AMERICAN-EURASIAN JOURNAL OF SUSTAINABLE AGRICULTURE
ISSN: 1995-0748, EISSN: 1998-1074
2015, volume(9),issue(4):pages(60-73)
Published Online June 2015 in http://www.aensiweb.com/AEJSA/

Effect of bio-fertilizers on growth, yield, water relations, photosynthetic pigments and carbohydrates contents of Origanum vulgare L. plants grown under water stress conditions

Soha El-Sayed Khalil and Abdel-Salam Ali El-Noemani

Water Relation & field Irrigation Dept., National Research Centre, 33 El-Tahrir St., Dokki, 12622 Giza, Egypt.

Received 28 February 2015; accepted 26 May 2015; published 21 June 2015

Address For Correspondence:
Prof. Dr. Abdel-Salam Ali El-Noemani , National Research Centre, Water Relations and Field Irrigation Department, Agricultural & Biological Research Division, 33 El-Tahrir St., Dokki, 12622 Cairo, Egypt.
Mobile: +201204463278 – Fax: +20233370931, E-mail: a.elnoemani@gmail.com

ABSTRACT

Two experiments were carried out at the greenhouse of the National Research Centre, Dokki, Cairo, Egypt during the two successive seasons of 2012/2013 and 2013/2014 to investigate the effect of water stress (75, 55 and 35% depletion of the available soil moisture) and bio-fertilizer treatments {Arbuscular Mycorrhizal Fungus (AMF), yeast application (at rate of 8 g/L)} and/or maintained as uninoculated controls) on growth, yield, water relations, photosynthetic pigments concentration and carbohydrates content of Origanum vulgare L. plant. It was concluded that water stress induced gradual and progressive declines in most of the previously mentioned characters; and drastic increases in the contents of chlorophyll a, Chlb, total chlorophyll and reducing sugars. Inoculation oregano plants with mycorrhizal fungi (AMF) and/or active yeast showed positive effects on all studied characters, except the contents of chlorophyll a, b and total chlorophyll (Chla+b). Dual inoculation with mycorrhizae and yeast provided higher tolerance to drought conditions compared with the individual treatment. Mycorrhizal colonization showed generally more pronounced effects than yeast treatment.

KEY WORDS
Available soil water, Arbuscular Mycorrhizal Fungus, yeast, oil %, total sugars.

INTRODUCTION

Plants are exposed to a variety of biotic or abiotic stresses, such as drought, salt loading and freezing that influence their development, growth and productivity. One of the major abiotic stresses that affect plant productivity is water stress resulting from drought and salinity [39]. On the other hand, plants vary greatly in their capability to tolerate stress conditions, hence some of them are unable to endure stress so wilt and die (sensitive plants), while others (tolerant plants) can tolerate stress by undergoing certain physiological changes in their tissues which thus maintain their cell water potential turgidity at normal level, in spite of soil drought [114] Drought is one of the most important environmental factors inhibiting photosynthesis and decreasing growth and productivity of plants. It is one of the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% [20, 132]. Regarding the rate of root growth, their number and the ratio of root/ shoot growth, [15, 60] and have found direct relationships between each of those growth criteria and increasing rate of water stress. Concerning the effect of water stress on each of the fresh and dry weight of shoot and roots, conflicting results were obtained. Some results indicated that both roots and shoot weights were decreased [46]. However others showed that only root weights were increased with vigorous drought, but shoot decreased [57].

The genus *Origanum* belongs to the family of *Lamiae* (*Labiatae*) and includes many species that are commonly found as wild plants in the Mediterranean areas [119]. Because of special compositions of essential oil, the leaves of *Origanum* plants are widely used as a very popular spice for food production. *Origanum vulgare* L. is the widest spread among all the species within the genus which distributed all over, Europe, West and Central Asia up to Taiwan, North Africa, and America [36]. Traditionally, leaves and flowers of oregano are used in Lithuania mostly for their beneficial properties to cure cough, sore throats, relieve digestive complaints, and probably stimulate the appetite [54].

The volatile oil of oregano has been used traditionally for respiratory disorders, indigestion, dental caries, rheumatoid arthritis and urinary tract disorders [31]. Recently, this spice plant has drawn more attention of consumers due to the antimicrobial, antifungal, insecticidal and anti-oxidative effect of this herb on human health [70, 122, 13].

Bio-fertilizers are products containing living cells of different types of microorganisms [22] that have an ability to convert nutritionally important elements from unavailable to available form through biological processes [130] and are known to help with expansion of the root system and better seed germination. Bio-fertilizers differ from chemical and organic fertilizers that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. Some microorganisms have positive effects on plant growth promotion [22]. They also increase germination and vigour in young plants, leading to improved crop stands [22].

Various *Pseudomonas* species have shown to be effective in controlling pathogenic fungi and stimulating plant growth by a variety of mechanisms, including production of side rophores, synthesis of antibiotics, production of phytohormones, enhancement of phosphate uptake by the plant, nitrogen fixation, and synthesis of enzymes that regulate plant ethylene levels [3]. Arbuscular mycorrhizal fungi (AMF) are a major component of rhizosphere micro-flora in natural ecosystems and have been reported to form obligate symbiotic associations with most angiosperm plants, including several medicinal species [129]. The ability of AMF to enhance host plant uptake of relatively immobile nutrients, in particular P, and several micronutrients, has been the most recognized beneficial effect of mycorrhiza. Therefore, mycorrhizas are multifunctional in (agro) ecosystems, potentially improving physical soil quality (through the external hyphae), chemical soil quality (through enhanced nutrient uptake), and biological soil quality (through the soil food web) [21]. Also, Candida or yeast is a microorganism that has an objective of increasing their number and of accelerating certain microbial process to increase the availability of nutrients elements in the form which can be easily taken by plants. Thus greater attention has been directed on the use of microorganisms as bio stimulants to provide nutrients for higher plants without any pollution to the environment [45].

The objective of this work was mainly to investigate the response of growth, yield, water relations, photosynthetic rate, and total sugars content of *Origanum vulgare* L. plant to AM fungi and/or yeast application under different soil moisture levels.

MATERIALS AND METHODS

Growth conditions:

A pot experiment was conducted during the two growing seasons of 2012/2013 and 2013/2014 in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt. The seedlings were directly transplanted on the 15th of November of each season in earthenware pots, 40 cm diameter and 40 cm height with perforated bottoms, and were filed with 10 kg of sandy soil. Each pot was maintained to field capacity, one day before starting the water treatments, so that the soil moisture amount at each pot is uniform.

Soil type:

The mechanical and chemical analyses of the soil were determined according to a standard method described by [67].

Table 1: Physical and chemical analyses of the tested soil.

(a) Physical analyses						
Particle size distribution				Textural class	$CaCO_3$ %	O.M%
Coarse sand %	Fine sand %	Silt %	Clay %			
5.38	78.53	10.08	6.01	Sandy	1.20	0.80

(b) Chemical analyses												
pH	EC dS/ m	Ion concentration in paste extract (m mol/ L)								Available (mg/kg)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO3^{--}$	$HCO3^{-}$	Cl^{-}	$SO4^{--}$	N	P	K
8.1	0.83	3.90	2.70	1.85	0.55	0.00	1.30	4.55	3.15	58.6	9.50	190.51

Treatments:

1. Water Treatments:

The following three water treatments were applied throughout the entire growth period of the plant:

W1 = water stress is maintained around 75% depletion of the available soil water and the soil water is maintained to field capacity when this depletion level is reached.

W2 = water stress is maintained around 55% depletion of the available soil water and the soil water is maintained to field capacity when this depletion level is reached.

W3 = water level is maintained around 35% depletion of the available soil water and the soil water is maintained to field capacity when this depletion level is reached.

All pots were weighed every 1 to 3 days on a beam balance. The pots were then irrigated to restore the soil to the appropriate moisture regime by adding a calculated amount of water. The general principal stated by [19] was used for the water treatment application. All plants received the soil moisture levels after four weeks from transplanting.

2. Bio-fertilizers treatments:

Bio-fertilizers obtained from General Organization for Agriculture Equalization Fund (GDAEF) Ministry of Agriculture, Egypt.

a. AM fungi inoculum:

Mycorrhizal inoculum bulked in an open-pot culture of *Zea mays* L. and consisted of soil, spores, mycelia, and colonized root fragments. The AM species was *Glomus intraradices Schenck*,.Mycorrhizal inoculum were mixed with sand (1:10 w/w) and supplied as soil inoculation into root absorption zone of plants. Tengrams of inoculum were added to the appropriate pots at sowing time just below the *Origanum vulgare* L. seedlings. The inoculum material contained 275 spores gm^{-1} on oven dry bases, whereas 10g of autoclaved soil was used for the non-mycorrhizal treatment [77].

b-Yeast application:

Yeast (*Candida tropicalis*) solution was prepared according to method described by [118] at rate of 8g/L. The plants were treated by these treatments two times during the crop life as a soil application, the first was after 45 days from transplanting and the second was two weeks later. The composition of yeast employed in the experiment was described in Table (2) as found by [88].

Design of the Experiments:

This experiment included 12 treatments which were the combination between three soil moisture levels (75, 55, 35% depletion of the available soil moisture) and four bio-fertilizers treatments (B0= (control),B1=AMF treatment, B2=yeast treatment and B3=AMF + yeast treatment) treatments were arranged in a randomized complete blocks design with five replicates, The different soil moisture treatments were assigned at random in the main plots, while sub-plots were devoted to the different bio-fertilizers treatments.

Data Collection:

The following characters were either measured or computed on three oregano plants: Plant height (cm), number of branches/plant, stem diameter, fresh and dry weights of the whole plant. The relative water content percent was measured on fresh leaves according to [134]. The determination of total soluble solids concentration in the cell sap of fresh leaves was also estimated by using refractometer, the corresponding values of osmotic pressure (Atm) were then obtained from tables given by [42]. The fresh leaves were used also for the estimation of chlorophyll, reducing, non-reducing and total soluble sugars. The photosynthetic pigments of fresh leaves, chlorophyll a and b and total chlorophyll (a + b) as well as carotenoids contents were determined using for such purpose the 4^{th} leaf from the growing point of the plant using the spectrophotometric method recommended by [82]. Total soluble, reducing and non-reducing sugars were extracted in 0.2 M phosphate buffer with pH 7 and analyzed according to the method of [99]. Essential oil content was determined by hydro-distillation for 3 hours by submitting fresh herb (100 g) for each replicate at each cut in modified Clevenger apparatus [38]. Essential oil percentage of each replicate was determined and expressed as (%).

Statistical analysis:

The collected data were subjected to statistical analysis of variance using the normal (F) test and the means separation were compared by using Least Significant Difference (LSD) at 5% level according to [120].

RESULTS AND DISCUSSION

Growth and yield:

Throughout the present investigation, results in both seasons and cuts indicated that water stress conditions had a significant inhibitory effect on all morphological characters and yield components (Table 3). However, the decrease was more pronounced under the highest stress level W1. By increasing the severity of drought from W3 to W1, plant height (cm) showed significant reduction in its values. Such reduction in plant height in response to water stress may be due to blocking up of xylem and phloem vessels thus hindering any translocation through them [62]. Similar results were obtained by [83, 23, 11, 114, 130, 62]. Data on hand, illustrated also that, number of branches/plant increased markedly with increasing soil moisture level in both cuts, this may be due to that drought reduced cycling- dependent kinase activity, results in slower cell division as well as inhibition of growth [107]. This was supported by the results of [96] on *Calendula officinalis* L. and [124] on *Cichorium intybw* L. Data in the same table regarding number of branches/plant also revealed that the difference between the moderate stress level W2 and the lowest stress level W3 was insignificant in both cuts. In addition, data obtained from the same table revealed significant reduction in stem diameter under water limitation (W1). Similar results were also reported for other medicinal plants such as chamomile [99] caraway [72], peppermint [66], basil and Borage *Borago officinalis* L. [120]. As expected, plants that received more soil moisture water (W3) produced a higher amount of fresh and dry matters compared to the other water treatments in both cuts. In agreement with our results, [101] in oregano, [53] in chamomile and [62] on Roselle reported that drought stress reduced herbage yield of tested plants. Decrease in fresh and dry weights was as a result of reduction in plant height and leaf area. Reduction in fresh and dry weights of the plant may also be due to a decrease in plant growth, photosynthesis and canopy structure during the water stress as reported by [111]. Moreover, the pronounced effect of decreased irrigation on overall growth and yield of oregano plant may be attributed to the lower availability of sufficient moisture around the root and thus a lesser proliferation of root biomass resulting in the lower absorption of nutrients and water leading to production of lower biomass [115]. Moreover, [37] reported that the inhibited vegetative growth and yield in highly water stress conditions might be due to; (a) an osmotic stress due to a lowering of the external water potential, or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition.[73] reported that drought stress inhibits the dry matter production largely through its inhibitory effects on leaf expansion, leaf development, and consequently reduced light interception [88]. Similar results were obtained by [84] on Mexican marigold, [6] on coriander, and [51**] on** different medicinal plants. Water stress also decreased significantly the oil percentage, where W3 treatment caused the most pronounced increase in the essential oil percent; however, this percentage decreased at W1 treatment. Reduction in essential oil % was due to reduction in herbage yield as shown in Table 3. This indicated that essential oil yield is positively related to soil water content and herbage yield [115]. Our results were consistent with those of [109, 12]. An early morphological and yield response to drought stress is the avoidance mechanism through adjustment of plant growth rate such as a reduction in shoot height, stem diameter and total dry mass. A common adverse effect of water stress on plant growth and yield were the reduction in fresh and dry biomass production [12].

Table 2: The composition of active yeast, minerals and vitamins.

<table>
<tr><td colspan="4">The composition of active yeast</td></tr>
<tr><td colspan="2">Protein</td><td colspan="2">47%</td></tr>
<tr><td colspan="2">Carbohydrates</td><td colspan="2">33%</td></tr>
<tr><td colspan="2">Minerals</td><td colspan="2">8%</td></tr>
<tr><td colspan="2">Nucleic acids</td><td colspan="2">8%</td></tr>
<tr><td colspan="2">Lipids</td><td colspan="2">4%</td></tr>
<tr><td colspan="4">The composition of minerals</td></tr>
<tr><td>Na</td><td>0.12 mg/g</td><td>Cu</td><td>8.00 μ/g</td></tr>
<tr><td>Ca</td><td>0.75 mg/g</td><td>Se</td><td>0.10 μ/g</td></tr>
<tr><td>Fe</td><td>0.02 mg/g</td><td>Mn</td><td>0.02 μ/g</td></tr>
<tr><td>Mg</td><td>1.65 mg/g</td><td>Cr</td><td>2.20 μ/g</td></tr>
<tr><td>K</td><td>21.0 mg/g</td><td>Ni</td><td>3.00 μ/g</td></tr>
<tr><td>P</td><td>13.5 mg/g</td><td>Va</td><td>0.04 μ/g</td></tr>
<tr><td>S</td><td>13.5 mg/g</td><td>Mo</td><td>0.40 μ/g</td></tr>
<tr><td>Zn</td><td>0.17 mg/g</td><td>Sn</td><td>3.00 μ/g</td></tr>
<tr><td>Si</td><td>0.03 mg/g</td><td>Li</td><td>0.17 μ/g</td></tr>
<tr><td colspan="4">The composition of vitamins</td></tr>
<tr><td colspan="2">Thiamine</td><td colspan="2">60 - 100 μ/g</td></tr>
<tr><td colspan="2">Riboflavin</td><td colspan="2">35 - 50 μ/g</td></tr>
<tr><td colspan="2">Niacin</td><td colspan="2">300 - 500 μ/g</td></tr>
<tr><td colspan="2">Pyridoxine HCL</td><td colspan="2">28 μ/g</td></tr>
<tr><td colspan="2">Pantorhenate</td><td colspan="2">70 μ/g</td></tr>
<tr><td colspan="2">Biotin</td><td colspan="2">1.3 μ/g</td></tr>
<tr><td colspan="2">Cholin</td><td colspan="2">40 μ/g</td></tr>
<tr><td colspan="2">Folic acid Vit. B12</td><td colspan="2">5 - 13 μ/g</td></tr>
<tr><td colspan="2">Vit.B12</td><td colspan="2">0.001μ/g</td></tr>
</table>

In the present study, each of the two bio fertilizers single or in combination induced mostly significant increases in growth and yield parameters of oregano plants compared to control ones. Results showed that duel inoculation of seedlings with AMF + yeast (B3) showed significantly higher plant height, number of branches, stem diameters, fresh and dry weights and oil% than non-inoculated controls and more effective than single inoculation (Table 3). On comparing between the different types of bio-fertilizer in their effectiveness, mycorrhizal plants showed higher growth and yield parameters compared with control plants and yeast treatment (Table 3). Moreover, increased growth, yield and development in AM plants, compared to non-mycorrhizal ones, were reported by [34, 64, 62]. The mycorrhiza induced significant increase in shoot nutrient contents (N, P, K, Ca, Mg, Fe, Cu and Mn) and Phosphorus content. This higher nutrient uptake in mycorrhizal plants might be attributed to the contribution of fungal external mycelia which explore a large volume of soil and thus absorb more nutrients [41] and act as antagonists against some plant pathogens [27].. Moreover, it has been demonstrated that plants inoculated with Arbuscular mycorrhizal fungi utilize more soluble phosphate from organic phosphate than non-inoculated plants [8]. The main explanation is that mycorrhizas developed an extrametrical mycelium, which increased the root phosphate absorbing sites [18]. Furthermore, active yeasts exhibit plant growth promoting characteristics, including pathogen inhibition [29]; phytohormone production [89]; phosphate solubilization [82]; N and S oxidation [32], side rophore production [105] and stimulation of mycorrhizal-root colonization [127]; [82]. In another study, [112] reported that *Zingiber officinale* plants inoculated with *Glomus mosseae* significantly increased plant growth and yield. Moreover, [59] reported that bio-fertilizers significantly increased plant height, root length, root growth, alkaloid content and N, P, K, Ca and Mg uptake in *Catharanthus roseus* in comparison to the uninoculated control. In addition, [93] found an increase in shoot, root dry weight, N, P and potassium (K) content and essential oils in *Ocimum basilicum* inoculated with bio-fertilizers; which also enhanced the root, shoot, growth and dry weight [7]. Such response resulting in improved plant growth was also obtained by [116] for *Pelargonium graveolens* inoculated with *Glomus fasciculatum* and *Azotobacter chroococcum* and *Pseudomonas* spp. and for *Withania somnifera* [10]. Furthermore, [104] reported that the essential oils of MAPs have antimicrobial properties. The inoculation of plants results in higher colonization rate in the rhizosphere by the microorganisms and as a result of a plant's defense response, the essential oil content increases.

Data of interaction between water stress and bio-fertilizer treatments illustrated that the maximum growth and yield parameters of oregano plants were mostly observed under the combined effect of the highest soil moisture level and duel inoculation of AMF + yeast treatment (W3XB3) and with significant differences compared with the other treatments. The data of interaction revealed also that the difference between AMF + yeast under moderate soil moisture level (W2XB3) and same treatment under the highest soil moisture level (W3XB3) was mostly insignificant in all growth parameters and yield components of the two cuts (Table 3). Similar to our findings, [55] found that inoculation of *Catharanthus roseus*plants with *Pseudomonas* strains stimulated the shoot, root growth and yield under drought stress. Also, [14] observed significant increases in shoot length, shoot weight, number of leaves and nodes, root dry weight and essential oil yield of *Origanum majorana* inoculated with *P. fluorescens* and *Bradyrhizobium sp*. under drought stress conditions.

Water relations:

A. Osmotic pressure:

It is apparent from data in Table 4 that the cell sap osmotic pressure of oregano leaves was increased as water stress increased from W3 upto W1. Where, the highest significant increase in cell sap osmotic pressure was observed under the lowest soil moisture level W1 as compared to the other water treatments during the two growing seasons. Osmotic adjustment is generally thought to be the major mechanism to maintain cell turgor in many species as the water potential decreases, enabling water uptake and the maintenance of plant metabolic activity and therefore growth and productivity [79]. Osmotic adjustment (OA) is recognized as an effective component of drought resistance in several medicinal plants [78]. OA involves the net accumulation of solutes in a cell in response to a fall in the water potential of the cell's environment. As a consequence of this net accumulation, the osmotic potential of the cell is lowered, which in turn attracts water into the cell and tends to maintain turgor pressure. Similar effect of water stress on osmotic pressure was also observed by (Sensoy *et al.*, 2007; Zeng *et al.*, 2009; Shanan *et al*., 2011 and Khalil and Hussein, 2012).

The use of different types of beneficial microorganisms proved significant increase in osmotic pressure values of oregano plants; where all bio-fertilizers treatment revealed significant reduction on osmotic pressure values compared to control ones. The maximum reduction in osmotic pressure values was obtained under the dual inoculation of AMF and yeast (B3) compared to control and the other bio-fertilizer treatments, followed by single inoculation with AMF. The positive effect of bio-fertilizer could be attributed to their effect on supplying plants with their requirements of various nutrients as well as their effect on lowering soil PH; which could facilitating the availability of soil nutrients and improving physical characters which in turn favor root development [61]. Similar results were detected by [123, 49, 52, 97, 28].

The effect of the interaction between different soil moisture levels and different microorganisms inoculation indicated that the lowest values of cell sap osmotic pressure for the two cuts in both seasons were obtained when oregano plants were grown under the highest soil moisture level W3 and inoculated with AMF +

yeast (W3XB3) compared with the other treatments, followed by the same treatment under the moderate soil moisture level (W2XB3); where the difference between the two treatments was insignificant. While the highest means were obtained under the combined effect of (W1XB0) compared with the other treatments. In our experiment, mycorrhiza improved plant water relations under drought conditions corresponding of mycorrhiza's contribution in P uptake to AMF-plants and act to synthesis of certain phytohormones as like as ABA and cytokinin.

Table 3: Effect of water stress, bio-fertilizers treatments and their interactions on *Origanum vulgare* L. growth and yield (combined analysis of 2012/2013 and 2013/2014 seasons).

Characters		Plant height (cm)		No of branches/ plant		Stem diameter (cm)		F.W (g)		D.W (g)		Oil%	
Treatments		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Water stress treatments													
W1		40	56	6	7	0.7	1.2	29	58	17	39	0.56	0.60
W2		45	57	10	12	1.3	1.7	53	75	24	41	0.58	0.61
W3		45	59	10	13	1.5	1.9	58	77	25	42	0.60	0.64
L.S.D $_{0.05}$		0.2	0.3	1.3	1.1	0.1	0.1	2.7	1.0	0.1	0.3	0.01	0.01
Bio fertilizers treatments													
B0		41	51	6	9	0.7	1.4	42	59	18	29	0.51	0.55
B1		43	63	10	11	1.4	1.7	49	72	25	45	0.62	0.66
B2		42	53	9	11	1.1	1.5	45	66	21	41	0.55	0.57
B3		46	61	11	13	1.5	1.8	52	82	26	47	0.62	0.67
L.S.D $_{0.05}$		1.2	2.2	1.4	0.5	0.2	0.1	2.1	4.6	2.5	1.3	0.03	0.05
Water stress X bio-fertilizers													
W 1	B0	38	49	5	6	0.5	0.9	23	50	12	28	0.49	0.60
	B1	40	60	7	8	0.7	1.2	31	66	20	44	0.60	0.61
	B2	39	50	6	6	0.7	1.1	28	52	15	40	0.54	0.64
	B3	42	65	7	8	0.9	1.5	35	65	22	45	0.60	0.01
W 2	B0	42	50	7	9	0.7	1.5	50	63	20	29	0.49	0.52
	B1	45	65	11	12	1.6	1.9	55	74	27	45	0.63	0.66
	B2	43	55	10	13	1.3	1.5	52	72	23	41	0.56	0.57
	B3	48	59	12	15	1.7	2.0	56	90	28	48	0.62	0.68
W 3	B0	43	55	7	12	0.8	1.7	53	65	22	30	0.55	0.61
	B1	45	65	11	13	1.8	2.0	60	75	27	46	0.64	0.66
	B2	44	55	10	13	1.4	1.8	55	74	24	43	0.56	0.59
	B3	49	60	13	15	1.8	2.0	64	92	29	49	0.64	0.68
L.S.D $_{0.05}$		3.5	4.1	2.7	3.1	0.9	1.0	4.1	3.7	2.4	3.1	0.09	0.10

These results were in agreement with the observation of [71, 26]. The reduction in osmotic pressure values in AMF oregano plants may be due to that roots of AMF inoculated plants were longer with increasing fungal hyphae growth which caused increase in phosphorous and water absorption [100, 6]. Furthermore, the positive response of oregano plants to yeast application may be attributed to that yeast contains macro and micro nutrition; also it has growth regulators and vitamins [48, 45, 61].

B-Relative water content (RWC) %:

For the RWC % of oregano leaves, results in Table 4 showed that during plant development, increasing stress caused an observed adverse action on relative water content. Plants subjected to the lowest soil moisture level W1 showed the lowest significant means of RWC %. The present results agreed with those obtained by [125, 34, 102, 1, 61]. Such decrease in RWC % under the lowest soil moisture level may be attributed to decline in osmotic and water potentials with concomitant preliminary decrease in RWC %, also decreased water quantity which caused decrease in relative turgidity is due to decreased photosynthesis as a consequence of stomata closure; this caused reduced translocation and hence reduced turgor pressure [68].

The use of different types of beneficial microorganisms proved significant increases in RWC% compared with control plants (Table 4). Data in the same table indicated also that, plants inoculated with AMF + yeast (B3) recorded the highest significant values for the RWC % compared to un-inoculated, followed by single inoculation with AMF. The results reached by [17, 16, 58, 74, 126, 28] confirmed our data. The favorable effects of the combination between mycrohiza + yeast may be explained based on the beneficial effects of them on the improvement of soil physical and biological properties and also, the chemical characteristics resulting in more release of available nutrient elements to be absorbed by plant roots and its effect on the physiological processes such as photosynthesis activity as well as the utilization of carbohydrates. A similar suggestion was made by [43] on rocket plants. Furthermore, this simulative effect may be related to the good equilibrium of nutrients and water in the root medium [2] or to the beneficial effects of mycorrhiza on vital enzymes and hormonal, stimulating effects on plant growth and yield.

The combined effect of the three soil moisture levels and beneficial microorganisms inoculation in both seasons revealed that the highest significant means for RWC% were recorded by the interaction between the lowest stress level and AMF + yeast inoculation (W3XB3) compared with the other treatment. The increase in RWC values of bio-fertilized plants than respective uninoculated control plants may be due to the associated increase in the hydraulic nature of branch root junctions, which facilitate the radial flow of water, or due to changes in root morphology such as root dry weight and root branching [93] or due to enhanced root length and area compared to uninoculated controls, resulting in root systems with longer and thinner roots. Plants with very fine roots are more effective for water and mineral uptake due to higher root specific surface area [78]. Moreover, [137] reported that root proliferation enhancement and enhanced water uptake in inoculated drought-stressed rice plants which may be induced by IAA.

Table 4: Effect of water stress, bio-fertilizer treatments and their interactions on water relations of *Origanum vulgare* L. plant (combined analysis of 2012/2013 and 2013/2014 seasons).

Characters / Treatments		Osmotic pressure		RWC%	
		1st cut	2nd cut	1st cut	2nd cut
Water stress treatments					
W1		7.23	7.27	38.97	37.11
W2		6.30	6.42	52.56	55.64
W3		6.06	6.17	60.99	62.94
L.S.D 0.05		0.04	0.02	2.00	1.78
Bio fertilizers treatments					
B0		7.23	7.41	44.53	48.15
B1		6.44	6.34	53.05	53.60
B2		6.56	6.69	49.99	50.95
B3		5.90	6.04	55.79	54.89
L.S.D 0.05		0.08	0.11	1.02	0.96
Water stress X bio fertilizers					
W1	B0	8.41	8.49	30.53	31.59
	B1	6.86	6.94	43.69	39.18
	B2	7.24	7.13	35.73	34.36
	B3	6.41	6.51	45.92	43.30
W2	B0	6.86	7.13	50.98	53.44
	B1	6.41	6.04	52.97	56.30
	B2	6.22	6.61	51.99	55.05
	B3	5.72	5.88	54.31	57.78
W3	B0	6.41	6.61	52.08	59.43
	B1	6.04	6.04	62.49	65.31
	B2	6.22	6.32	62.24	63.43
	B3	5.56	5.72	67.14	65.59
L.S.D 0.05		2.17	3.01	4.07	5.14

Photosynthetic pigments content:

As presented in Table 5 we observed a sharp significant increase in photosynthetic pigments content of oregano plant with increasing stress level in both cuts of the two growing seasons. Where increasing stress level caused significant increase in the total content of Chla, Chlb, Chla+b as well as Carotenoids contents, so as to reach their maximum records under the lowest soil moisture level W1 (Table 5). These results were fortified by those of [25, 56, 50, 62].The increase in photosynthetic pigments under water stress conditions may be due to the decline in relative water content (RWC) of plant's leaves under drought conditions [136]. Accumulation of Carotenoids for osmotic regulation in drought-stressed leaves in many crops has been reported by [65, 40].

Furthermore, the level of photosynthetic pigment contents (i.e. Chla, Chlb and Chla+b) in both cuts showed a significant decrease in response to bio-fertilizer treatments as compared to control ones. Where the highest Chla, chlb and Chl a+b were obtained under control treatment, while the lowest means were observed under the combined treatment of AMF + yeast (B3) comparable to those of untreated plants (Table 5). The reduction in Chla, Chlb and Chla + b may be due to the ameliorative effects of these microorganisms on plant water relations under water stress conditions and the increase in the RWC % which reduced the effect of stress on photosynthetic pigments content and decreased its concentrations. Reversed trend was obtained for Carotenoids content, where all bio-fertilizer treatments increased significantly the Carotenoids content of oregano leaves compared with control treatment. The maximum Carotenoids content was observed in the combined treatment of AMF + yeast (B3), followed by AMF (B1) treatment. The increase in Carotenoids content as response to bio fertilizers treatments were fortified by those of [5] on fennel plants, [96] on tuberose, [4] on marigold plants. Carotenoids act as an accessory pigment (absorb sun light when chla and chlb cannot absorb sun light) [44].

Regarding the effect of interaction between the studied factors, data in Table 5 showed also that the highest concentration of Chla, Chlb and Chla+b were observed in control treatment of the lowest soil moisture level (W1XB0) compared with the other treatments. However, the maximum Carotenoids concentration was obtained under the lowest soil moisture level combined with duel inoculation of AMF+yeast (W1XB3) compared to the other treatments. While, the lowest means were observed in single inoculation of active yeast (B2) under the highest soil moisture level (B2XW3).The increase in Carotenoids content due to bio fertilizer treatments under drought conditions may be due to that Carotenoids have essential functions in photosynthesis and photo protection. Besides their structural roles, they are well known for their antioxidant activity by quenching Chl3and O2, inhibiting lipid per-oxidation, and stabilizing membranes [24, 33, 91]. They also play a critical role in the assembly of the light-harvesting complex and in the radiation less dissipation of excess energy. Furthermore, it played important role as photo protective and antioxidant system to drought stress [122, 86].

Table 5: Effect of water stress, bio-fertilizer treatments and their interactions on photosynthetic pigments content of *Origanum vulgare* L. plant (combined analysis of 2012/2013 and 2013/2014 seasons).

Characters		Chl. a		Chl. b		Chl. a+b		Carotenoids	
Treatments		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Water stress treatments									
W1		6.44	7.71	17.51	15.29	23.95	22.99	5.58	7.62
W2		5.42	6.06	16.10	15.03	21.53	21.09	5.30	6.39
W3		4.54	5.83	13.64	15.21	17.92	20.53	4.58	6.06
L.S.D $_{0.05}$		0.07	0.12	0.03	0.05	1.25	0.33	0.05	0.21
Biofertilizers treatments									
B0		6.03	7.00	18.05	16.41	24.08	23.41	4.69	4.59
B1		5.34	6.39	15.06	14.29	20.27	20.68	5.32	7.59
B2		5.22	6.56	17.12	16.28	22.16	22.85	5.12	6.67
B3		5.27	6.16	12.77	13.71	18.03	19.21	5.48	7.90
L.S.D $_{0.05}$		0.03	0.08	1.77	1.01	2.01	0.92	0.02	0.06
Water stress X bio-fertilizers									
W1	B0	7.31	8.31	20.27	17.67	27.57	25.98	5.17	5.44
	B1	6.56	7.51	18.56	13.53	25.12	21.04	5.72	8.54
	B2	5.44	7.75	19.56	17.50	24.99	25.25	5.53	7.63
	B3	6.45	7.24	11.67	12.47	18.12	19.70	5.91	8.85
W2	B0	5.73	6.50	17.24	16.44	22.97	22.95	4.76	4.48
	B1	5.30	6.00	16.34	14.05	21.24	20.05	5.48	7.19
	B2	5.35	6.12	15.89	15.89	21.70	22.01	5.18	6.29
	B3	5.28	5.60	14.93	13.74	20.21	19.34	5.76	7.60
W3	B0	5.06	6.18	16.64	15.13	21.70	21.31	4.15	3.84
	B1	4.15	5.66	10.29	15.30	14.44	20.96	4.75	7.05
	B2	4.89	5.82	15.91	15.46	19.80	21.28	4.65	6.09
	B3	4.07	5.65	11.70	14.94	15.76	18.59	4.77	7.25
L.S.D $_{0.05}$		0.21	0.16	2.07	3.18	4.01	3.94	0.08	0.10

Carbohydrate contents:

It is evident from Table 6 that the values of reducing sugars extracted from the leaves of oregano plants in both cuts and seasons were increased progressively and significantly by increasing the stress level. In contrast to soluble sugars, non-reducing and total sugars values in shoot of oregano plants declined significantly in response to water stress application, such decline was most pronounced with increasing the severity of drought (Table 6). Concerning reducing sugars our results were shown to be similar to those of [30, 35, 106, 138] using different plants. As to those of non-reducing and total sugars they were found to be consistent with the results of each of [132, 79, 134]. We attributed the above decline in total insoluble sugars which accompanied an increase in soluble sugars to soil water deficiency which triggers certain chemical stimulus (mostly ABA) through stomata conductance, CO2 concentration in leaf tissues, electron transport system, CO2 fixation, rate of photosynthesis and eventually quantity of photosynthates, thus causing decline in growth rates. These conditions, in the meantime, enhances some plants to increase their respiration rates as a prerequisite to produce both ATP to activate stressed cells and osmotic soluble substances which reduces cell osmotic potential thus increasing cell water uptake. Our view was fortified by each of [75, 9, 102, 15, 85, 47].

Data tabulated in Table 6 showed also that the bio-fertilizer treatments had a significant effect on reducing, non-reducing and total sugars of oregano leaves in seasons and cuts (Table 6).Thus, all bio-fertilizer treatmentsindividually or combined proved significant increases in the mean values of reducing, non-reducing and total sugars compared with control treatment. So, the maximum mean values were obtained in AMF + yeast (B3) treatment. Whereas, the least means were obtained in control treatments. Improvement in reducing, non-reducing and total sugars in all biofertilizers treated plants may be due to increased carbon fixation, activation of enzymes. Increased reducing and non-reducing sugar contents in different bio-fertilized plants were observed by [69, 80, 90, 103].

Concerning the effect of interaction between different soil moisture levels and different bio-fertilizer treatments, the data of interaction revealed that the highest significant effect on non-reducing and total sugars were obtained under the combined effect of the lowest stress level W3 and the B3 treatment (W3XB3) compared with other treatments. An opposite situation being apparent for reducing sugars where the highest significant means were observed under the highest stress level W1 combined with B3 treatment (W1XB3) compared to the other treatments.

Table (6): Effect of water stress, bio-fertilizer treatments and their interactions on carbohydrates content of *Origanum vulgare* L. plant. (combined analysis of 2012/2013 and 2013/2014 seasons).

Characters		Reducing sugars		Non reducing sugars		Total sugars	
Treatments		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Water stress treatments							
W1		2.75	3.10	1.68	1.55	4.43	4.65
W2		2.48	2.63	2.50	2.65	4.98	5.23
W3		1.55	1.63	3.53	3.70	5.08	5.33
LSD 0.05		0.06	0.02	0.08	0.12	0.04	0.07
Bio fertilizers treatments							
Cont		1.90	2.17	2.27	2.27	4.17	4.43
AMF		2.33	2.57	2.57	2.73	4.90	5.23
Yeast		2.30	2.30	2.57	2.57	4.87	4.87
AMF + Yeast		2.50	2.77	2.87	2.97	5.37	5.73
LSD 0.05		0.09	0.12	0.04	0.10	0.07	0.11
Water stress X biofertilizers							
W1	Cont	2.50	2.70	1.50	1.30	4.00	4.00
	AMF	2.90	3.20	1.60	1.60	4.50	4.80
	Yeast	2.70	3.00	1.70	1.50	4.40	4.50
	AMF+Yeast	2.90	3.50	1.90	1.80	4.80	5.30
W2	Cont	2.30	2.50	2.20	2.20	4.50	4.70
	AMF	2.40	2.70	2.50	2.90	4.90	5.40
	Yeast	2.50	2.40	2.40	2.50	4.90	4.90
	AMF+Yeast	2.70	2.90	2.90	3.00	5.60	5.90
W3	Cont	0.90	1.30	3.10	3.30	4.00	4.60
	AMF	1.70	1.80	3.60	3.70	5.30	5.50
	Yeast	1.70	1.50	3.60	3.70	5.30	5.20
	AMF+Yeast	1.90	1.90	3.80	4.10	5.70	6.00
LSD 0.05		0.41	0.50	0.72	0.90	0.36	0.25

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