, sealed, and labeled in plastic bags. Soil samples were dried at 40°C in an air-forced oven. The dried materials were sieved to remove stones and plant residues before being processed in a stainless-steel mill and sieved at 2-mm intervals. The sieved soils were collected and kept until they could be analyzed further. Chemical tests of the soil were carried out according to Jackson's methodology Jackson (1967).

Certain Enzyme Extraction and Assaying Activities:

Superoxide dismutase (SOD) was assayed according to the method of Dhindsa *et al.* (1980). Peroxidase (POD) was measured as described by Polle *et al.* (1994). Catalase (CAT) was determined by the procedure described by Aebi (1984).

Statistical Analyses:

At the 0.05, 0.01, and 0.001 levels of probability, all data were statistically examined using one-way ANOVA and post hoc-LSD tests (the least significant difference) (Snedecor and Cochran 1982). The biochemical analysis data is the average of three replicates.

RESULTS**Variation in Soil Properties of among Different habitats.**

Tables 1, 2, and 3 show the mean soil parameters of the five different habitats that sustain the sampled plants. The means of soil characteristics reveal a highly significant variance in soil physical attributes (Table 1), a moderate significance difference, and a

non-significant difference. The most common forms of texture were loamy sand in Wadi Gebal and Musa's Gorge, and sandy clay loam in Wadi Graginya and Wadi El-Arbae'en, while the soil texture of W.AbuTuweita is sandy loam (Table 1).

Variations in the average soil chemical characteristics among the five wadis were recorded as follows:

Maximum values in PH, EC, TDS, CaCO₃, Na⁺, K⁺, and Mg⁺⁺ were found in Musa's Gorge, with values of 8.1, 158.81, 348.22, 31.30, 24.16, 74.44, and 5.16, respectively, whereas current minimum values in water content were found at 0.20.

Wadi Gebal has high water content, Cl, and HCO₃ values of 1.41, 18.50, and 14.47, respectively, whereas it has low EC, TDS, CaCO₃, Na, and SO₄ values of 107.15, 225.21, 12.08, 16.52, and 59.86.

With a score of 5.93, Wadi Graginya showed the highest result in organic matter percent. Wadi AbuTuweita reported a maximum value of 28 for Ca and the lowest values of 6.28, 2.58, 11.51, and 12.87 for PH, Mg, Cl, and HCO₃, respectively. Wadi El-Arbae'en, a high value of 70.37 was observed in SO₄, while the lowest values of 4.33, 21.19, and 18.5 were reported in organic matter, K, and Ca.

A statistical investigation of chemical and physical soil parameters indicated substantial variances in several chemical and physical soil variables among various wadis. Non-significant changes in HCO₃ were found, while significant and high-significant variances in other chemical and physical property parameters were found across the five wadis tested.

Table 1: A one-way analysis of variance (ANOVA) was used to compare the physical properties of soil in diverse habitats.

Habitat	Physical properties					
Wadi Name	Fine gravel	Coarse sand	Fine sand	Silt	Clay	Texture
Musa's Gorge	17.25 ± 0.25	31.11±0.20	21.22±0.23	16.27±0.25	10.8±0.25	Loamy sand
W. Gebal	10.5 ± 0.20	32.21±0.27	20.27±0.28	18.57±0.27	16.3±0.20	Loamy sand
W. Graginya	13.11±0.16	28.5±0.22	22.67±0.24	16.40±0.21	17.8±0.25	Sandy clay loam
W.AbuTuweita	7.45±0.28	17.1±0.25	33.67±0.27	20.47±0.24	17.3±0.28	Sandy loam
W. El-Arbaeen	11.5±0.23	23.01±0.20	24.09±0.25	21.87±0.29	18.7±0.29	Sandy clay loam
F ratio	221.5	498.75	401.87	111.61	160.2	--
P-value	***	***	***	***	***	--

Each statistic represents the average of 3 replicates with standard errors. *** = significance at P < 0.001.

Table 2: A one-way analysis of variance (ANOVA) was used to investigate the chemical properties of soil in diverse habitats

Habitat	Chemical properties					
Wadi Name	pH	EC ($\mu\text{s}/\text{cm}$)	T.D.S (ppm)	Water Content (%)	Organic matter (%)	CaCO ₃ (%)
Musa's Gorge	8.1±0.05	158.81±0.70	348.22±0.84	0.20±0.06	4.72±0.17	31.3±0.13
W. Gebal	7.51±0.06	118.30±0.78	244.22±0.83	1.41±0.08	4.89±0.14	17.18±0.16
W. Graginya	6.89±0.03	107.15±0.71	225.21±0.84	0.45±0.09	5.93±0.15	12.08±0.15
W. AbuTuweita	6.28±0.06	127.22±0.71	252.20±0.85	0.34±0.07	4.55±0.16	14.23±0.15
W. El-Arbaeen	6.38±0.4	116.60±.72	233.21±0.89	0.50±0.08	4.33±0.18	16.12±0.17
F ratio	5.837	1012.4	3604.07	30.78	21.66	2367.82
P-value	**	***	***	***	***	***

Each statistic represents the average of 3 replicates with standard errors. ** = significance at $P < 0.01$, *** = significance at $P < 0.001$.

Table 3: A one-way analysis of variance (ANOVA) was used to investigate the chemical properties (water-soluble ions) of soil in diverse habitats.

Habitat	Chemical properties (Water Soluble Ions)						
Wadi Name	Na ⁺ (ppm)	K ⁺ (ppm)	Ca ⁺⁺ (meq/L)	Mg ⁺⁺ (meq/L)	Cl ⁻ (meq/L)	HCO ₃ ⁻ (meq/L)	SO ₄ ⁻ (meq/l)
Musa's Gorge	24.16±0.16	74.44±0.28	27±0.17	5.16±0.14	12.30±0.03	13.87±0.66	67.04±0.27
W. Gebal	17.12±0.15	35.30±0.14	24±0.20	2.76±0.12	18.50±0.02	14.47±0.72	68.96±0.29
W. Graginya	16.52±0.14	24.38±0.25	22±0.25	1.98±0.16	16.20±0.01	12.97±0.70	59.86±0.28
W. AbuTuweita	19.76±0.12	38.77±0.45	28±0.24	2.58±0.20	11.51±0.02	12.87±0.66	62.41±0.26
W. El-Arbaeen	18.50±0.11	21.19±0.28	18.5±0.22	2.75±0.21	17.10±0.04	12.97±0.75	70.37±0.23
F ratio	316121.1	3283.33	211.04	149.77	38103.21	1.185	1204.83
P value	***	***	***	***	***	NS	***

Each statistic is based on the average of 3 replicates with standard errors. NS = non-significant, whereas *** = significant at $P < 0.001$.

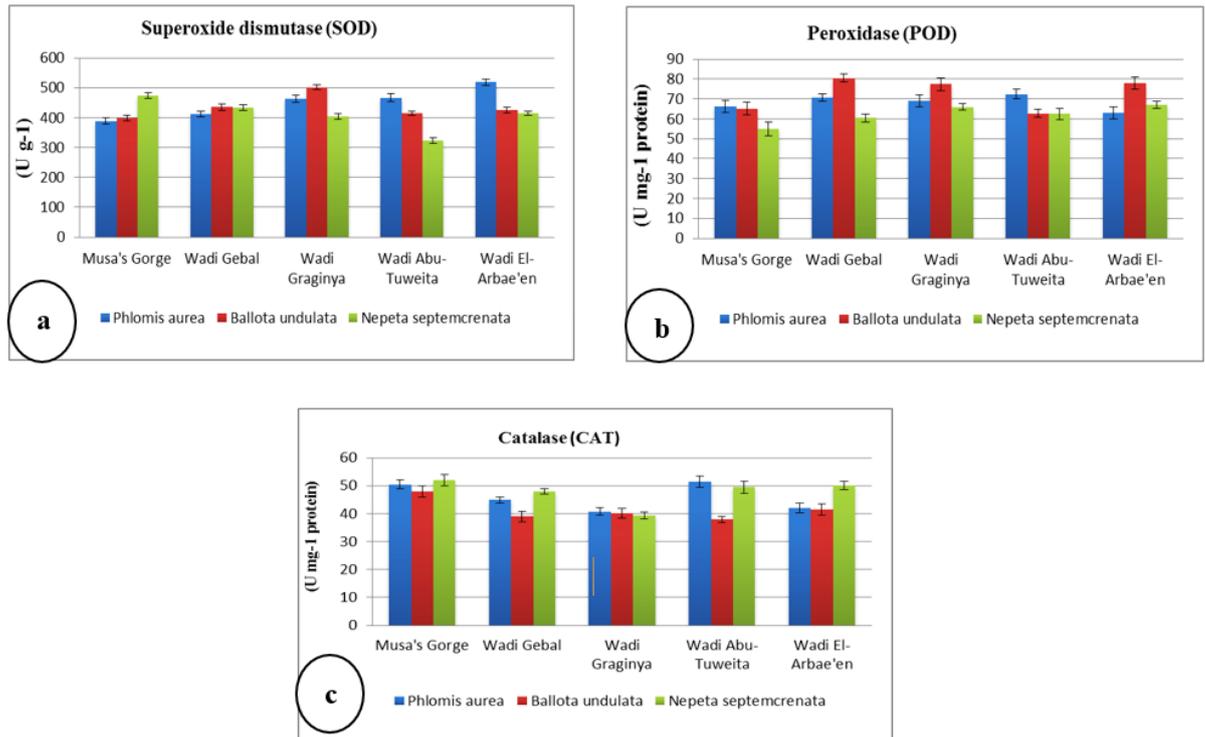
Antioxidant Enzymes:

Figure 1(a,b and c) depict the mean of antioxidant enzyme activity. These findings suggest that the largest value of superoxide dismutase (SOD) was found in *phlomis aurea* plants in W. El-Arbaeen (fig 1). *Phlomis aurea* plants exhibited the highest maximum value (518.4 U/g⁻¹) in W. El-Arbaeen and the lowest minimum value (388.42 U/g⁻¹) at Musa's Gorge at the species level. W. Graginya had the highest value (501.7U/g⁻¹) while Musa's Gorge had the lowest value (398.98U/g⁻¹). Plants of *Nepeta septemcrenata* had the highest value (473.2 U/g⁻¹) at Musa's Gorge and the lowest value (323.7 U/g⁻¹) at W. AbuTuweita.

In *phlomis aurea* plants, peroxidase (POD) had the highest value in W. AbuTuweita (72.45 /U mg⁻¹ protein) and the lowest value in W. El-Arbaeen (63.13

U/ mg⁻¹ protein). W. Gebal plants had the highest value (80.43 U/ mg⁻¹ protein) while W. Abu Tuweita plants had the lowest value (62.73 U/ mg⁻¹ protein). Near W. El-Arbaeen, *Nepeta septemcrenata* plants had the highest value (67.04 U/ mg⁻¹ protein) and the lowest value (54.88 U/mg⁻¹ protein) at Musa's Gorge.

Phlomis aurea plant samples collected in W. AbuTuweita had a maximum value of (CAT) 51.45 U/ mg⁻¹ protein and a minimum value of 40.77 U/ mg⁻¹ protein. At Musa's Gorge, *Ballota undulata* plants had the highest value (47.94 U/mg⁻¹ protein) and the lowest value (37.92 U/ mg⁻¹ protein), whereas, at W. AbuTuweita, had the lowest value (37.92 U/ mg⁻¹ protein). At Musa's Gorge, plants of *Nepeta septemcrenata* exhibited the highest value (52.01 U /mg⁻¹ protein) and the lowest value (39.42 U/ mg⁻¹ protein) (Fig.1c).



Figs.1. Variation in antioxidant enzyme activity (SOD), (POD), and (CAT) of plant species collected from Saint Katherine's five habitats.

DISCUSSION

Despite their ecological importance and numerous intriguing qualities, these unique species' ecophysiology has received little attention, particularly at the SKP high elevations (Hegazy 2016., Helal *et al.* 2020). As a result, understanding how such adaptations affect plant development is important for researchers who want to forecast how endemic species may respond to climate change in the future.

Elevation affects landscape topography, geology, rainfall volume, and, as a result, soil moisture and texture, groundwater depth, hydrology, evaporation, soil type, and vegetation itself (Knoop and Walker 1985). According to the findings, the majority, if not all, of the metabolic components evaluated were significantly altered by changes in elevation rankings and habitats. The metabolic components of the plants differed substantially throughout the elevation ranks of the three species studied. Several other researchers obtained similar results. Plants in semi-arid environments have many physiological features that allow

them to adapt to adverse conditions such as drought and strong light (Kramer 1983., Tanaka-Oda *et al.* 2010).

Plants in high mountain environments are subjected to unfavorable or even dangerous abiotic conditions, which change growth dynamics and endanger their survival. This is especially true for species that are endangered or endemic El-Keblawy and Khedr (2017). In the SKP Mountains, there is a significant contrast between habitats. The spread and intensity of plant growth are reduced (Moustafa and Zaghloul 1996). This might be ascribed to the preponderance of adverse climatic conditions in the highest region of the SKP Mountains. The current study's results (Table 2) for chemical and physical soil parameters indicated considerable variations amongst wadis in a variety of chemical and physical soil variables. Changes in HCO_3^- were found to be non-significant, but there were considerable and extremely significant differences in other chemical and physical property parameters across the five wadis studied. Low temperature and oxygen

deficiency (Neina 2019., Santiago 2000). water precipitation, light intensity, and UV radiation (Soethe *et al.* 2008., Sharma *et al.* 2014). are additional adverse biotic features of the SKP as a high elevation habitat. In general, a complex interplay between abiotic stresses and plant development has resulted in numerous physiological features through adaptation, acclimation, and speciation, which may differ between plant species (Chen *et al.* 2019., Pandey *et al.* 2017).

Abiotic stress disrupts cellular homeostasis in the plant species under consideration, resulting in the generation of free radicals, which can cause oxidative damage (Kusvuran 2012, Kusvuran *et al.* 2016). Furthermore, high light intensity and UV-B radiation are among the most aggressive stressors in the SKP's high alpine area (El-Ghani *et al.* 2017). Under these circumstances, electrons released from excited chlorophylls may be transported from photosystem I of the photosynthetic process to O₂ to produce superoxide radicals, triggering a chain reaction of free radical liberation (Logan 2007). At low concentrations, hydrogen peroxide acts as a signaling molecule to induce the defense responses of plants under stress, but at high concentrations, it can cause significant disruption in metabolism through damage to lipids of membranes and nucleic acids, conformational changes of enzymic proteins, destruction of thiol-containing compounds, and so on (Sharma and Dubey 2007).

All sorts of abiotic or biotic stressors enhance the formation of hazardous oxygen derivatives. Plants that thrive under oxidative stress actively activate antioxidant systems, which aid in adaptability. Through the scavenging of free radicals, antioxidant defense mechanisms can counteract oxidative stress (Sharma *et al.*, 2012) The results (Figures 1–3) showed that plant species grown in diverse environments, where plants thrive under relatively low-stress conditions, rely on antioxidant enzymes and antioxidant chemicals to scavenge free radicals (Hasanuzzaman *et al.* 2019., Gong *et al.* 2018). The findings of the

current study (Table 1) suggested that the highest value of superoxide dismutase (SOD) was discovered in *Phlomis aurea* plants, indicating a reversal trend of change in the activity level of antioxidant enzymes (catalase, superoxide dismutase, and peroxidase) in all species. In W. El-Arbaeen, *Phlomis aurea* plants had the highest maximum value (518.4 U/g-1). W. Gebal had the highest value (80.43 U/ mg-1 protein) of *Ballota undulata* plants in this regard. Plants of *Nepeta septemcrenata* also had the highest value (52.01 U /mg-1 protein) in Musa's Gorge. Following these findings, Yang (Srivastava 2008). discovered that plant cells and tissues exhibit a variety of metabolic reactions to environmental stress, some of which may be adaptive (El-Esawi 2017). The simultaneous activity of enzymatic and non-enzymatic antioxidants regulates the number of reactive oxygen species (ROS) in plants. Catalase (CAT), superoxide dismutase (SOD), and peroxidase activity (POD) are three enzymatic antioxidants that are essential ROS scavengers (Chai and Wong 2012). The mechanisms of action of various antioxidants in the alleviation of oxidative stress vary (Elkeilsh *et al.* 2020).

Environmental differences (such as light, temperature, soil qualities, and altitude) significantly contribute to the antioxidant activity of indigenous medicinal plants (Liu *et al.* 2016). Many substantial differences in the antioxidant activity and soil chemical characteristics of three indigenous targeted species gathered from five distinct environments were discovered in this investigation. To respond to high abiotic stress, *Phlomis aurea* relies mostly on raising the activity of its antioxidant enzymes.

Conclusions

Environmental factors play a significant role in variation in vegetation distribution and plant community structure, with this variation in plant community structure producing a noticeable difference in most environmental factors such as gradients in elevation, temperature, and rainfall between different locations. It was revealed that poor environmental conditions

induced by abiotic factors including moisture, salinity, and mineral elements can limit plant growth and development, as well as cause physiological and biochemical abnormalities. Finally, the wide range of antioxidant activity in these plants may result in significant variances in their efficacy as herbal medications.

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ARABIC SUMMARY

دراسات فسيولوجية بيئية حول استجابات الإجهاد التأكسدي للنباتات المستوطنة (العورور والقاصه و الزيتيه) في جبل سانت كاترين، مصر.

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في هذا البحث تم تقييم مضادات الأكسدة الانزيمي وعلاقتها بالظروف الكيميائية والفيزيائية لثلاث من النباتات المتوطن m في منطقة سانت كاترين في مصر. في هذا البحث تم تقييم نشاط انزيمات الكاتاليز والديزيموتيز وكذلك نشاط انزيم البيروكسيداز كمضادات للشوارد الحرة للاوكسجين للتخفيف من الظروف البيئية . تم قياس الخواص الكيميائية والفيزيائية مثل EC، TDS، TSS، pH، HCO₃⁻، CO₃⁻، Cl⁻، SO₄. تم اختيار خمسة مواقع في جبل سانت كاترين في مصر. أثناء عملية أخذ العينات، بينت التحاليل الاحصائية للمكونات الفيزيائية والكيميائية اختلافات كبيرة في العديد من متغيرات التربة الكيميائية والفيزيائية بين الوديان المتنوعة (المواقع الخمس سابقة الذكر) . كانت التغيرات في HCO₃ غير عالية القيمة المعنويه ، بينما لوحظ اختلافات جوهرية وعالية الأهمية في مقاييس الخواص الكيميائية والفيزيائية الأخرى بين الوديان الخمسة التي تم فحصها. وجدت الدراسة الحالية أنه في النظام البيئي المجهد، تتغلب النباتات المختارة على الإجهاد عن طريق تغيير أنشطة إنزيمات الإجهاد، مما يدل على وجود ميكانيكية التكيف للنمو في بيئة مجهد وملائمة لثلاثة أنواع نباتية هي (العورور والقاصه و الزيتيه) للمصفوفات البيئية كمؤشرات، لاسيما في ظل الظروف المناخية المتغيرة.

الكلمات المفتاحية: العورور، القاصه، الزيتيه، مضاد الاكسده، العوامل الفيزيائية والكيميائية