



International Journal of Botany

ISSN: 1811-9700

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>



Research Article

Soil Moisture and Glutamic Acid Affect Yield, Volatile Oil and Proline Contents of Oregano Herb (*Origanum vulgare* L.)

¹Aisha M.A. Ahmed, ¹Iman M. Talaat and ²Khalid A. Khalid

¹Department of Botany, National Research Centre, 12311 Dokki, Cairo, Egypt

²Department of Medicinal and Aromatic Plants, National Research Centre, 12311 Dokki, Cairo, Egypt

Abstract

Background and Objective: Oregano herb has various pharmaceutical properties. Soil Moisture (SM) deficit has harmful effects on aromatic plants. So, the aim of this study was to decrease the harmful effect of SM deficit on oregano plants by adapting them through the use of glutamic acid (GLU). **Materials and Methods:** Oregano plants were divided into two main groups. The first group was subjected to different levels of SM: 100, 75, 50 and 25% corresponding to the Field Water Capacity (FWC), while second group was subjected to the same SM levels but GLU was added at 0.3 g L⁻¹ as foliar spray. Fresh Mass (FM), Dry Mass (DM), Volatile Oil (VO) percentage and milliliter 100 plant⁻¹, constituents of VO and proline (PRO) were identified. **Results:** The SM with GLU treatments increased the FM and DM of herb, VO and constituents of VO and PRO contents compared with the treatments of SM without GLU. Greatest FM, DM and VO were obtained with the treatment of 75% SM × GLU. The values were 59.2, 73.9, 23.8 and 29.8 g plant⁻¹ and 7 and 9 mL 100 plant⁻¹ of both seasons. The maximum values of PRO were resulted under the treatment of 25% SM × GLU with the values of 2.3 and 2.7 μmol g⁻¹. The highest amounts of major components (carvacrol, p-cymene and γ-terpinene) were recorded with the treatments of 25% SM × GLU with the values of 42.9, 24.1 and 17.6%. About 25 and 50% of SM × GLU treatments resulted in the highest values of monoterpene hydrocarbons, MCH (47.5%) and oxygenated monoterpenes, MCHO (47.1%) while 75% of MS without GLU treatment produced the highest amounts of sesquiterpene hydrocarbons, SCH (6.2%) and oxygenated sesquiterpenes, SCHO (5.4%). **Conclusion:** It can use GLU to decrease the harmful effect of SM deficits.

Key words: Soil moisture, glutamic acid, fresh mass, dry mass, volatile oil, proline

Received: September 12, 2016

Accepted: November 09, 2016

Published: December 15, 2016

Citation: Aisha M.A. Ahmed, Iman M. Talaat and Khalid A. Khalid, 2017. Soil moisture and glutamic acid affect yield, volatile oil and proline contents of oregano herb (*Origanum vulgare* L.). Int. J. Bot., 13: 43-51.

Corresponding Author: Aisha M.A. Ahmed, Department of Botany, National Research Centre, 12311 Dokki, Cairo, Egypt

Copyright: © 2017 Aisha M.A. Ahmed *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Origanum vulgare L., family Lamiaceae is commonly named oregano. It is cultivated and distributed in many places of the world of temperate climates of North Africa, Europe, Asia and America¹⁻². It is used to treat cough, sore throats and relieve digestive complaints³. The VO of oregano has different antimicrobial⁴ and antioxidant activities⁵.

The SM deficit limits the production of the agricultural lands in the world⁶. Yield and metabolites of agricultural crops can be affected by SM factor⁷. In the aspect of aromatic plants, drought causes significant changes in growth, yield and some metabolite products such as PRO and VO compositions⁸.

The chemical constituents of cumin herb were significantly affected by SM deficit conditions⁹. Morphological measurements (FM and DM) were reduced as SM decreased but the contents of PRO were increased of some aromatic plants such as basil species, calendula, lemon balm, apple geranium and black cumin herbs¹⁰⁻¹⁴.

The SM was effective in changing the yield (FM and DM) and VO composition of oregano plants¹⁵. In some previous studies, Fatima *et al.*¹⁶ found that citronella grass VOs were increased under SM deficit factor. The VOs and its main constituents of basil species, calendula, lemon balm, apple geranium and black cumin were promoted under SM deficit conditions¹⁰⁻¹⁴. By contrast, rosemary and anise VOs were decreased¹⁷⁻¹⁸. On the other hand the Achillea VO yield was increased under limited SM but the main constituent was decreased¹⁹.

Accumulations of amino acids were detected in plants under a biotic stress factors such as SM deficit which has different roles in plants such as acting as osmolyte, regulation of ion transport, modulating stomatal opening, detoxification of heavy metals, synthesis and activity of some enzymes, gene expression and redox-homeostasis²⁰. On the other hand, FM and DM of croton plants increased with GLU treatments²¹. The FM and DM of datura, lemon grass and basil were significantly increased under some amino acids treatments²²⁻²⁵. Amino acids promoted FM, DM, VO and main constituents of VO (farnesene, bisabolol oxide B, α -bisabolol, chamazulene and bisabolol oxide) extracted from chamomile flowers²⁶. Amino acids had significant effects on the FM and DM, VO and its constituents (2,2-dimethylbutanoic acid, isobutyl isobutyrate, α -isophorone, thymol, fenchyl acetate and linalool) of khella plants²⁷.

Some studies are available where accumulation of GLU in cotton and rice under SM deficit factor²⁸⁻²⁹. Thus, this study aimed to reduce the hazards effects of SM deficit on oregano plants by adapting them through the use of GLU.

MATERIALS AND METHODS

Experimental: Experiments were carried out in a greenhouse at National Research Centre, Egypt, during 2015 and 2016. Oregano seedlings were obtained from the Institute of Medicinal and Aromatic Plants, Egypt. Uniform seedlings were transplanted into plastic pots (30 cm diameter and 50 cm height). In the 1st week of June during both seasons, the pots were transferred to a greenhouse adjusted to natural conditions. Each pot was filled with 10 kg of air-dried soil. Three weeks after transplanting, the seedlings were thinned to three plants per pot. Pots were divided into two main groups. The first group was subjected to different levels of SM: 100, 75, 50 and 25% corresponding to the Field Water Capacity (FWC) determined in the field (by weight). The second group was subjected to the same treatments but GLU was added at 0.3 g L⁻¹ as foliar spray. All agricultural practices were conducted according to the main recommendations by the Ministry of Agriculture, Egypt. Physical and chemical properties of the soil used in this study were determined according to Jackson³⁰ and Cottenie *et al.*³¹ are presented in Table 1.

Harvesting: At full bloom, the plants were harvested twice (first and second harvests) during the growing seasons by cutting the plants 5 cm above the soil surface. Total FM and DM (g plant⁻¹) were recorded.

Volatile oil isolation: The FM (aerial part) was collected from each treatment during the 1st and 2nd harvests in both seasons; air dried and weighed to extract the Volatile Oil (VO), then 100 g from each replicate of all treatments was subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus³². The VO content was calculated as a relative percentage (v/w). In addition, total VOs (mL 100 plant⁻¹) were calculated by using the DM. The VOs extracted from oregano were collected during the 1st and 2nd harvests in both seasons from each treatment and dried over anhydrous sodium sulfate to identify the chemical constituents.

Table 1: Physical and chemical properties of the soil used

Clay (%)	Silt (%)	Sand (%)	OM (%)	N (%)	P (%)	K (%)	pH	EC (dsm ⁻¹)
38.0	36.0	26.0	1.3	0.3	0.1	0.1	7.7	0.6

OM: Organic matter, EC: Electronic conductivity

GC-MS analysis: The GC-MS analysis was carried out with an agilent 5975 GC-MSD system. The DB-5 column (60 m×0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL min⁻¹). The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C min⁻¹ that was kept constant at 220°C for 10 min and followed by elevating the temperature to 240°C at a rate of 1°C min⁻¹. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was m/z 35–450.

Gas chromatography analysis: Gas Chromatography (GC) analysis was carried out using an agilent 6890N GC system using Flame Ionization Detector (FID) temperature of 300°C. To obtain the same elution order with GC-MS, simultaneous auto injection was done on a duplicate of the same column at the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of components: Identification of the VOs components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their Retention Index (RI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, Mass Finder 3 Library)³³⁻³⁴ and in-house "Baser Library of Volatile Oil Constituents" built up by genuine compounds and components of known oils. Additionally, the previous study of Joulain and Koenig³⁵ were also used for the identification.

PRO determination: The PRO was determined at both seasons in fresh leaves according to Bates *et al.*³⁶ as follows: Samples: Fully expanded (sun) leaves from pot-grown oregano plants were sampled, purified PRO was used to standardize the procedure for quantifying sample values. Reagents: Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 mL glacial acetic and 20 mL, 6 M phosphoric acid with agitation until dissolved. Kept cool (stored at 4°C) the reagent remains stable 24 h; there are following procedure: (1) Approximately 0.5 g of plant material was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and the homogenate was filtered through whatman No. 2 filter paper, (2) Two milliliter of filtrate was reacted with 2 mL acid ninhydrin and 2 mL of glacial acetic acid in test tube for 1 h at 100°C and the reaction terminated in an ice bath, (3) The reaction mixture was extracted with 4 mL toluene, mixed vigorously with test tube stirrer 15-20 sec and (4) The chromophore containing toluene was separated from aqueous phase, warmed to room

temperature and the absorbance read at 520 nm using to standard curve and calculated on a fresh weight basis as follow:

$$\frac{(\mu\text{g PRO/mL} \times \text{mL toluene} / 15.5 \mu\text{g}/\mu\text{mol}) \{(\text{g sample})/5\}}{\mu\text{moles PRO/g of fresh material}}$$

Statistical analysis: In this experiment, 2 factors were considered: SM: 100, 75, 50 and 25% and GLU (with and without). For each treatment there were 5 replicates, each of which had 10 pots; in each pot 3 individual plants were planted. The experimental design followed a complete random block design. According to Snedecor and Cochran³⁷ the averages of data were statistically analyzed by using 2 ways analysis of variance (ANOVA). Significant values determined according to p-values (p<0.05 = significant, p<0.01 = moderate significant and p<0.001 = highly significant). The applications of that technique were according to the STAT-ITCF program³⁸.

RESULTS

Effect of SM, GLU and their interactions on the FM and DM:

The SM with or without GLU affected yield of herb (FM and DM) during both seasons (Table 2). In general, FM and DM decreased under the various SM levels, especially at 50 and 25% FWC. The SM with GLU treatments caused an increase in FM and DM compared with SM without GLU treatments. The heaviest FM and DM were recorded at the treatment of 75% FWC×GLU with the values of 59.2, 73.9 and 23.8, 29.8 g plant⁻¹ during 1st and 2nd seasons, respectively. The changes in FM and DM were highly significant for SM with or without GLU except the DM at 2nd season were insignificant for the interactions between SM and GLU.

Effect of SM, GLU and their interactions on VO composition:

The deficit of SM (less than 100%) caused an increase in VO (%) during both seasons (Table 3). The VO (%) was increased under SM levels with GLU compared with SM without GLU treatments. The highest VOs (%) were detected at the lowest SM level (25%)×GLU with the value of 0.5% at both seasons. The VO yields (mL 100 plant⁻¹) affected by the amount of SM with or without GLU. The SM×GLU caused an increase in VO yield comparison with SM without GLU. About 75% of SM×GLU treatment recorded the highest yield of VO with the values of 7 and 9 mL 100 plant⁻¹ during both seasons. The changes in VO (% or yield) were significant or highly significant for SM or GLU treatments while it was insignificant for SM×GLU treatments (Table 3).

Quantity and quality of constituents present with SM and GLU levels in oregano VO were investigated. Twenty two components were detected, ranged from 99-99.9% of total VO and classified into four chemical classes i.e., monoterpene

hydrocarbons (MCH), oxygenated monoterpenes (MCHO), sesquiterpene hydrocarbons (SCH) and oxygenated sesquiterpenes (SCHO) (Table 4). Monoterpenes (MCH+MCHO) was the major class (more than 85%).

Table 2: Effect of SM, GLU and their interactions on the yield (FM and DM)

Treatments		Yield of herb (g plant ⁻¹)							
		FM				DM			
		1st season		2nd season		1st season		2nd season	
GLU (0.3 g L ⁻¹)	SM	M	SD	M	SD	M	SD	M	SD
Without GLU	100	36.2	±3.0	42.7	±2.0	12.6	±2.0	19.2	±0.2
	75	36.9	±3.5	55.2	±5.0	16.7	±1.0	23.4	±0.4
	50	30.2	±1.0	41.7	±1.0	12.5	±0.5	18.5	±0.5
	25	15.8	±1.0	24.8	±5.7	7.6	±0.4	11.3	±0.4
Overall without GLU		29.8	±9.0	41.1	±11.7	12.4	±3.5	18.1	±4.6
With GLU	100	40.3	±1.4	62.9	±2.1	19.8	±1.4	25.9	±5.0
	75	59.2	±6.0	73.9	±3.0	23.8	±1.0	29.8	±0.9
	50	31.4	±1.0	49.2	±1.1	13.4	±3.0	22.1	±0.1
	25	21.3	±1.0	32.5	±0.7	8.7	±0.4	13.4	±0.5
Overall with GLU		38.1	±14.8	54.6	±11.3	16.4	±6.3	22.8	±3.6
Overall SM	100	38.3	±4.8	52.8	±11.2	16.2	±4.2	22.6	±4.8
	75	48.1	±12.6	64.6	±10.9	20.3	±4.4	26.6	±3.6
	50	30.8	±1.1	45.5	±4.5	13.0	±0.6	20.3	±2.0
	25	18.6	±3.8	28.7	±5.4	8.2	±0.8	12.4	±1.5
F-value									
SM			78.0***		117.2***		40.5***		42.8***
GLU			34.2***		89.5***		20.8***		23.1***
SM×GLU			11.9***		5.9***		4.4***		1.5

GLU: Glutamic acid, M: Mean, SM: Soil moisture, SD: Standard deviation, FM: Fresh mass, DM: Dry mass

Table 3: Effect of SM, GLU and their interactions on VO (percentage and mL 100 plant⁻¹) and PRO contents

Treatments		VO content											
		Percentage				mL 100 plant ⁻¹				PRO (µmol g ⁻¹)			
		1st season		2nd season		1st season		2nd season		1st season		2nd season	
GLU (0.3 g L ⁻¹)	SM	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Without GLU	100	0.1	±0.0	0.1	±0.0	1.0	±0.0	2.0	±1.0	0.7	±0.2	0.8	±0.1
	75	0.2	±0.1	0.2	±0.1	3.0	±0.1	5.0	±1.0	0.9	±0.1	1.1	±0.1
	50	0.3	±0.1	0.3	±0.1	4.0	±0.1	6.0	±1.0	1.0	±0.2	1.4	±0.2
	25	0.4	±0.1	0.4	±0.1	3.0	±0.1	5.0	±0.7	1.3	±0.1	1.7	±0.3
Overall without GLU		0.3	±1.4	0.3	±1.7	2.8	±1.4	4.5	±0.4	1.0	±0.3	1.3	±0.4
With GLU	100	0.2	±0.1	0.2	±1.0	4.0	±1.4	5.0	±2.0	1.0	±0.4	1.2	±0.1
	75	0.3	±0.1	0.3	±0.1	7.0	±1.0	9.0	±2.0	1.6	±0.2	1.7	±0.1
	50	0.4	±0.1	0.4	±0.1	5.0	±2.0	8.0	±2.0	2.2	±0.2	2.2	±0.2
	25	0.5	±0.1	0.5	±0.1	4.0	±1.0	7.0	±2.7	2.3	±0.5	2.7	±0.1
Overall with GLU		0.4	±0.2	0.4	±1.4	5.0	±1.7	7.5	±0.5	1.8	±0.2	2.0	±0.3
Overall SM	100	0.2	±0.1	0.2	±0.1	2.5	±1.8	3.5	±1.9	0.9	±0.2	1.0	±0.3
	75	0.3	±0.1	0.3	±0.1	5.0	±2.6	7.0	±2.6	1.3	±0.4	1.4	±0.3
	50	0.4	±0.1	0.4	±0.1	4.5	±1.0	7.5	±2.2	1.6	±0.7	1.8	±0.5
	25	0.5	±0.1	0.5	±0.1	3.5	±0.7	6.0	±1.4	1.8	±0.7	2.2	±0.7
F-value													
SM			7.5***		7.5***		3.9*		6.3***		15.2***		42.0***
GLU			5.0*		5.0*		14.0***		23.7***		68.6***		101.1***
SM×GLU			0.0		0.0		1.8		0.2		3.4*		2.6*

GLU: Glutamic acid, M: mean, SM: Soil moisture, SD: Standard deviation, VO: Volatile oil

Table 4: Effect of SM, GLU on the VO constituent

Constituents (%)	RI	SM treatments												F-value				
		Without GLU						With GLU										
		100	75	50	25	100	75	50	25	100	75	50	25					
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	
α-Thujene	923	0.4	±0.1	0.7	±0.1	1.1	±0.1	0.9	±0.1	1.1	±0.1	0.7	±0.2	1.0	±0.1	1.2	±0.3	1.2
α-Pinene	933	1.2	±0.2	1.1	±0.1	1.5	±0.5	1.6	±0.1	1.5	±0.5	0.9	±0.1	0.5	±0.1	0.1	±0.0	1.9
β-Pinene	980	0.7	±0.1	1.2	±0.2	0.8	±0.1	1.1	±0.1	0.9	±0.1	1.4	±0.4	1.3	±0.3	0.9	±0.1	1.6
Myrcene	991	3.1	±0.1	2.4	±0.4	2.1	±0.1	2.2	±0.0	1.8	±0.2	0.5	±0.1	0.8	±0.1	0.8	±0.1	0.9
α-Phellandrene	1005	1.9	±0.1	0.8	±0.1	0.8	±0.1	1.1	±0.1	0.9	±0.2	0.7	±0.2	0.7	±0.1	0.9	±0.2	2.3
p-Terpinene	1018	1.4	±0.4	1.8	±0.2	1.5	±0.5	1.5	±0.7	1.4	±0.4	1.7	±0.3	1.2	±0.2	1.3	±0.4	0.1
p-Cymene	1025	19.6	±0.4	20.0	±2.0	20.5	±0.5	21.1	±0.1	21.3	±0.3	22.4	±0.4	23.3	±0.3	24.1	±0.1	0.4
Limonene	1031	2.1	±0.1	1.9	±0.1	2.0	±0.5	1.6	±0.1	1.2	±0.2	2.5	±0.5	0.1	±0.0	0.6	±0.0	2.5
γ-Terpinene	1062	13.8	±0.2	14.1	±0.1	15.3	±0.3	15.9	±0.1	14.8	±0.2	15.8	±0.4	16.3	±0.3	17.6	±0.8	0.1
Linalool	1098	1.1	±0.1	1.3	±0.3	1.4	±0.4	1.4	±0.6	1.5	±0.5	1.7	±0.3	1.6	±0.4	1.4	±0.6	0.6
Borneol	1165	0.3	±0.2	0.2	±0.1	0.5	±0.1	0.5	±0.1	0.4	±0.1	0.7	±0.3	0.5	±0.1	0.5	±0.1	2.8*
Terpinen-4-ol	1177	1.1	±0.1	1.1	±0.1	1.0	±0.2	1.2	±0.2	1.4	±0.1	0.5	±0.1	0.7	±0.2	0.4	±0.1	0.6
α-Terpineol	1189	0.2	±0.1	0.3	±0.1	0.2	±0.1	0.4	±0.7	0.4	±0.1	0.6	±0.4	0.6	±0.4	0.5	±0.1	0.9
Bornyl acetate	1285	1.1	±0.1	1.5	±0.5	1.2	±0.2	1.5	±0.1	2.4	±0.9	1.7	±0.2	1.4	±0.4	0.7	±0.3	2.7
Thymol	1290	1.8	±0.2	1.5	±0.5	1.5	±0.5	1.6	±0.1	1.3	±0.3	0.8	±0.2	0.5	±0.1	0.4	±0.1	0.4
Carvacrol	1298	38.1	±0.7	38.4	±0.2	38.9	±0.1	40.1	±0.1	39.1	±0.1	39.8	±0.2	41.8	±0.2	42.9	±0.1	0.4
β-Elementene	1375	1.7	±0.3	1.6	±0.4	1.4	±0.4	0.6	±0.1	0.9	±0.1	0.8	±0.2	0.1	±0.0	0.1	±0.0	1.1
β-Caryophyllene	1418	2.8	±0.4	2.9	±0.1	1.4	±0.4	0.3	±0.1	0.5	±0.1	0.8	±0.1	0.9	±0.1	0.5	±0.3	4.2*
Germacrene D	1480	1.6	±0.4	1.7	±0.3	1.4	±0.4	1.7	±0.4	1.5	±0.5	1.7	±0.3	0.9	±0.1	0.8	±0.1	0.5
Caryophyllene oxide	1581	1.2	±0.1	1.4	±0.4	1.6	±0.4	1.3	±0.4	1.4	±0.4	1.8	±0.2	1.6	±0.4	1.5	±0.7	0.3
Viridiflorol	1590	2.4	±0.1	2.5	±0.5	2.7	±0.3	1.7	±0.4	2.1	±0.1	1.3	±0.3	1.8	±0.1	1.0	±0.7	0.8
α-Cadinol	1653	1.4	±0.3	1.5	±0.5	0.9	±0.1	0.6	±0.5	0.9	±0.1	0.6	±0.4	1.7	±0.3	1.4	±0.6	0.8
MCH	-	44.2	±0.6	44.0	±2.0	45.6	±0.4	47.0	±2.8	44.9	±0.1	46.6	±0.4	45.2	±0.2	47.5	±0.7	6.2*
MCHO	-	43.7	±0.4	44.3	±0.3	44.7	±0.3	46.7	±0.5	46.5	±0.5	45.8	±0.2	47.1	±0.1	46.8	±0.3	0.9*
SCH	-	6.1	±0.1	6.2	±0.2	4.2	±0.2	2.6	±0.6	2.9	±0.1	3.3	±0.3	1.9	±0.1	1.4	±0.6	3.0*
SCHO	-	5.0	±1.0	5.4	±0.2	5.2	±0.2	3.6	±0.6	4.4	±0.4	3.7	±0.3	5.1	±0.1	3.9	±0.1	2.1*
Total identified	-	99.0	-	99.9	-	99.7	-	99.9	-	98.7	-	99.4	-	99.3	-	99.6	-	-

GLU: Glutamic acid, M: Mean, SM: Soil moisture, SD: Standard deviation, MCH: Monoterpene hydrocarbons, MCHO: Oxygenated monoterpenes, SCH: Sesquiterpene hydrocarbons, SCHO: Oxygenated sesquiterpenes, RI: Retention index; α: alpha, β: beta, γ: gamma, p: Para

Carvacrol, p-cymene and γ -terpinene were detected as major components which gave the highest values with all SM or GLU \times SM treatments. All major components increased under SM or the interaction between SM and GLU treatments compared with control (100% of FWC). The highest amounts of major components were recorded with the treatments of 25% SM \times GLU with the values of 42.9, 24.1 and 17.6%, respectively. The highest amounts of MCH (47.5%) and MCHO (47.1%) were obtained from the treatments of 25 and 50% of SM \times GLU. The greatest values of SCH (6.2%) and SCHO (5.4%) were obtained from the treatment of 75% of MS without GLU. The changes in all constituents were insignificant for investigated treatments except the components of borneol and β -caryophyllene were significant. The changes in all chemical classes were significant for different treatments.

Effect of SM, GLU and their interactions on the PRO content:

Treating oregano plants by various levels of SM, GLU and their interactions promoted the accumulation of PRO. The highest amount (2.3 and 2.7 $\mu\text{mol g}^{-1}$) of PRO content produced from the treatment of 25% SM content \times GLU during both seasons.

DISCUSSION

The decrease of herb yield (FM and DM) under low SM levels (50 and 25% of FWC) during both seasons may be due to exposure to injurious SM causing reduction of turgor which would result in reducing plant growth and development of cells, especially in the herb (stems and leaves)³⁹. Low SM reduces plant cell development, so the leaves and plant size will be smaller⁴⁰. When the size of leaf is smaller, the capacity to trap light reduces too and the capacity of total photosynthesis reduces, i.e., photosynthesis is restricted in low SM cases, with a subsequent inhibition in plant growth and performance⁴⁰. Low SM resulted in significant reduces in fresh and dry mater of Japanese mint, basil species, calendula, lemon balm, apple geranium and black cumin^{41,10-14}. On the other hand, under SM deficit the available water does not move into the root cells. Water loses in transpiration and not be completely replaced, resulting in turgor loss. In the guard cells which surrounding the stomatal pore, the turgor decreases, the cells fill the pore and the stomatal pore reduces, so the transpiration reduces. The uptake of CO₂ and the carbon assimilation rate of the plant are reduced when the stomata are closed. The duration of water deficit affected in reducing the crop production and causing injury to chloroplasts. There may also be an interaction with other

stresses, such as heat stress, when transpiration is reduced that will also contribute to the strain on the plant⁴². The effect of SM on VO contents, its constituents and chemical classes may be due to the influence on enzyme role and metabolism activities of VO productivity⁴³. The VO contents of *Parthenium argentatum*, peppermint, hyssopus and anise were enhanced and there were significant quantitative variations among the VO in terms of chemical constituents⁴⁴⁻⁴⁷. *Bunium persicum* VO and its constituents were affected by soil SM treatments⁴⁸. Regarding to the oregano VO composition, similar constituents were found by Said-Al Ahl¹⁵ and Teixeira *et al.*⁴⁹, they said that the major constituents of VO extracted from oregano herb were carvacrol, p-cymene and γ -terpinene as well as the components belong to different classes (MCH, MCHO, SCH and SCHO). The accumulations of PRO were promoted by decrease of SM levels during the first and second seasons. These results are in accordance with those obtained by Slama *et al.*⁵⁰ as well as Blum and Ebercon⁵¹, they reported that PRO is regarded as a source of energy, carbon and nitrogen for recovering tissues under soil moisture deficits.

The positive effects of GLU on FM, DM, VO and PRO contents under SM deficit confirmed by some previous studies, i.e., GLU promoting the auxin synthesis in plants⁵², auxins play an important role in plant development such as growth of root system, vascular tissue differentiation, auxiliary bud formation, apical dominance and flower initiation under stress factors⁵³. Azimi *et al.*⁵⁴ revealed that amino acids have a positive effect on plant, root development, yield and significantly mitigates injuries caused by environmental stresses. Amino acids are crucial to sustain cellular functions under the soil moisture deficits⁵⁵. Amino acids can improve the yield and PRO content under SM deficits⁵⁶⁻⁵⁷. The VOs (percentage, yield and constituents) of chamomile and khella were significantly affected by amino acids treatments²⁶⁻²⁷. Omer *et al.*⁵⁸ indicated that amino acids increase the VO content and major constituents of chamomile. Saburi *et al.*⁵⁹ reported that basil VO was improved with the treatments of amino acids.

CONCLUSION

It can be concluded that SM resulted in a highly significant reduction of FM and DM of oregano herb while PRO, VO and main constituents of VO were increased. The GLU \times SM recorded higher values of all measurements than SM treatments. Adapting oregano plants to SM conditions through the use of GLU is very important especially in arid and semi arid regions for increasing the yield and active constituents such as VO of oregano plants.

SIGNIFICANT STATEMENT

Previous studies indicated that reducing SM limits the quantity and quality of oregano herb. In this study the effect of GLU on oregano herb were carried out under SM stress factor. Results showed that GLU promotes yield and active principal of oregano herb under SM deficit. It means, using GLU to decrease the harmful effect of SM deficits.

REFERENCES

1. Ietswaart, J.H., 1980. A Taxonomic Revision of the Genus *Origanum* (Labiatae). 1st Edn., Springer, Netherlands, ISBN: 978-90-6021-463-3, Pages: 154.
2. Goliaris, A.H., P.S. Chatzopoulou and S.T. Katsiotis, 2003. Production of new Greek oregano clones and analysis of their essential oils. *J. Herbs Spices Med. Plants*, 10: 29-35.
3. Radusiene, J., L. Ivanauskas, V. Janulis and V. Jakstas, 2008. Composition and variability of phenolic compounds in *Origanum vulgare* from Lithuania. *Biologija*, 54: 45-49.
4. Raduoiene, J., A. Judpintiene, D. Peeilyte and V. Janulis, 2005. Chemical composition of essential oil and antimicrobial activity of *Origanum vulgare*. *Biologija*, 4: 53-58.
5. Jaloszynski, K., A. Figiel and A. Wojdylo, 2008. Drying kinetics and antioxidant activity of oregano. *Acta Agrophys.*, 11: 81-90.
6. Delfine, S., F. Loreto, P. Pinelli, R. Tognetti and A. Alvino, 2005. Isoprenoids content and photosynthetic limitations in rosemary and spearmint plants under water stress. *Agric. Ecosyst. Environ.*, 106: 243-252.
7. Charles, D.J., R.J. Joly and J.E. Simon, 1990. Effects of osmotic stress on the essential oil content and composition of peppermint. *Phytochemistry*, 29: 2837-2840.
8. Petropoulos, S.A., D. Daferera, M.G. Polissiou and H.C. Passam, 2008. The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. *Sci. Hort.*, 115: 393-397.
9. Bettaieb, I., S. Knioua, I. Hamrouni, F. Limam and B. Marzouk, 2011. Water-deficit impact on fatty acid and essential oil composition and antioxidant activities of cumin (*Cuminum cyminum* L.) aerial parts. *J. Agric. Food Chem.*, 59: 328-334.
10. Khalid, K.A., 2006. Influence of water stress on growth, essential oil and chemical composition of herbs (*Ocimum* sp.). *Inter. Agrophys.*, 20: 289-296.
11. Khalid, K.A. and J.A.T. da Silva, 2010. Yield, essential oil and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions. *Sci. Hortic.*, 126: 297-305.
12. Khalid, K.A., J.A.T. da Silva and W. Cai, 2010. Water deficit and polyethylene glycol 6000 affects morphological and biochemical characters of *Pelargonium odoratissimum* (L.). *Sci. Hortic.*, 125: 159-166.
13. Khalid, K.A. and W. Cai, 2011. The effects of mannitol and salinity stresses on growth and biochemical accumulations in lemon balm. *Acta Ecol. Sinica*, 31: 112-120.
14. Khalid, K.A. and A.M.A. Ahmed, 2017. Growth and certain biochemical components of black cumin cultivated under salinity stress factor. *J. Mater. Environ. Sci.*, 8: 7-13.
15. Said-Al Ahl, H.A.H., E.A. Omer and N.Y. Naguib, 2009. Effect of water stress and nitrogen fertilizer on herb and essential oil of oregano. *Int. Agrophys.*, 23: 269-275.
16. Fatima, S., A.H.A. Farooqi and S. Sharma, 2000. Effect of drought stress and plant density on growth and essential oil metabolism in citronella java (*Cymbopogon winterianus*) cultivars. *J. Med. Aromatic Plant Sci.*, 22: 563-567.
17. Singh, M. and S. Ramesh, 2000. Effect of irrigation and nitrogen on herbage, oil yield and water-use efficiency in rosemary grown under semi-arid tropical conditions. *J. Med. Aromatic Plant Sci.*, 22: 659-662.
18. Zehtab-Salmasi, S., A. Javanshir, R. Omidbaigi, H. Alyari and K. Ghassemi-Golezani, 2001. Effects of water supply and sowing date on performance and essential oil production of anise (*Pimpinella anisum* L.). *Acta. Agron. Hung.*, 49: 75-81.
19. Tatar, O., A. Konakchiev, T. Tsonev, V. Velikova and E. Gesheva *et al.*, 2016. Plant-soil water status-induced changes in physiological and biochemical properties of yarrow. *J. Essential Oil Bearing Plants*, 19: 1776-1787.
20. Rai, V.K., 2002. Role of amino acids in plant responses to stresses. *Biol. Planta.*, 45: 481-487.
21. Mazher, A.A.M., S.M. Zaghloul, S.A. Mahmoud and H.S. Siam, 2011. Stimulatory effect of kinetin, ascorbic acid and glutamic acid on growth and chemical constituents of *Codiaeum variegatum* L. *Plant. Am. Eurasian J. Agric. Environ. Sci.*, 10: 318-323.
22. Moursy, H.A.I., M.S. Hussein and K.M. El-Bahr, 1988. Effect of some alkaloid precursors on the growth and alkaloid production of *Datura stramonium* L. cultured *in vitro*. *Egypt. J. Bot.*, 31: 153-165.
23. Gamal El-Din, K.M., S.A. Tarraf and L.K. Balbaa, 1997. Physiological studies on the effect of some amino acids and micronutrients on growth and essential oil content in lemon-grass (*Cymbopogon citratus* Hort.) Mansoura Univ. *J. Agric. Sci.*, 22: 4229-4241.
24. Talaat, I.M. and A.A. Youssef, 2002. The role of the amino acids lysine and ornithine in growth and chemical constituents of Basil plants. *Egypt. J. Applied Sci.*, 17: 83-95.
25. Youssef, A.A., R.A. El-Mergawi and M.S.A. Abd El-Wahed, 2004. Effect of putrescine and phenylalanine on growth and alkaloid production of some *Datura* species. *J. Agric. Sci. Mansoura Univ.*, 29: 4037-4053.
26. El-Din, K.M.G. and M.S.A. Abd El-Wahed, 2005. Effect of some amino acids on growth and essential oil content of chamomile plant. *Int. J. Agric. Biol.*, 7: 376-380.

27. Talaat, I.M., H.I. Khattab and A.M. Ahmed, 2014. Changes in growth, hormones levels and essential oil content of Ammi visnaga L. plants treated with some bioregulators. Saudi J. Biol. Sci., 21: 355-365.
28. Hanower, P. and J. Brzozowska, 1975. Effects of osmotic stress on composition of free amino acids in cotton leaves. Phytochemistry, 14: 1691-1694.
29. Yang, C.W., C.C. Lin and C.H. Kao, 2000. Proline, ornithine, arginine and glutamic acid contents in detached rice leaves. Biol. Planta., 43: 305-307.
30. Jackson, M.L., 1973. Soil Chemical Analysis. 1st Edn., Prentice Hall Ltd., New Delhi, India, Pages: 498.
31. Cottenie, A., M. Verloo, L. Kiekens, G. Velgh and R. Camerlynck, 1982. Chemical Analysis of Plant and Soils. Laboratory of Analytical and Agrochemistry, State University of Gent, Belgium, Pages: 63.
32. Clevenger, J.F., 1928. Apparatus for the determination of volatile oil. J. Am. Pharmaceut. Assoc., 17: 345-349.
33. McLafferty, F.W. and D.B. Stauffer, 1989. The Wiley/NBS Registry of Mass Spectral Datum. Wiley, New York, ISBN: 9780471602682, Pages: 7872.
34. Koenig, W.A., D. Joulain and D. H. Hochmuth, 2004. Terpenoids and Related Constituents of Essential Oils. Mass Finder Publishing, Hamburg, Germany.
35. Joulain, D. and K.A. Koenig, 1998. The Atlas of Spectra Data of Sesquiterpene Hydrocarbons. 1st Edn., EB Verlag Publishing, Hamburg, Germany.
36. Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.
37. Snedecor, G.W. and W.G. Cochran, 1990. Statistical Methods. 11th Edn., Iowa State University Publishing, Ames, Iowa, USA.
38. Foucart, A., 1982. Analyse Factorielle, Programmatiol sur Micro-Ordinateur. Masson, Paris, ISBN-13: 978-2225764509, Pages: 243.
39. Kaufmann, M.R. and A.N. Eckard, 1971. Evaluation of water stress control with polyethylene glycols by analysis of guttation. Plant Physiol., 47: 453-456.
40. Hsiao, T.C., 1973. Plant responses to water stress. Annu. Rev. Plant Physiol., 24: 519-570.
41. Misra, A. and N.K. Srivastava, 2000. Influence of water stress on Japanese mint. J. Herbs Spices Med. Plants, 7: 51-58.
42. Bohnert, H.J. and R.G. Jensen, 1996. Strategies for engineering water-stress tolerance in plants. Trends Biotechnol., 14: 89-97.
43. Burbott, A.J. and W.D. Loomis, 1969. Evidence for metabolic turnover of monoterpenes in peppermint. Plant Physiol., 44: 173-179.
44. Nik, Z.B., M. Mirza and M. Ghaffari, 2008. Effect of drought stress on growth and essential oil contents in *Parthenium argentatum* Gray. J. Essential Oil Bearing Plants, 11: 423-429.
45. Khorasaninejad, S., A. Mousavi, H. Soltanloo, K. Hemmati and A. Khalighi, 2011. The effect of drought stress on growth parameters, essential oil yield and constituent of Peppermint (*Mentha piperita* L.). J. Med. Plants Res., 5: 5360-5365.
46. Tavakoli, M. and Z. Aghajani, 2016. The effects of drought stress on the components of the essential oil of *Hyssopus officinalis* L. and determining the antioxidative properties of its water extracts. J. Applied Environ. Biol. Sci., 6: 31-36.
47. Saeedfar, S., M. Negahban and M.M. Soorestani, 2015. The effect of drought stress on the essential oil content and some of the biochemical characteristics of anise hyssop (*Agastache foeniculum* [Pursh] Kuntze). Eur. J. Mol. Biotechnol., 8: 103-114.
48. Saeidnejad, A.H., M. Kafi, H.R. Khazaei and M. Pesarakli, 2013. Effects of drought stress on quantitative and qualitative yield and antioxidative activity of *Bunium persicum*. Turk. J. Bot., 37: 930-939.
49. Teixeira, B., A. Marques, C. Ramos, C. Serrano and O. Matos *et al.*, 2013. Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. J. Sci. Food Agric., 93: 2707-2714.
50. Slama, I., T. Ghnaya, K. Hessini, D. Messedi, A. Savoure and C. Abdelly, 2007. Comparative study of the effects of mannitol and PEG osmotic stress on growth and solute accumulation in *Sesuvium portulacastrum*. Environ. Exp. Bot., 61: 10-17.
51. Blum, A. and A. Ebercon, 1976. Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. Crop Sci., 16: 428-431.
52. Taiz, L. and E. Zeiger, 2010. Plant Physiology. 5th Edn., Sinauer Associates Inc., Sunderland, MA., USA.
53. Zhao, Y., 2010. Auxin biosynthesis and its role in plant development. Annu. Rev. Plant Biol., 61: 49-64.
54. Azimi, M.S., J. Daneshian, S. Sayfzadeh and S. Zare, 2013. Evaluation of amino acid and salicylic acid application on yield and growth of wheat under water deficit. Int. J. Agric. Crop Sic., 5-8: 816-819.
55. Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra, 2009. Plant drought stress: Effects, mechanisms and management. Agron. Sustain. Dev., 29: 185-212.
56. Kasraie, P., M. Nasri, M. Khalatbari, A. Pazoki and R. Monem, 2012. The effects of time spraying amino acid on water deficit stress on yield, yield component and some physiological characteristics of grain corn (TWC647). Ann. Biol. Res., 3: 4282-4286.
57. Pranckietiene, I., E. Mazuolyte-Miskine, V. Pranckietis, R. Dromantiene, G. Sidlauskas and R. Vaisvalavicius, 2015. The effect of amino acids on nitrogen, phosphorus and potassium changes in spring barley under the conditions of water deficit. Zemdirbyste-Agriculture, 102: 265-272.

58. Omer, E.A., H.A.H. Said-Al Ahl, A.G. El Gendy, K.A. Shaban and M.S. Hussein, 2013. Effect of amino acids application on production, volatile oil and chemical composition of chamomile cultivated in saline soil at Sinai. *J. Applied Sci. Res.*, 9: 3006-3021.
59. Saburi, M., M.R.H.S. Hadi and M.T. Darzi, 2014. Effects of amino acids and nitrogen fixing bacteria on quantitative yield and essential oil content of basil (*Ocimum basilicum*). *Agric. Sci. Dev.*, 3: 265-268.