

ANTIOXIDANTS RESPONSE TO SEASONAL CHANGES IN *THYMUS CAPITATUS* (L.)

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This paper aims to study the impact of microhabitats and seasonal variations on antioxidants components in *Thymus capitatus* plants growing in nature. *T. capitatus* is a perennial xerophyte growing naturally at calcareous hills and wadis closest to the Mediterranean region. Freshly terminal shoots of *T. capitatus* and associated soil were collected from Wadi Habis (west Matrouh), Egypt. The plant samples were studied from three microhabitats; upstream, wadi-runnel and wadi-bed during winter and summer, 2018. Soil physical analysis was done. In plant samples, water content, malondialdehyde (MDA), specific activities of peroxidase (POD), polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL) and proline content, total phenols, anthocyanin, chlorophyll a, chlorophyll b, and carotenoids, were determined. The specific activity of PPO was increased in the summer season while water content increased in winter. The other parameters were not changed significantly by seasonal variations. Regarding microhabitats effect, MDA attained the highest value in those at upstream, proline content was found higher at wadi-runnel, while PPO, POD, PAL, chlorophyll a, chlorophyll b, carotenoids, anthocyanin and total phenols were the highest in those at wadi-bed. Our results indicated that *T. capitatus* is highly tolerant to drought stress via keeping stability on MDA content.

Keywords: antioxidant enzymes, anthocyanin, total phenols; *Thymus capitatus*

INTRODUCTION

In Egypt, *Thymus capitatus* (L.) Link is one among three species of *Thymus* genus included in Lamiaceae family, growing in calcareous hills at the Mediterranean region. It is a low shrub, much branched stems, small linear leaves 3-8 × 0.5-1 mm, flowers in ovoid heads 0.6-1.5 cm (Boulos, 2002). *T. capitatus* is rich with essential oils in its aerial parts with ~2.6% of their dry weight (Tagnaout et al., 2022). Recently, the essential oils of *Thymus* species

are widely used in food industries to increase the shelf-life (Martínez et al., 2018), perfumes, cosmetic and medicinal purposes (Hazzit et al., 2009) and in some biological properties *viz.* antioxidant, antifungal and antibacterial (Masaada et al., 2015; Casiglia et al., 2019; Benoutman et al., 2022 and Tagnaout et al., 2022). The use of this plant is common as a natural source of antioxidants, as plants are rich in phenolic and flavonoid compounds (Jabri-Karouia et al., 2012 and Benoutman et al., 2022).

Drought stress can cause an imbalance between photochemical and biochemical processes in the leaves, which includes reactive oxygen species (ROS) production and generation of oxidative stress (Fang and Xiong, 2015 and Zhang et al., 2019). However, the ROS at little concentrations are responsible to drive normal metabolism in different plant cell categories (Baxter et al., 2014), the higher concentrations of them can cause deleterious damage in nucleic acids, proteins, lipid membrane, cell structure and metabolism (Mittler and Blumwald, 2015). Malondialdehyde (MDA) is an indicator for lipid peroxidation and oxidative damage, resulting in disruption of metabolic function and loss of cellular integrity (Tsikas, 2017 and Abd El-Maboud, 2019). The excessive ROS must be counteracted by antioxidants to resist damage development and cell death. Plants are equipped with two antioxidant defense systems enzymatic and non-enzymatic to protect their cells from ROS (Hoque et al., 2008 and Zhang et al., 2019). The enzymatic antioxidant joins superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, peroxidase and polyphenol oxidase (Murshed et al., 2013; Abd El-Maboud et al., 2018 and Guo et al., 2018). The non-enzymatic antioxidant encompasses photosynthetic pigments, ascorbate, proline, glutathione, betaine and phenolic compounds (Abd El-Maboud and Eisa, 2016 and Abd El-Maboud, 2019). Indeed, non-enzymatic antioxidant has been emphasized as the only effective means to stop the harmful effects of hydroxyl radicals on cellular structures (Bose et al., 2014). Altitudinal gradients and seasonal changes effects on antioxidant capacity and total phenols in the three Lamiaceae; *Salvia officinalis*, *Sideritis perfoliata* L. subsp. *perfoliate* and *Mentha spicata* have been studied (Chrysargyris et al., 2021). Also, the seasonal effect on some internal antioxidant compounds of some halophytes has been done (Abd El-Maboud, 2019).

Although there are some studies confirmed that *T. capitatus* has antioxidant properties (Masaada et al., 2015; Benoutman et al., 2022 and Tagnaout et al., 2022), the information generated is not enough, especially in the antioxidant enzymes system. Thus, our study aims to determine enzymatic and non-enzymatic antioxidants as affected by seasonal changes in *T. capitatus* growing naturally at Wadi Habis (west Matruh, Egypt) during winter and summer seasons.

MATERIALS AND METHODS

Fresh shoot of *T. capitatus* was collected from three different microhabitats; upstream portion, runnel and wadi-bed along Wadi Habis (west Matruh, Egypt). Plant samples were taken randomly from three plants in three replications for each habitat. Also, soil samples were collected from three replications at 0-20 cm depth, dried and powdered smoothly using wooden holder and passed through 2 mm sieves. The soil of Wadi Habis has sandy clay loam texture except at upstream has sandy loam texture, pH ranged from 7.6 at upstream to 8.0 at wadi bed, low electrical conductivity, and moisture content was lower than 2% in the dry season and increased up to 14.87% at wadi bed in winter as shown in Table (1). The geographical position of the three studied sites were 31° 21.852 N, 27° 01.638 E, 89 m alt for upstream, 31° 22.721 N, 27° 02.008 E, 46 m alt for runnel and 31° 22.861N, 27° 02.228 E, 20 m alt for wadi bed. Wadi Habis belongs to the subtropical Mediterranean climate (ranges from mild and wet winter to dry summer), wherever average precipitation around 140 mm falls usually from October to February (Abdel Moghith et al., 2015). Fresh plant samples were washed with tap water followed by distilled water before subjected to laboratory analyses to determine lipid peroxidation, polyphenoloxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL) specific activities and contents of chlorophyll (Chl.) a and b, carotenoid, anthocyanin, proline and total phenolics.

Table (1). Soil characters supporting *T. capitatus*.

Habitat	Season	Moisture content (%)	pH	EC (ds m ⁻¹)	Sand %	Silt %	Clay %	Texture
Upstream	Winter	11.45	7.6	0.261	73.48	11.84	14.68	Sandy loamy
	Summer	1.49	7.8	0.347	70.29	13.97	15.74	Sandy loamy
Wadi-runnel	Winter	13.41	7.8	0.702	61.18	18.50	20.32	Sandy clay loam
	Summer	1.65	7.8	0.800	61.00	18.55	20.45	Sandy clay loam
Wadi-bed	Winter	14.87	7.9	0.460	63.82	13.21	22.97	Sandy clay loam
	Summer	1.85	8.0	0.852	63.22	13.54	23.24	Sandy clay loam

1. Enzymes Extraction

Fresh leaves were weighed and ground in 0.1 M sodium phosphate buffer, pH = 7, including 1% polyvinylpyrrolidone and 0.1 mM EDTA. Then spun at 4500 rpm for 15 min at 4°C and the supernatant obtained was used for measuring PPO, POD and PAL activities. Also, soluble protein in the enzyme extract was estimated as described by Lowry et al. (1951) using bovine serum albumin as standard.

2. Malondialdehyde (MDA) Concentration

The level of lipid peroxidation was determined in term of MDA content as reported by Heath and Packer (1968). Half g fresh samples were amalgamated by 2.5 ml of 0.1% TCA, and then centrifuged at 5000 rpm for 10 min at 4°C. For 0.5 ml of supernatant, 1.5 ml 5% TBA (dissolved in 20% TCA) was added. The mixture was incubated in water bath at 95°C for 25 min, cooled then centrifuged and the absorbance was read at 532 and 600 nm. The results were expressed as $\mu\text{mol g}^{-1}$ FW.

3. Peroxidase (POD) Assay

Peroxidase activity was assayed as mentioned by Hammerschmidt et al. (1982). The assay mixture consisted of 50 mM phosphate buffer (pH = 6.6), 0.3% H_2O_2 and 1% guaiacol mixed as 8:1:1, respectively. An amount of 2.9 ml of the assay mixture was taken in a test tube, and then 0.1 ml of enzyme extract was added to start the reaction. The absorbance reading was taken down every 30 s for 3 min at 470 nm using spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech). The change rate in absorbance per minute was totalized and one unit of enzyme was expressed as $\Delta\text{OD} = 0.01$. The POD activity was viewed as unit mg^{-1} protein.

4. Polyphenoloxidase (PPO) Assay

The reaction mixture contained 0.1 ml enzyme extract, 0.6 ml of 20 mM catechol and 2.3 ml phosphate buffer (0.1 M, pH = 6.5). The absorbance was read at 420 nm immediately and after 1 min as described by Oktay et al. (1995), using spectrophotometer (UV- Vis spectrophotometer UV 9100 B, Lab Tech). The quantity of enzyme that caused an increase in absorbance by 0.001 per min was termed as a unit of PPO activity and expressed as unit mg^{-1} protein.

5. Phenylalanine Ammonia Lyase (PAL) Assay

PAL assay was quantified according to Lister et al. (1996). The reaction mixture included 0.1 ml enzyme extract, 1.9 ml of 0.05 M Tris-HCl buffer (pH 8.8) and 1 ml of 0.02 M L-phenylalanine. Then allowed to proceed for 1 h at 37°C. After that the reaction was terminated by adding 0.2 ml of 6 M HCl. A unit of enzyme activity was equivalent to the amount of enzyme that preceded an increase in absorbance of 0.01 per hour at 290 nm using spectrophotometer (UV- Vis spectrophotometer UV 9100 B, Lab Tech). Data expressed as unit mg^{-1} protein.

6. Chlorophyll a, b and Carotenoids

Chlorophyll a, b and carotenoids were extracted and assayed according to A.O.A.C. (1990). Fresh leaf samples (0.5 g) were homogenized in a mortar with 85% acetone then the homogenate was filtered. The residue was washed several times with acetone till completely extracted. Then spectrophotometer (UV- Vis spectrophotometer UV 9100 B, Lab Tech) was used to read the optical densities at 662, 644 and 440.5 nm for Chl. a, Chl. b

and carotenoids, respectively. The results were quantitatively determined and calculated as mg 100 g⁻¹ FW.

7. Total Anthocyanin Content

Total anthocyanin content was estimated as described in Connor et al. (2002). Fresh leaves were extracted with 1% HCl diluted in methanol to get an absorbance between 0.200 and 1.000 at 530 nm. Data were expressed as mg cyanidin-3-glucoside (c3g) equivalents per 100 g fresh weight using a molar extinction coefficient (29.600).

8. Proline Determination

Proline concentration was evaluated using a ninhydrin colorimetric method of Troll and Lindsley (1955) as modified by Petters et al. (1997). Half g of frozen tissues was ground and homogenized in 5 ml of 0.1 M sodium phosphate buffer (pH = 6), then centrifuged for 10 min at 4500 rpm. An amount of 0.2 ml of the extract was reacted with 1 ml of ninhydrin solution (2.5 g dissolved in 100 ml of orthophosphoric acid, acetic acid and water with volume as 15: 60: 25, respectively) for 1 h in boiling water. Thereafter, the developed dye was extracted with 1 ml toluene and vigorously vortexed for 15 s. The toluene phase was aspirated and measured at 515 nm by spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech), using L-proline as standard. Proline concentration was expressed as µg proline g⁻¹ FW.

9. Total Phenols

Total phenolics determination was carried out using Folin-Denis reagent as reported in (Shahidi and Nacz, 1995). A known weight of plant samples was extracted by 80% ethanol, 1 ml of the extract was added to 0.5 ml of Folin reagent, mixed well, accompanied with addition of 1 ml of saturated Na₂CO₃ and 3 ml of distilled water. After 1 h, absorbance of blue color was read at 725 nm by spectrophotometer (UV- Vis spectrophotometer UV 9100 B, Lab Tech) using catechol as a standard. The results were expressed as mg g⁻¹ FW.

10. Statistical Analysis

Two ways-Anova was carried out using Infostat software (<https://www.infostat.com.ar>) for all biochemical parameters. Fisher's least significant difference at $P \leq 0.05$ was used to distinguish the difference in tested traits by locations and seasons. The significantly different mean values were marked by different alphabets.

RESULTS

Data in Tables (2 and 3) show that summer season caused a significant increase in specific activity of PPO, while a reduction in water content in *T. capitatus*. On the other hand, MDA, specific activities of POD and PAL,

proline, phenols, chl. a, chl. b, total chl. and anthocyanin had no obvious difference between winter and summer seasons.

In the present paper, microhabitats had significant change in all studied parameters as represented in Tables (2 and 3). Water content increased as elevation decreased, recorded the highest content in those growing at wadi-bed. MDA attained the highest amount in those at up-stream followed by runnel and wadi-bed. While specific activities of POD and PAL, chl. a and b, anthocyanin and phenols recorded the highest values in *T. capitatus* growing at wadi-bed. Proline content was the highest in *T. capitatus* growing at runnel while the lowest in those at wadi-bed.

Regarding the interaction between microhabitats and seasons, the water content was the highest in *T. capitatus* growing at wadi-bed in winter, while MDA value was the highest in those at up-stream either in winter or summer season. The specific activities of POD and PAL recorded the highest value in those at wadi-bed during summer as represented in Table (2). The highest proline content was observed in *T. capitatus* at runnel during summer. For total phenols, anthocyanin, chl a, chl b, total chl and carotenoids the higher accumulation was in those growing at wadi-bed without significant change between winter and summer as shown in Table (3).

Table (2). Water content, MDA and specific activities of POD, PPO and PAL in *T. capitatus*

Item	Water content (%)	MDA ($\mu\text{m g}^{-1}$ fr. wt)	POD (unit mg^{-1} protein)	PPO (unit mg^{-1} protein)	PAL (unit mg^{-1} protein)
Seasons effect					
Winter	62.80 ^b	8.58 ^a	2685.95 ^a	16199.00 ^a	4477.87 ^a
Summer	42.26 ^a	8.45 ^a	2726.14 ^a	17658.76 ^b	4371.30 ^a
Locations effect					
Upstream	47.67 ^a	9.92 ^c	2161.05 ^a	16399.43 ^a	3397.22 ^a
Runnel	50.76 ^b	8.43 ^b	2484.17 ^a	15477.49 ^a	4231.82 ^b
Wadi-bed	59.17 ^c	7.21 ^a	3472.92 ^b	18909.73 ^b	5644.72 ^c
Locations * Seasons					
Upstream*W	59.19 ^d	10.09 ^d	2197.88 ^a	16572.45 ^b	3501.31 ^{ab}
Upstream*S	36.14 ^a	9.76 ^{cd}	2124.22 ^a	16226.42 ^{ab}	3293.13 ^a
Wadi-runnel*W	59.13 ^d	8.55 ^{bc}	2496.60 ^a	13354.94 ^a	4328.77 ^b
Wadi-runnel*S	42.39 ^b	8.30 ^{ab}	2471.74 ^a	17600.03 ^b	4134.86 ^{ab}
Wadi-bed*W	70.09 ^e	7.11 ^a	3363.38 ^b	18669.61 ^b	5603.52 ^c
Wadi-bed *S	48.25 ^c	7.30 ^{ab}	3582.47 ^b	19149.84 ^b	5685.92 ^c

W= Winter, S= Summer and different letters mean significant, while same letters mean non-significant change

Table (3). Proline, total phenols, anthocyanin and photosynthetic pigments in *T. capitatus*

Item	Proline ($\mu\text{g g}^{-1}$ fr. wt)	Phenols (mg g^{-1} fr. wt)	Anthocyanin [$\text{mg (100 g}^{-1})$ fr. wt]	Chl. a [$\text{mg (100 g}^{-1})$ fr. wt]	Chl. b [$\text{mg (100 g}^{-1})$ fr. wt]	Total Chl. [$\text{mg (100 g}^{-1})$ fr. wt]	Carotenoid [$\text{mg (100 g}^{-1})$ fr. wt]
Seasons effect							
Winter	106.29 ^a	7.56 ^a	96.57 ^a	7.69 ^a	5.50 ^a	13.19 ^a	4.71 ^a
Summer	105.29 ^a	7.70 ^a	99.09 ^a	7.65 ^a	4.96 ^a	12.62 ^a	4.67 ^a
Locations effect							
Upstream	103.09 ^b	6.08 ^a	89.28 ^a	5.27 ^a	3.12 ^a	8.40 ^a	3.32 ^a
Wadi-runnel	133.89 ^c	7.78 ^b	92.27 ^a	7.88 ^b	5.08 ^b	12.96 ^b	4.72 ^b
Wadi-bed	80.38 ^a	9.02 ^c	111.94 ^b	9.86 ^c	7.49 ^c	17.35 ^c	6.03 ^c
Locations * Seasons							
Upstream*W	104.81 ^{ab}	6.06 ^a	89.16 ^a	5.37 ^a	3.25 ^a	8.62 ^{ab}	3.39 ^a
Upstream*S	101.38 ^b	6.10 ^a	89.40 ^a	5.18 ^a	2.99 ^a	8.17 ^a	3.25 ^a
Wadi-runnel*W	131.83 ^c	7.62 ^b	90.41 ^a	8.01 ^{bc}	5.31 ^{ab}	13.32 ^{cd}	4.75 ^{ab}
Wadi-runnel *S	135.96 ^c	7.94 ^b	94.13 ^{ab}	7.74 ^b	4.86 ^{ab}	12.60 ^{bc}	4.70 ^{ab}
Wadi-bed*W	82.24 ^a	9.00 ^c	110.13 ^{bc}	9.68 ^{bc}	7.94 ^c	17.62 ^e	6.00 ^b
Wadi-bed*S	78.52 ^a	9.06 ^c	113.75 ^c	10.04 ^c	7.03 ^{bc}	17.07 ^{de}	6.06 ^b

W= Winter, S= Summer and different letters mean significant, while same letters mean non-significant change

DISCUSSION

T. capitatus is a xerophytic species growing naturally in alkaline soil, either sandy loam or sandy clay loam soil in the northwestern coast of Egypt. The reduction in water content in the studied species during summer (drought) is in line with many authors. Khaleghi et al. (2019) noticed RWC reduced by 26.58% and 42.01% after 8 days of drought stress in *Maclura pomifera* subjected to 50% and 30% FC respectively, as compared to control plants (100% FC). Moreover, Bano et al. (2021) reported relative water content in mung bean cultivars has been reduced significantly by drought stress. The high level of water content in *T. capitatus* at wadi-bed due to the high clay amount that efficiently withhold water and avail more water to be absorbed by roots then transferred to shoots.

Plants affected by abiotic stress including drought led to lipid peroxidation via accumulation of MDA and / or H_2O_2 . In the present study, *T. capitatus* is a tolerant plant which does not affect by seasonal changes (keeping stability in MDA content). Oxidative stress represented by MDA accumulation was not affected under abiotic stress including drought in some tolerant potato cultivars (Demirel et al., 2020). However, many authors found significant increase in MDA in plants exposed to water deficit stress (Abd El-Maboud et al., 2018; Khaleghi et al., 2019 and Elkelish et al., 2021). The reduction in MDA contents in *T. capitatus* at wadi-bed may reflect the motivation of ROS scavenging capacity via higher accumulation of PPO, POD

and PAL specific activities, total phenols and anthocyanin accumulation. The photosynthesis process in *T. capitatus* is still stable by seasonal variations. Bano et al. (2021) found non-significant change in chl a, b and a/b ratio in mung bean cultivars under normal or water deficit conditions. The stability of proline content agrees with those found by Kumar et al. (2017), who declared that neither water stress nor salinity stress caused significant differences in proline content of *Nerium oleander* leaves. Also, seasonal variation did not produce significant change in proline accumulation of some Amaranths species (Abd El-Maboud, 2019). However previous studies on different plant species reported a significant increase in proline content under drought conditions (Khaleghi et al., 2019; Elkelish et al., 2021 and Sathish et al., 2022).

The PPO in *T. capitatus* exhibited higher specific activity respond to drought stress during summer converted their increased capacity of antioxidant and resilience to environmental stresses. So, this enzyme has a significant role to keep the balance in plant cell exposed to abiotic stress. Our results are supported with the study of Elkelish et al. (2021), who were dictating APX, CAT and PPO manifested higher activities under drought stress conditions. In addition to Abd El-Maboud et al. (2018), who found a linear relationship between water stress and PPO activity in leaves of *Periploca angustifolia*. On the other side, Bano et al. (2021) declared that POD activity increased in both drought-resistance and drought-sensitive mung bean cultivars under drought stress.

Carotenoids can initiate plant development and help plants to withstand reversible conditions. They not only play a vital role in photoprotection of photosynthesis but also as a precursor in signaling during plant growth under abiotic stress (Ashraf and Harris, 2013). They are accessory pigments, synthesized in plants to collect light energy and transfer into chl., then convert to chemical energy. They perform a critical role in photosynthesis, photoprotection, and biosynthesis of phytohormones (abscisic acid and strigolactone) in plant cells (Sathasivam et al., 2020). Indeed, they can be considered as the first line of defense against 1O_2 toxicity in the plants chloroplasts as compared the others (Triantaphylidès and Havaux, 2009). The reduction in photosynthetic pigments in plants at upstream are in harmony with those obtained by Cui et al. (2018), who stated a significant negative correlation between both chl. a and chl. b with elevation in *Leymus secalinus*. Furthermore, *T. capitatus* response to oxidative stress at upstream (the highest MDA accumulation) by reducing photosynthetic metabolites. In this direction, Yamauchi et al. (2008) found a negative interrelationship between MDA accumulation and electron transfer in heat-stressed plants and suggested that the task of PSII might be injured by MDA modification of PSII proteins. Moreover, plants in upstream as compared to wadi-bed showed significant differences in total phenols and anthocyanin contents. With decreased elevation, the amount of phenols and anthocyanin increased and reached the

optimum values at wadi-bed. The results are also in line with the study reported by Zargoosh et al. (2019), who stated that total phenols decreased with increasing altitude in *Scrophularia striata* growing at Dehloran area. However, the same authors found the increment of phenols in *S. striata* growing at Badreh area. Phenolics play significant role in plant growth, especially in lignin and pigment biosynthesis (Bhattacharya et al., 2010). Furthermore, they not only restrict free radicles distribution but membrane peroxidation reactions as well (Cramer et al., 2011). Phenolic compounds have oxidoreductive properties, which enable them to serve as reducing agents, hydrogen donors, and singlet oxygen quenchers (Chang et al., 2001). In addition, these compounds are also significantly participated in plant lignin and pigment biosynthesis (Bhattacharya et al., 2010). Besides they are commonly used as additives to save food from degradation (Soong and Barlow, 2006). In the present study, anthocyanin content and total phenols content in *T. capitatus* are exhibited positive correlation with each other. Anthocyanins are glycosylated polyphenolic compounds which are considered as a large group of plant secondary metabolites (Tanaka et al., 2008). Anthocyanins are photo protective agents which minimize photo-oxidative damage by absorbing excess UV and visible light, scavenging free radicals and stress signals (Guo et al., 2008 and Kovinich et al., 2015).

CONCLUSION

Our data infer that drought tolerance of *T. capitatus* might be associated with reducing oxidative injury by stimulation of the antioxidant systems. *T. capitatus* showed low lipid peroxidation on account of the higher accumulation of antioxidant compounds beside antioxidant enzymes activity. Stability of MDA by seasonal changes reflects that *T. capitatus* is highly tolerant to drought stress. *T. capitatus* is rich with anthocyanin and phenolics that give the plant more priority to be introduced for sustainable development programs in arid and semi-arid areas closest to the Mediterranean regions.

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استجابة مضادات الأكسدة للتغيرات الموسمية في نبات الزعتر

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يهدف هذا البحث إلى دراسة تأثير كلاً من الموائل الدقيقة والاختلافات الموسمية على مضادات الأكسدة الداخلية في نبات الزعتر النامي في الطبيعة. فهذا النبات الجفافي المعمر ينمو بالتلال والوديان المتاخمة لمنطقة البحر الأبيض المتوسط. في هذه الدراسة تم تجميع نموات طرفية من المجموع الخضري للنبات وأيضاً عينات تربة مرافقة من ثلاثة موائل دقيقة من وادي حابس غرب مطروح وهي أعلى الوادي، شق أو مجرى الوادي وبطن الوادي خلال شتاء وصيف عام ٢٠١٨. تم تحليل التربة فيزيائياً وتقدير كل من المحتوى المائي للنبات، المالون داي ألدهيد، النشاط النوعي لإنزيمات البولي فينول أكسيداز، البيروكسيداز والفينيل أمونيا لبيز، محتوى البرولين، الفينولات الكلية، الأنثوسيانين، كلوروفيل أ وب والكاروتينويد. لوحظ ازدياد النشاط الإنزيمي للبولي فينول أكسيداز في فصل الصيف وازدياد المحتوى المائي للنبات في فصل الشتاء، أما باقي الصفات فلم تتأثر معنوياً بتأثير الموسم. بالنسبة لتأثير الموائل الدقيقة فلوحظ ازدياد المالون داي ألدهيد في النباتات أعلى الوادي، والبرولين في نباتات مجرى الوادي بينما كان أعلى تركيز لكل من النشاط النوعي لأنزيمات البولي فينول أكسيداز، البيروكسيداز والفينيل أمونيا لبيز، الفينولات الكلية، الأنثوسيانين، محتوى كلوروفيل أ وب والكاروتينويد في نباتات بطن الوادي. وأثبتت النتائج قدرة تحمل نبات الزعتر للإجهاد الجفافي متمثلاً في عدم تأثر المالون داي ألدهيد (دلالة على أكسدة الليبيدات) بالاختلافات الموسمية.