Italian Journal of Agrometeorology
Rivista Italiana di Agrometeorologia

Firenze University Press
www.fupress.com/ijam



**Citation:** S. Khajeh Hosseini, F. Fanoodi, S.A. Tabatabaei, R. Yazdani Biouki, J. Masoud Sinaki (2020) Drought stress response of hyssop (Hyssopus officinalis L.) as influenced via the antitranspirants and osmolytes materials. *Italian Journal of Agrometeorology* (2): 35-44. doi: 10.13128/ijam-985

**Received:** June 27, 2020

**Accepted:** August 18, 2020

**Published:** January 25, 2021



**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Competing Interests:** The Author(s) declare(s) no conflict of interest.

# Drought stress response of hyssop (*Hyssopus officinalis* L.) as influenced via the antitranspirants and osmolytes materials

Sarah Khajeh Hosseini[1], Farzad Fanoodi[2,*], Sayed Ali Tabatabaei[3], Rostam Yazdani Biouki[4], Jafar Masoud Sinaki[5]

[1] *PhD Student, Department of Agriculture, Damghan Branch, Islamic Azad University, Damghan, Iran*
[2] *Department of Agriculture, Damghan Branch, Islamic Azad University, Damghan, Iran*
[3] *Seed and Plant Improvement Research Department, Yazd Agricultural and Natural Resource and Education Center, AREEO, Yazd, Iran*
[4] *Assistant Professor, National Salinity Research Center, Agricultural Research, Education and Extension Organization (AREEO), Yazd, Iran*
[5] *Department of Agriculture, Damghan Branch, Islamic Azad University, Damghan, Iran*
* Corresponding author. E-mail: farzadfanoodi@yahoo.com

**Abstract.** Drought or insufficient water is considered as one of the most common limiting factors in global agricultural production. This study evaluated the effects of drought stress and foliar application of kaolin, chitosan, and glycine amino acid at different application times in Hyssop (*Hyssopus officinalis* L.) for two-year. The results demonstrated that extreme drought affects the growth and yield parameters of hyssop, such as decrease morphological traits as well as yield components, protein content and increase enzymatic antioxidants. The foliar application, especially with kaolin at flowering, caused the greatest changes in general, greatly enhanced the number of lateral branches, shoot dry weight, protein content by (13.62%), (6.15%), (7%) in the first year, respectively. In addition, chitosan had a beneficial effect on shoot dry weight too. Also, kaolin reduced the effects of oxidative stress in the plant by increasing its antioxidant capacity to. So that the enzymatic antioxidants CAT and POX enhanced by (32.48%), (43.35%) in the first year and by (5.95%), (13.18%) in the next year, respectively.

**Keywords**. Amino acid, dry matter production, enzymatic antioxidants, transpiration.

## INTRODUCTION

Hyssop (*Hyssopus officinalis* L.) belongs to the Lamiaceae family and is considered as a native plant from southern Europe and Near East to the region surrounding the Caspian Sea and cultivated in central and southern European countries including Russia, Spain, France, Yugoslavia, Netherland, Hungary and Italy (Mitic and Dordevic, 2000). The essential oil and extracts isolated from *Hyssopus officinalis* were shown to have biological and pharmacological activities including anti-bacterial (Michalczyk et al., 2012), antioxidant (Kizil et al., 2010) and antiplatelet (Tognolini et al., 2006). despite hyssop having a slightly bitter taste, it is often used as a minty flavour and condiment in food industries (Fathiazad et al., 2011).

Plants often encounter unfavourable conditions, which interrupts their growth and productivity. Among the various abiotic stresses, drought is considered as the major factor which limits crop productivity worldwide (Tardieu et al., 2014). Also, drought causes the inhibition of shoot growth, adjustment of leaf area, stomatal closure, and reduction of transpiration, inhibition of photosynthesis, shifts in carbon and nitrogen metabolism, synthesis of compatible solutes, and secondary oxidative stress (Xoconostle-Cazares et al., 2011). Further, oxidative stress arises from a significant increase in the concentration of reactive oxygen species (ROS) (Ermak and Davies, 2002 ). ROS are highly reactive and can alert normal cellular metabolism through oxidative damage to membranes, proteins, and nucleic acids. Further, they cause protein denaturation, DNA mutation (Baby and Jini, 2011).To counter the deleterious effects of ROS, plants have developed the scavenging mechanism of ROS categorized as enzymatic antioxidants, acting as a defence mechanism to regulate the ROS levels (Hossain et al., 2013). The enzymes of the antioxidant system protect cells by eliminating ROS including catalase (CAT), superoxide dismutase (SOD), and numerous peroxidases (POX), among which ascorbate peroxidase (APX) can be mentioned (Jozwiak and Politycka, 2019).

Reduced transpiration rate is considered as one of the effective ways to reduce the unfavourable effects of drought stress in plants (Neyestani and Azimzadeh, 2005). Foliar application of antitranspirants (ATS) is a promising tool for regulating transpiration to maintain a favourable plant water status (Goreta et al., 2007). ATs were classified into three types based on their active role. The first is related to metabolic materials which are chemical compounds which can prevent stomata from opening fully by affecting the guard cells around the stomata pore, leading to a decrease in the loss of water vapour from plant leaves (Anjum et al., 2011); Second, film-forming materials which are emulsions of wax, latex or plastics dry on the foliage to form thin transparent films which hinder the escape of water vapour from the leaves (Faralli et al., 2016) Finally, reflecting materials which reduce the absorption of the radiant energy, as well as leaf temperatures and transpiration rate (Glenn et al., 2003). Reflective materials such as kaolin clay and chitosan can reduce the absorption of radiant energy (heat), lower leaf temperatures, and decrease transpiration (Jifon and Syvertsen, 2003). Kaolin is a white, non-porous, non-swelling, low-abrasive, fine-grained, plate-shaped, and alumino silicate mineral $[Al_4Si_4O_{10}(OH)_8]$ (Glenn and Puterka, 2005). Besides, chitosan is produced from chitin, an important component of crustacean shells such as crab, shrimp and crawfish, and is mainly made of (1–4)-2-amino-2-deoxy-β-D-glucan (Ma et al., 2013).

Increasing the synthesis and accumulating osmolytes in plants is regarded as one of the methods which prolong water uptake and reduces osmotic stress due to drought stress (Tang and Newton, 2005). Major osmolites organic compounds include amino acids, polyols, sugars and methylamines (Pessarakli, 2011). The use of external sources of osmolyte organic compounds in environmental stressors can modulate the destructive effects of stresses on plants (Namvar et al., 2018). Further, glycine amino acid as osmolyte is a soluble nitrogenous compound which is accumulated under stress in plants (Galeshi, 2015; Hussein and Terry, 2002). Glycine is a proteinogenic amino acid and the smallest biological amino acid in the cells with a molar mass of 75 g $mol^{-1}$. Further, it is a hydrophilic and non-polar amino acid, which can have an acidic or basic reaction in different mediums due to its chemical structure (Souri and Hatamian, 2019).

By considering the above studies, the present research aimed to evaluate the growth, yield changes and antioxidant activities in hyssop (*Hyssopus officinalis* L.) treated with the chitosan, kaolin and glycine amino acid under drought stress condition and different application times.

## MATERIALS AND METHODS

### *Experimental design*

The experiment was conducted during a two-year factorial split-plot experiment based on completely randomized block design with three replications at the research field of Yazd Agricultural and Natural Resources Center, Iran (31° N latitude and 54° E longitude 1220 m above sea level) in 2017 and 2018. The presented temperature and rainfall rate of the site during the crop growing periods of the experiments is depicted in Fig 1.

The experimental treatments included irrigation at three levels ( 25, 50, 75 % of the available water discharge from the soil) as control, mild and intense stress as main treatments and spraying treatments in three levels of water (control), kaolin (2.5%, Sepidan WP 95, Aluminium silicate, LD 50> 5000 $mg.kg^{-1}$), chitosan (0.4 $g.l^{-1}$, Sigma-Aldrich, Medium molecular weight, 75-85% deacetylated chitin, LD 50> 10.000 $mg.kg^{-1}$), glycine amino acid (2.5 per thousand, Merck, Molar mass: 75.067 $g{\cdot}mol^{-1}$, Density: 1.1607 $g.cm^{-3}$, LD 50>2600 $mg.kg^{-1}$) and time of spraying (vegetative and flowering, just flowering) were considered as subplots. Soil fertilizers were added to the experimental farmland based on the soil test during farm preparation (Tab. 1). To sup-

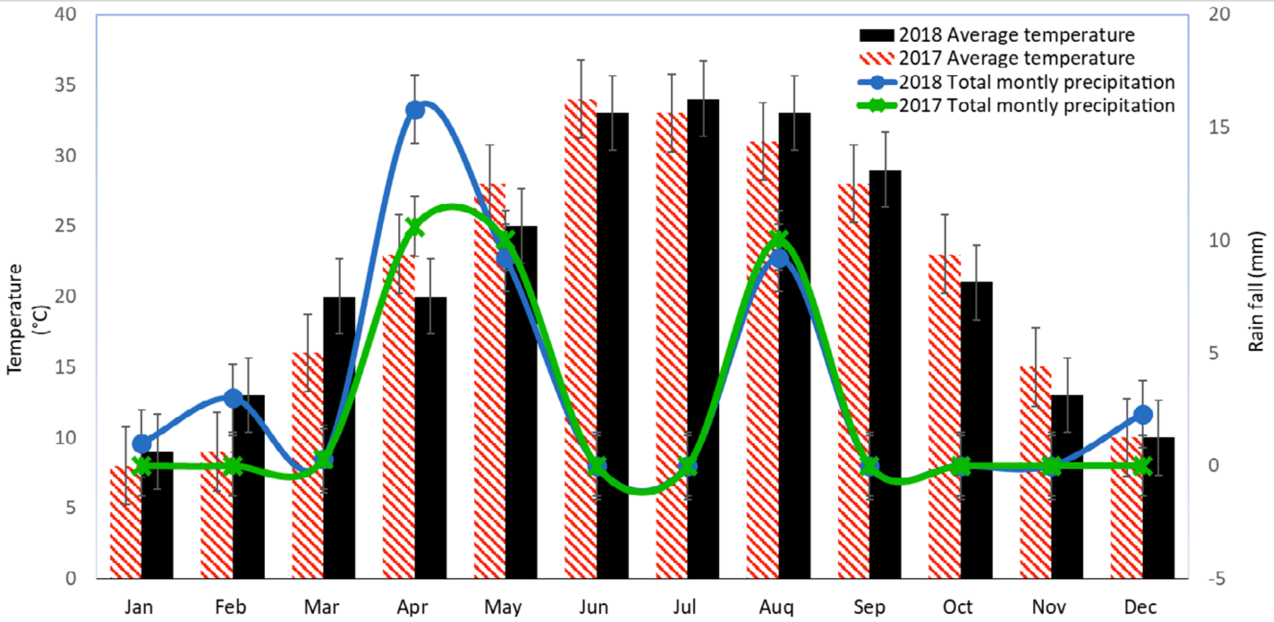


**Fig. 1.** Meteorological conditions during 2017 and 2018.

ply the needed nitrogen based on the results of soil analysis, urea fertilizer (46% nitrogen) was used in two stages including pre-planting to the soil and mid-vegetative period to the plant at the rate of 100 kilograms per hectare.

Each subplot sizes into the main plot were 3×4 m and consisted of six planting rows. The distance between the plants and rows was 20 and 50 cm, respectively. The distance between the main plot and the blocks was 1.5 and 3 m, respectively. The seeds were sown by hand. All of the plots were regularly irrigated after sowing until seedling establishment. Drought stress after complete plant establishment was increased by irrigation intervals of 7 days (control, 25% of the available water discharge from the soil), 9 days (medium stress, 50% of the available water discharge from the soil), and 11 days (severe stress, 75% of the available water discharge from the soil), which was done based on soil moisture recordings by the TDR model (TRASE System 1). Weeds were controlled by hand during crop growth and development as required. Phonological stages of the hyssop plant were determined based on the 50% of the plants in reaching a specified developmental stage, and accordingly, the time interval to each stage based on the day after planting was recorded.

### *Collection of plant samples*

Sampling from each experimental unit was performed by considering marginal effects at the end of the flowering stage each year, The number of lateral branches and leaves, as well as leaves and shoot dry weight was determined to evaluate the growth and yield changes. In addition, the water soluble protein content was determined according to Bradford's (1976) to evaluate antioxidant changes. Further, enzymatic antioxidants such as Catalase (CAT), and peroxidase (POX) were measured by using (Dhindsa et al., 1981) and (Mac-Adam et al., 1992), respectively.

### *Statistical analysis*

After checking the data distribution normality (Kolmogorov-Smirnov and Shapiro-Wilk test) assumption,

**Tab. 1.** The main characteristics of the experimental farm in 0–30 cm soil depth.

| Potassium (ppm) | Phosphorous (ppm) | N.Total (%) | Sand (%) | Silt (%) | Clay (%) | Organic Carbon (%) | pH | Electrical conductivity ( $dS.m^{-1}$) | Soil texture |
|---|---|---|---|---|---|---|---|---|---|
| 360 | 41.6 | 0.01 | 76 | 20 | 5 | 0.221 | 7.67 | 1.68 | Loamy sand |

the studied traits were statistically analyzed by ANOVA by using the SAS 9.4 procedure PROC GLM. The mean comparison of data was done by LSD range test at 5% level of significance.

## RESULTS

### *Morphological traits and yield components*

#### Number of the leaves, leaves dry weight

Analysis of variance indicated positive effects of using antitranspirant and osmolytes materials on morphological traits and yield components of hyssop under limited irrigation (Tab. 2). Due to the results of this study, the number of leaves was significantly affected by antitranspirant and leaves dry weight was significantly affected by osmolytes materials. According to Fig. 2, the highest number of the leaves was observed in the interaction effects of control irrigation with water spraying (4779 per plant) and control irrigation with chitosan spraying (4381 per plant) in the first year and the highest leaves dry weight was observed in the interaction effects of control irrigation and glycin spraying in vegetative and flowering (230.8 $g.m^{-2}$) in the second year (Tab. 3).

#### Number of lateral branches, Shoot dry weight

Among the materials used, glycin and kaolin had the most influence on the number of lateral branches and

**Tab. 2.** The results Analysis on hyssop (*Hyssopus officinalis* L.) traits evaluated under, Drought stress (D), Foliar application (F) and Foliar application Time (FT).

| Treatment | df | Mean sum of squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number of leaves | | Number of lateral branches | | Leaves dry weight | | Shoot dry weight | |
| | | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| R | 2 | 2804010$^{ns}$ | 3035819$^{ns}$ | 4051$^{**}$ | 2452$^{ns}$ | 410.4$^{ns}$ | 303.51$^{ns}$ | 326.9$^{**}$ | 21.11$^{ns}$ |
| D | 2 | 49883736$^{**}$ | 21573726$^{*}$ | 8372$^{**}$ | 51646$^{**}$ | 3757$^{**}$ | 1249.61$^{ns}$ | 1522$^{**}$ | 23867.18$^{**}$ |
| Error a | 4 | 614629 | 2229893 | 167.1 | 766 | 108.2 | 930.00 | 11.70 | 321.17 |
| FT | 1 | 153735$^{ns}$ | 4917248$^{*}$ | 3472$^{*}$ | 6884$^{*}$ | 0.125$^{ns}$ | 2188.90$^{*}$ | 48.35$^{ns}$ | 1326.98$^{ns}$ |
| F | 3 | 112359$^{ns}$ | 7049457$^{**}$ | 1124$^{ns}$ | 16081$^{**}$ | 1312$^{**}$ | 2426.48$^{**}$ | 56.24$^{ns}$ | 5063.73$^{**}$ |
| D × FT | 2 | 198809$^{ns}$ | 1542008$^{ns}$ | 1629$^{ns}$ | 2865$^{ns}$ | 25.29$^{ns}$ | 9699.45$^{**}$ | 115.5$^{*}$ | 1634.31$^{*}$ |
| D × F | 6 | 647572$^{**}$ | 4665474$^{**}$ | 2382$^{*}$ | 7607$^{**}$ | 647.6$^{**}$ | 7873.52$^{**}$ | 137.1$^{**}$ | 1997.43$^{**}$ |
| F × FT | 3 | 59826$^{ns}$ | 6213617$^{**}$ | 2683$^{*}$ | 2696$^{ns}$ | 110.1$^{ns}$ | 10583.34$^{**}$ | 22.46$^{ns}$ | 2073.92$^{**}$ |
| FT × F × D | 6 | 222147$^{ns}$ | 16340720$^{**}$ | 2800$^{**}$ | 18355$^{**}$ | 35.16$^{ns}$ | 5604.08$^{**}$ | 47.24$^{ns}$ | 2112.67$^{**}$ |
| Error b | 42 | 125985 | 918659 | 728.8 | 1449 | 43.60 | 384.16 | 27.21 | 473.49 |
| C.V | | 13.70 | 19.08 | 18.80 | 18.57 | 16.99 | 21.81 | 18.68 | 20.23 |

| Treatment | df | Mean sum of squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Protein | | CAT | | POX | |
| | | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| R | 2 | 0.1596$^{**}$ | 1.1660$^{**}$ | 0.0824$^{ns}$ | 0.4777$^{**}$ | 0.0664$^{**}$ | 0.0005$^{*}$ |
| D | 2 | 2.5706$^{**}$ | 1.4440$^{**}$ | 2.4956$^{**}$ | 0.3620$^{**}$ | 0.0316$^{**}$ | 0.0151$^{**}$ |
| Error a | 4 | 0.0003 | 0.0904 | 0.0004 | 0.0098 | 0.0002$^{ns}$ | 0.0004 |
| FT | 1 | 9.3615$^{**}$ | 0.0021$^{ns}$ | 0.0652$^{**}$ | 0.0791$^{*}$ | 0.0739$^{**}$ | 0.0009$^{ns}$ |
| F | 3 | 17.2600$^{**}$ | 4.9409$^{**}$ | 0.2432$^{**}$ | 0.0217$^{ns}$ | 2.5934$^{**}$ | 0.0056$^{*}$ |
| D × FT | 2 | 0.3773$^{**}$ | 5.9961$^{**}$ | 0.105$^{**}$ | 0.0731$^{*}$ | 0.6366$^{**}$ | 0.0157$^{**}$ |
| D × F | 6 | 20.6942$^{**}$ | 6.2558$^{**}$ | 1.0897$^{**}$ | 0.1052$^{**}$ | 0.6463$^{**}$ | 0.0203$^{**}$ |
| F × FT | 3 | 3.2322$^{**}$ | 0.3547$^{**}$ | 0.0449$^{**}$ | 0.0423$^{ns}$ | 0.6972$^{**}$ | 0.0046$^{*}$ |
| FT × F × D | 6 | 6.4904$^{**}$ | 7.4057$^{*}$ | 0.1282$^{**}$ | 0.1314$^{**}$ | 1.2918$^{**}$ | 0.0129$^{**}$ |
| Error b | 42 | 0.0002 | 0.0871 | 0.0002 | 0.0208 | 0.0002 | 0.0017 |
| C.V | | 0.14 | 3.23 | 2.13 | 26.54 | 1.39 | 17.68 |

df- degree of freedom; R- Replication; CV – coefficient of variation; ns – not significant; $^{**}$ – significant at $p \leq 0.01$;$^{*}$ - significant at $p \leq 0.05$.

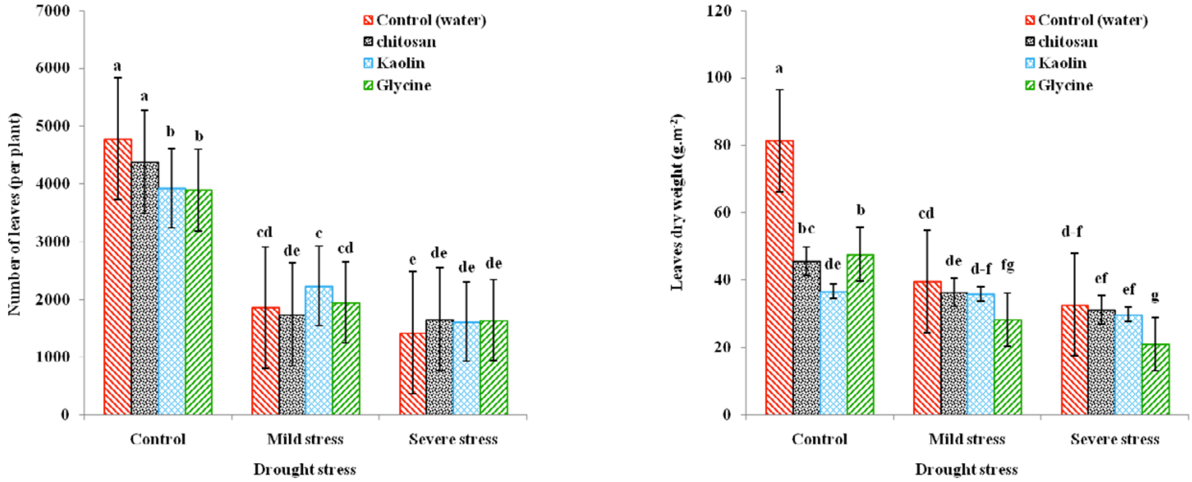


**Fig. 2.** The effect of drought stress and foliar application on leaves dry weight.

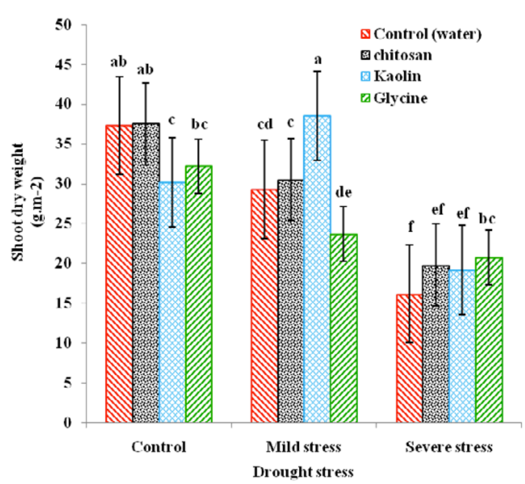


**Fig. 3.** The effect of drought stress and Foliar application on shoot dry weight.

shoot dry weight compared to control, respectively.The highest number of lateral branches was observed in interaction effects of control irrigation and glycin spraying in vegetative and flowering in 2017 (185 per plant) (Tab. 3) and the highest shoot dry weight was observed in interaction effects of mild stress and kaolin spraying (38.5g.m$^{-2}$) in the first year (Fig. 3).

## *Protein and enzymatic antioxidants*

Analysis of variance indicated the positive effects of using antitranspirant and osmolytes materials on protein content and enzymatic antioxidants of hyssop under limited irrigation (Tab. 2).The osmolytes materials used caused an increase in protein content in the first year. According to Tab. 3, the highest protein content (12.47%) was observed in the interaction effects of severe stress and glycin spraying at just flowering. The protein content, the CAT and POX activities were also affected by antitranspirants. The highest protein content (12.08%) in 2018 and the highest CAT enzyme activity content (1.593 U. mg protein$^{-1}$. min $^{-1}$) in 2018, was observed in interaction effects of severe stress and kaolin spraying at just flowering (Tab. 3).The kaolin affected the POX enzyme activity content too. So that the highest POX activities (2.382 U. mg protein$^{-1}$. min $^{-1}$) was obtained in interaction effects of control irrigation and kaolin spraying at vegetative and flowering in the first year (Tab. 3). Among the antitranspirant materials used chitosan showed better results of increasing studied antioxidants traits compared with control too. As the results showed, at the severe stress level, the foliar application of chitosan in just flowering had the highest of activity CAT (0.868 U. mg protein$^{-1}$. min $^{-1}$) and POX (0.438 U. mg protein$^{-1}$. min $^{-1}$) in the second year (Tab. 3).

# DISCUSSION

## *Grow and yield*

Plants are exposed to environmental stresses during their growth. Drought stress is considered as one of the major abiotic stresses leading to a high reduction in plant growth and yield, which can affect the absorption and transfer of nutrients to the plant (Askari et al., 2018). In the present study, the results indicated that the severe drought stress caused a reduction on growing and yielding

**Tab. 3.** The influence of drought stress (D), foliar application (F) and Foliar application time (FT) on hyssop (*Hyssopus officinalis* L.) traits evaluated.

| Treatment | | | Number of leaves (per plant) | | Leaves dry weight (g.m$^{-2}$) | | Number of lateral branches (per plant) | | Shoot dry weight (g.m$^{-2}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| D | FT | F | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| Control | Flowering | Control(water) | NS | 2389$^{j}$ | NS | 68.5$^{e-j}$ | 170$^{ab}$ | 75$^{M}$ | NS | 79.0$^{h-j}$ |
| | | Chitosan | NS | 5330$^{d-g}$ | NS | 68.3$^{e-j}$ | 179$^{ab}$ | 230$^{b-f}$ | NS | 78.8$^{h-j}$ |
| | | Kaolin | NS | 4780$^{e-h}$ | NS | 52.9$^{h-j}$ | 164$^{a-c}$ | 160$^{g-l}$ | NS | 67.38$^{ij}$ |
| | | Glycine | NS | 5644$^{d-f}$ | NS | 68.7$^{e-j}$ | 141$^{a-e}$ | 225$^{b-f}$ | NS | 73.3$^{ij}$ |
| | Vegetation and flowering | Control(water) | NS | 4753$^{f-h}$ | NS | 63.9$^{f-j}$ | 153$^{a-d}$ | 226$^{b-f}$ | NS | 73.7$^{ij}$ |
| | | Chitosan | NS | 4440$^{f-i}$ | NS | 55.7$^{g-j}$ | 136$^{b-f}$ | 154$^{i-l}$ | NS | 64.2$^{ij}$ |
| | | Kaolin | NS | 2985$^{ij}$ | NS | 44.4$^{ij}$ | 167$^{a-c}$ | 134$^{lm}$ | NS | 47.9$^{j}$ |
| | | Glycine | NS | 3903$^{g-j}$ | NS | 230.8$^{a}$ | 185$^{a}$ | 169$^{f-l}$ | NS | 84.8$^{f-i}$ |
| Mild stress | Flowering | Control(water) | NS | 9829$^{a}$ | NS | 183.2$^{b}$ | 140$^{b-e}$ | 281$^{b}$ | NS | 161.5$^{a-c}$ |
| | | Chitosan | NS | 6695$^{b-d}$ | NS | 143.6$^{c}$ | 79$^{g}$ | 218$^{c-h}$ | NS | 156.9$^{a-c}$ |
| | | Kaolin | NS | 3902$^{g-j}$ | NS | 64.93$^{f-j}$ | 163$^{a-c}$ | 143$^{j-l}$ | NS | 85.1$^{f-i}$ |
| | | Glycine | NS | 3974$^{g-i}$ | NS | 65.8$^{f-j}$ | 94$^{fg}$ | 127$^{lm}$ | NS | 84.5$^{f-i}$ |
| | Vegetation and flowering | Control(water) | NS | 3927$^{g-j}$ | NS | 43.6$^{ij}$ | 113$^{d-g}$ | 135$^{k-m}$ | NS | 143.5$^{b-d}$ |
| | | Chitosan | NS | 6354$^{c-e}$ | NS | 75.3$^{d-i}$ | 181$^{ab}$ | 202$^{f-j}$ | NS | 158.4$^{a-c}$ |
| | | Kaolin | NS | 6742$^{b-d}$ | NS | 86.9$^{d-g}$ | 140$^{b-e}$ | 158$^{h-l}$ | NS | 98.0$^{e-i}$ |
| | | Glycine | NS | 7300$^{cb}$ | NS | 79.4$^{d-h}$ | 100$^{e-g}$ | 222$^{b-g}$ | NS | 116.3$^{d-g}$ |
| Severe stress | Flowering | Control(water) | NS | 8009$^{b}$ | NS | 147.7$^{c}$ | 109$^{d-g}$ | 410$^{a}$ | NS | 183.7$^{a}$ |
| | | Chitosan | NS | 4557$^{f-i}$ | NS | 106.2$^{d}$ | 102$^{e-g}$ | 276$^{cb}$ | NS | 126.6$^{c-e}$ |
| | | Kaolin | NS | 3456$^{j-i}$ | NS | 84.6$^{d-h}$ | 122$^{c-g}$ | 188$^{f-l}$ | NS | 81.5$^{g-j}$ |
| | | Glycine | NS | 4850$^{e-h}$ | NS | 90.2$^{d-f}$ | 144$^{a-e}$ | 246$^{b-e}$ | NS | 163.9$^{ab}$ |
| | Vegetation and flowering | Control(water) | NS | 4976$^{e-h}$ | NS | 85.4$^{d-g}$ | 107$^{e-g}$ | 265$^{b-d}$ | NS | 93.3$^{e-i}$ |
| | | Chitosan | NS | 5303$^{d-g}$ | NS | 99.6$^{ed}$ | 160$^{a-c}$ | 268$^{cb}$ | NS | 120.3$^{d-f}$ |
| | | Kaolin | NS | 4107$^{f-i}$ | NS | 104.7$^{d}$ | 152$^{a-d}$ | 214$^{c-i}$ | NS | 129.0$^{b-e}$ |
| | | Glycine | NS | 2353$^{j}$ | NS | 42.6$^{j}$ | 178$^{ab}$ | 198$^{e-k}$ | NS | 109.6$^{d-h}$ |
| | | LSD (P=0.05) | NS | 1579.3 | NS | 32.296 | 44.5 | 62.73 | NS | 35.855 |

Different letters indicate significant differences at α = 0.05 (LSD test).

| Treatment | | | Protein (%) | | CAT (U. mg protein -1. min -1) | | POX(U. mg protein-1. min -1) | |
|---|---|---|---|---|---|---|---|---|
| D | FT | F | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| Control | Flowering | Control(water) | 11.98$^{c}$ | 9.50$^{ed}$ | 1.276$^{c}$ | 0.195$^{ij}$ | 0.773$^{m}$ | 0.145$^{ij}$ |
| | | Chitosan | 9.73$^{g}$ | 10.20$^{cb}$ | 0.558$^{k}$ | 0.490$^{d-g}$ | 1.265$^{g}$ | 0.127$^{c-h}$ |
| | | Kaolin | 8.24$^{n}$ | 7.02$^{j}$ | 0.963$^{de}$ | 0.600$^{b-f}$ | 0.565$^{n}$ | 0.27$^{b-e}$ |
| | | Glycine | 9.14$^{j}$ | 8.49$^{gh}$ | 1.493$^{b}$ | 0.219$^{h-j}$ | 0.42$^{q}$ | 0.121$^{j}$ |
| | Vegetation and flowering | Control(water) | 11.98$^{c}$ | 8.54$^{gh}$ | 1.276$^{c}$ | 0.644$^{a-f}$ | 0.773$^{m}$ | 0.203$^{e-i}$ |
| | | Chitosan | 7.41$^{p}$ | 8.21$^{hi}$ | 0.800$^{g}$ | 0.191$^{j}$ | 0.478$^{p}$ | 0.205$^{e-i}$ |
| | | Kaolin | 7.18$^{q}$ | 9.47$^{ed}$ | 0.975$^{d}$ | 0.505$^{d-g}$ | 2.382$^{a}$ | 0.267$^{b-f}$ |
| | | Glycine | 9.40$^{h}$ | 9.66$^{ed}$ | 0.943$^{e}$ | 0.429$^{f-i}$ | 1.105$^{i}$ | 0.24$^{c-f}$ |
| Mild stress | Flowering | Control(water) | 9.29$^{i}$ | 9.68$^{ed}$ | 0.4$^{l}$ | 0.502$^{d-g}$ | 1.431$^{f}$ | 0.2$^{f-i}$ |
| | | Chitosan | 10.15$^{e}$ | 10.17$^{cb}$ | 0.556$^{k}$ | 0.456$^{e-h}$ | 0.031$^{r}$ | 0.153$^{j-i}$ |
| | | Kaolin | 9.94$^{f}$ | 10.17$^{cb}$ | 0.057$^{p}$ | 0.632$^{a-f}$ | 1.481$^{e}$ | 0.333$^{b}$ |
| | | Glycine | 7.41$^{p}$ | 9.60$^{ed}$ | 0.405$^{l}$ | 0.335$^{g-j}$ | 1.505$^{d}$ | 0.231$^{c-g}$ |

| Treatment | | | Protein (%) | | CAT (U. mg protein -1. min -1) | | POX(U. mg protein-1. min -1) | |
|---|---|---|---|---|---|---|---|---|
| D | FT | F | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| | Vegetation and flowering | Control(water) | 9.29 i | 8.52 gh | 0.4 l | 0.755 a-c | 1.431 f | 0.273 b-d |
| | | Chitosan | 8.47 m | 7.87i | 0.337 m | 0.699 a-d | 0.876 k | 0.207 d-i |
| | | Kaolin | 8.55 l | 9.76 cd | 0.242 o | 0.490 d-g | 0.824 l | 0.234 c-f |
| | | Glycine | 6.74 r | 9.24 ef | 0.753 h | 0.688 a-e | 0.525 o | 0.202 f-i |
| Severe stress | Flowering | Control(water) | 8.70 k | 8.13 hi | 0.337 m | 0.703 a-d | 0.958 j | 0.275 bc |
| | | Chitosan | 8.47 m | 5.68 k | 0.884 f | 0.868 a | 0.563 n | 0.438 a |
| | | Kaolin | 7.67 o | 12.08 a | 1.593 a | 0.325 g-j | 1.582 c | 0.164 g-j |
| | | Glycine | 12.47 a | 8.73 g | 0.636 i | 0.794 ab | 1.143 h | 0.280 bc |
| | Vegetation and flowering | Control(water) | 8.70 k | 10.46 b | 0.337 m | 0.526 c-g | 0.958 j | 0.227 c-g |
| | | Chitosan | 12.03 b | 8.85 gf | 0.600 j | 0.815 ab | 0.491 p | 0.253 c-f |
| | | Kaolin | 4.57 s | 10.17 cb | 1.501 b | 0.590 b-f | 2.232 b | 0.225 c-g |
| | | Glycine | 10.22 d | 8.83 gf | 0.272 n | 0.583 b-f | 0.411 q | 0.207 d-i |
| | | LSD (P=0.05) | 0.02 | 0.49 | 0.026 | 0.238 | 0.023 | 0.608 |

CAT- Catalase; POX- Peroxidase; Different letters indicate significant differencesat α = 0.05 (LSD test).

components such as the number of leaves and reciprocally the leaves dry weight, and the number of lateral branches and reciprocally the shoot dry weight. However, the severe stress level had a less negative effect on all of the above traits in the second year compared to the non-stress condition, which is consistent with the other studies (Polanski et al., 2018).

The antitranspirants and osmolytes materials spraying on plant folige, at different growth stages could reduce the destructive effects of drought stress. In the present study, the foliar application, especially just flowering, had the best result on growth and yield. The foliar application of chitosan and water spraying had the highest number of the leaves at control stress level during the first year. In addition,water spraying caused the highest leaves dry weight in the first year. In general, chitosan may provide some amino compounds required for plant growth, leading to the increasing total nitrogen content in leaves or the higher ability of plants to absorb nitrogen from the soil as chitosan may increase key enzyme activity of nitrogen metabolism, as well as the transportation of nitrogen in the functional leaf. Further, chitosan can increase the availability, uptake and transport of essential nutrients via adjusting cell osmotic pressure, leading to plant growth and development (Abdul Manaim Mohammed et al., 2018). Regarding the second year, water spraying in just flowering had the highest number of the leaves at mild stress level, while the foliar application of glycin spraying in just flowering had the highest leaves dry weight at control stress level. Further more, the water spraying at severe stress level caused the highest number of lateral branches and reciprocally the leaves dry weight. In addition, the foliar application of glycin had the highest number of lateral branches at control stress level and kaolin spraying had the highest shoot dry weight at mild stress level during the first year. The positive effects of glycine foliar application on growing and yielding hyssop can be related to its ability for inducing osmotic regulation as compatible osmolyte contents in the plant. Generally, osmotic regulation results in expanding the cell, regulating the stomata and photosynthesis, and finally increasing the growth and yield in the plant (Galeshi, 2015). Further, the positive effects of kaolin spraying on growth and yield of hyssop can be attributed to its ability for anti-transpiration. Generally, these substances reduce transpiration in the plant and preserve leaves water potential through improving plant water conditions and dividing cell, which result in producing photosynthetic materials in canopy leading to an increase in the total dry matter accumulation and plant yield (Jan- Mohammadi et al., 2014)

*Antioxidant activity*

Drought induces oxidative stress in plants by generating reactive oxygen species (ROS) (A. Kasim et al., 2015). which can destroy proteins, lipids, carbohydrates, and nucleic acids (Bian and jiang, 2009). In this regard, the severe drought stress caused a reduction in protein content in the first year, compared to that of the control, which is consistent with the other studies (Rahimi et al., 2019). However, the protein was less affected by drought stress in both control and severe stress during the second year. On the other hand, the highest protein content was observed in mild stress, which can be related to the synthesis of more specific proteins during stress (Galeshi, 2015).which is consistent with the other studies (Ahrar et al., 2020). Generally, plants should include enzymatic antioxidant systems to protect cells from oxidative damage in order to keep the levels of active oxygen species under control (A. Kasim et al., 2015). Drought stress increased the activity of antioxidant enzymes such as CAT and POX. Based on the results, CAT activity decreased in the first year, while increased in the second year. However, POX increased in both years, which are in line with some other studies (Afsharmohammdian et al., 2016).

The foliar application, especially just flowering, improved the destructive effects of drought on the plant in two crop years. In fact, at severe stress level, the foliar application of glycin had the highest protein content at just flowering in the first year, while kaolin spraying at just flowering had the highest protein content in the second year. In addition, the foliar application of kaolin at severe drought stress in just flowering caused the highest of CAT activity content and spraying at vegetative and flowering at control drought stress caused the highest POX activity content in the first year. Glycin as an osmolyte and kaolin reduce the effects of oxidative stress in the plant by increasing its antioxidant capacity. Further, increased antioxidant activity by spraying kaolin was reported in other studies (Bernardo et al., 2017). Regarding the second year, foliar application of chitosan at severe drought stress in just flowering performed better, leading to the highest CAT and POX activity content. In general, the induction of antioxidant defence in plants by using chitosan can be related to the potential of this substance in neutralizing reactive oxygen species, which may be attributed to its specific structure including large numbers of accessible amine and hydroxyl groups which reacts with reactive oxygen species (Mahdavi et al., 2013). On the other hand, the ability of this substance can play a role in expressing a variety of genes involved in the plant defence response under stress conditions, which ultimately increase the production of secondary metabolites (Howlett, 2006).

**Tab. 4.** Correlation coefficients among traits of hyssop under different irrigation treatments in 2017.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 2 | 0.73$^{**}$ | 1 | | | | | |
| 3 | 0.48$^{**}$ | 0.24$^{*}$ | 1 | | | | |
| 4 | 0.67$^{**}$ | 0.57$^{**}$ | 0.45$^{**}$ | 1 | | | |
| 5 | 0.17$^{ns}$ | 0.35$^{**}$ | 0.20$^{ns}$ | 0.16$^{ns}$ | 1 | | |
| 6 | 0.40$^{**}$ | 0.38$^{**}$ | 0.10$^{ns}$ | 0.04$^{ns}$ | -0.16$^{ns}$ | | |
| 7 | 0.02$^{ns}$ | - 0.14$^{ns}$ | 0.15$^{ns}$ | - 0.05$^{ns}$ | -0.42$^{**}$ | 0.15$^{ns}$ | 1 |

| -1 | -0.9 | -0.8 | -0.7 | -0.6 | -0.5 | -0.4 | -0.3 | -0.2 | -0.1 | 0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

Red and blue colors show negative and positive, respectively
ns – not significant; ** – significant at p≤0.01; * - significant at p≤0.05.
1- The number of leaves; 2- leaves dry weight: 3- Number of lateral branches; 4- Shoot dry weight; 5-Protein content; 6- CAT activity; 7: POX activity.

**Tab. 5.** Correlation coefficients among traits of hyssop under different irrigation treatments in 2018.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 2 | 0.43$^{**}$ | | | | | | |
| 3 | 0.65$^{**}$ | 0.40$^{**}$ | | | | | |
| 4 | 0.49$^{**}$ | 0.41$^{**}$ | 0.54$^{**}$ | 1 | | | |
| 5 | 0.15$^{ns}$ | 0.08$^{ns}$ | 0.25$^{*}$ | 0.12$^{ns}$ | 1 | | |
| 6 | 0.26$^{*}$ | 0.04$^{ns}$ | 0.42$^{**}$ | 0.37$^{**}$ | 0.33$^{**}$ | 1 | |
| 7 | -0.07$^{ns}$ | 0$^{ns}$ | 0.17$^{ns}$ | 0.14$^{ns}$ | 0.13$^{ns}$ | 0.48$^{**}$ | 1 |

| -1 | -0.9 | -0.8 | -0.7 | -0.6 | -0.5 | -0.4 | -0.3 | -0.2 | -0.1 | 0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

Red and blue colors show negative and positive, respectively
ns – not significant; ** – significant at p≤0.01; * - significant at p≤0.05.
1- The number of leaves; 2- leaves dry weight: 3- Number of lateral branches; 4- Shoot dry weight; 5-Protein content; 6- CAT activity; 7: POX activity.

## CORRELATION BETWEEN TRAITS

In the first year, the calculation of correlation coefficients showed that the highest correlation was related to leaves number and leaves dry weight (r = 0.73$^{**}$). There after, the shoot dry weight had the highest correlation with leaves number (r = 0.67$^{**}$) and leaves dry weight (r = 0.57$^{**}$) (Tab. 4). There was a positive, weak and significant relationship between the antioxidant activity, the growth and yield such as CAT activity with leaves number (r = 0.40$^{**}$) and leaves dry weight (r = 0.38$^{**}$). While the correlation between the antioxidant activity of traits such as POX and protein was negative and significant. So that the amount of protein decreased with increasing POX enzyme. In the second year, the highest correlation was observed between the growth parameters such as the number of lateral branches and number of leaves (r = 0.65$^{**}$) and then between the growth parameters with the yield components such as the number of lateral branches with the shoot dry weight (r = 0.54$^{**}$) (Tab. 5). But there was a weak and significant relationship between the yield and growth as well as between the antioxidant activity and the growth and yield. Also There was a weak and significant relationship between the amount of protein, the CAT and POX. So on average, it can be concluded that with increasing the growth, the yield will increase and with increasing antioxidant activity in the plant, the growth and yield will decrease.

## CONCLUSION

The drought stress caused a reduction on growing and yielding components such as the number of leaves and reciprocally the leaves dry weight, and the number of lateral branches and reciprocally the shoot dry weight. The severe stress had a less negative effect on all of the above traits in the second year compared to the non-stress condition.The drought stress caused a reduction in protein content and CAT activity in the first year while both traits increased in the second year. the POX activity increased in two years too. The foliar application with chitosan, kaolin, and glycine amino acid, especially just flowering, reduced the adverse effects of water stress by improving growth and yield through influencing some traits such as the number of leaves, the leaves dry weight, the number of lateral branches, the shoot dry weight and the antioxidant activity through affecting some traits such as the protein content, activity contents of CAT and POX. Finally, it is recommended to increase plant production and reduce the effects of drought stresses in arid and semi-arid regions due to the beneficial effects of these substances and safety effects on the environment.

## REFERENCES

Abdul Manaim Mohammed sh., Sayed Ahmad H., Ahmad Al-Bouab, A., 2018. Effect of Chitosan, Putrescine and Irrigation Levels on the Drought Tolerance of Sour Orange Seedlings. Egyptian journal of horticulture, 45: 257-273.

Afsharmohammdian M., Ghanati F., Ahmadiani S., Sadrzamani K., 2016. Effect of drought stress on the activity of antioxidant enzymes and soluble sugars content of pennyroyal (*Mentha pulegium* L.). Nova Biological Reperta, 3: 228-237.

Ahrar A., Paknejad F., Tabatabaei S. A., Aghayari F., Soltani E., 2020. Evaluation of forage Amaranth (*Amaranthus hypochondriacus* L.) yield via comparing drought tolerance and susceptibility indices. Italian Journal of Agrometeorology 1: 31-40.

Kasim W., A.M. Hamada E., G. Shams El-Din N., Eskander S., 2015. Influence of seaweed extracts on the growth, some metabolic activities and yield of wheat grown under drought stress. International Journal of Agronomy and Agricultural Research, 7: 173-189.

Anjum S.A., Wang L., Farooq M., Xue L., Ali S., 2011. Fulvic acid application improves the maize performance under well-watered and drought conditions. Agronomy and Crop Science, 197:409-417.

Askari A., Ardakani M. R., Vazan S., Paknejad F., Hosseini Y., 2018. The effect of mycorrhizal symbiosis and seed priming on the amount of chlorophyll index and absorption of nutrients under drought stress in sesame plant under field conditions. Applied Ecology and Environmental Research, 16: 335-357.

Baby J., Jini D., 2011. Development of salt stress-tolerant plants by gene manipulation of antioxidant enzymes. Asian Journal of Agricultural Research. 5: 17-27.

Bernardo S., Dinis L.T., Luzio A., Pinto G., Meijon M., Valledor L., Conde A., Geros H., Correia C.M., Moutinho-Pereira J., 2017. Kaolin particle film application lowers oxidative damage and DNA methylation on grapevine (*Vitis vinifera* L.). Environmental and Experimental Botany, 139: 39-47.

Bian S., Jiang Y., 2009. Reactive Oxygen Species, Antioxidant Enzyme Activities and Gene Expression Patterns in Leaves and Roots of Kentucky Bluegrass in Response to Drought Stress and Recovery.Scientia Horticulturae, 120: 264-270.

Bradford M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annual Review of Biochemistry, 72: 248-254.

Dhindsa R.H., Plumb-Dhindsa R., Thorpe TA., 1981. Leaf senescence correlated with increased level of membrane permeability lipid peroxidation and decreased level of SOD and CAT. Journal of Experimental Botany, 32: 93-101.

Ermak G., Davies K.J., 2002. Calcium and oxidative stress: from cell signaling to cell death. Molecular Immunology, 38: 713-721.

Faralli M., Ivan G.G., Martin C.H., Roger D.B., Kevinm S.W., Fiona M.K.C., Peter S.K., 2016. Canopy application of film antitranspirants over the reproductive phase enhances yield and yield-related physiological traits of water-stressed oilseed rape (*Brassica napus*). Crop and Pasture. Science, 67: 751-765.

Fathiazad F., Mazandarani M., Hamedeyazdan S., 2011. Phytochemical analysis and antioxidant activity of *Hyssopus officinalis* L. from Iran. Advanced Pharmaceutical Bulletin, 1: 63-67.

Galeshi S., 2015.The Effect of environmental stresses on plants. (Drought, Salinity, Heat and Flooding), Gorgan University of Agricultural Sciences and Natural Resources Publication, 386 pages.

Glenn D.M., ErezA., Puterka G.J., Gundrum P.,2003. Particle films affect carbon assimilation and yield in Empire' apple. Journal of the American Society for Horticultural Science, 128: 356-362.

Glenn M., Puterka GJ., 2005. Particle films: A new technology for Agriculture. Horticultural Reviews, 31: 1-42.

Goreta S., Leskovar D. I., Jifon J. L., 2007. Gas exchange, water status, and growth of pepper seedlings exposed to transient water deficit stress are differentially altered by antitranspirants. American Society Horticulture Science, 132: 603-610.

Hossain M. A., Mostofa M. G., Fujita M., 2013. Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (*Brassica campestris* L.) seedlings, Molecular Plant Breeding, 4: 50-70.

Hussein SH., Terry N., 2002. Phytomonitoring the uniqe colonization of oil-contaminated saline environment by *Limoniastrum monopetalum* L. Boiss in Egypt. Journal of Environment International, 28: 127-135.

Howlett B., 2009. Secondary metabolite toxins and nutrition of plant pathogenic Fungi. Current Opinion in Plant Biology, 9: 371-375.

Jan-Mohammadi M., Mostafavi H., Kazemi H., 2014. Effect of chitosan application on the performance of lentil genotypes under rainfed conditions.Acta Technologica Agricalture, 4: 86-90.

Jifon J.L., Syvertsen J.P., 2003. Kaolin particle film applications can increase photosynthesis and water use efficiency of 'Ruby Red'grapefruit leaves. Journal of the American Society for Horticultural Science, 128: 107-112.

Jozwiak w., Politycka B., 2019. Effect of Selenium on Alleviating Oxidative Stress Caused by a Water Deficit in Cucumber Roots Plants, 8: 1-18.

Kizil S., Hasimi N., Tolan V., Kilinc E., Karatas H., 2010. Chemical composition, antimicrobial and antioxidant activities of hyssop (*Hyssopus officinalis* L.), Essential Oil. Notulae Botanicae Horti Agrobotanici Cluj-Napoca,38: 99-103.

Ma Z., Yang L., Yan H., Kennedy J.F., Meng X., 2013. Chitosan and oligochitosan enhance the resistance of peach fruit to brown rot. Carbohydrate Polymers, 94: 272-277.

Mac-Adam J.W., Nelson C.J., Sharp RE., 1992. Peroxidase activity in the leaf elongation zone of Tall Fescue. Plant Physiology, 99: 972-878.

Mahdavi B., Modarres Sanavy S. A. M., Aghaalikhani M., Sharifi M., 2013. Effect of chitosan on safflower (*Carthamus tinctorius* L.) Seed Germination and antioxidant enzymes activity under. water stress. Journal of Plant Research (Iranian Journal of Biology), 26: 352-365.

Michalczyk M., Macura R., Tesarowic zI., Banas J., 2012. Effect of adding essential oils of coriander (*Coriandrum sativum* L.) and hyssop (*Hyssopus officinalis* L.) on the shelf life of ground beef. Meat Science, 90: 842-850.

MiticV., Dordevic S., 2000. Essential oil composition of *Hyssopus officinalis* L. cultivated in Serbia FactaUniversitatis Series Physics. Chemistry and Technology, 2: 105-108.

Namvar A., Hashem H., Seyedsharifi R., 2018. Role of exogenous phytoprotectants in mitigation of adverse effects of abiotic stresses. Journal of Iranian plant Ecophysiological Research (plant Sciences Research), 12: 103-128.

Neyestani E., Azimzadeh M., 2005. Evaluation of drought tolerance of fifteen lentils (*Lens Culinaris* L.) genotype. Journal of Agricultural Land and Drought, 12:69- 77.

Pessarakli M., 2011. Handbook of Plant and Crop Stress, 1245 Pages.

Polanski Lech G., Khanifar A., Tadeusz W., 2018. Effect of salinity and drought stresses on growth parameters, essential oil constituents and yield in peppermint. African Journal of Agronomy, 6: 356-361.

Rahimi Y., Taleei A., Ranjbar M., 2019. Effect of water stress on Biochemical changes in *Mentha piperita* L. Iranian Journal of Field Crop Science, 50: 59-75.

Souri M.K., Hatamian M., 2019. Aminochelates in plant nutrition: a review. Journal of Plant Nutrition, 42: 67-78.

Tang W., Newton J.R., 2005. Polyamines reduced salt-induced oxidative damage by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation in Virginia pine. Plant Growth Regul, 46: 31-43.

Tardieu F., Parent B., Caldeira C.F., Welcker C., 2014. Genetic and physiological controls of growth under water deficit. Plant Physiology, 164: 1628-1635.

Tognolini M., Barocelli E., Ballabeni V., Bruni R., Bianchi A., Chiavarini M., Impicciatore M., 2005. Comparative screening of plant essential oils. Phenylpropanoid moiety as basic core for antiplate activity. Life Sci, 78: 1419-1432.

Xoconostle-Cázares B., Ramirez-Ortega FA., Flores-Elenes L., Ruiz-Medrano R., 2011. Drought tolerance in crop plants. American Journal of Plant Physiology, 5: 241–256.