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Original Article

The Effect of Vermicompost and PGPR on Physiological Traits of Lemon Balm (*Melissa officinalis* L.) Plant under Drought Stress

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Abstract

In this research, effect of vermicompost and plant growth promoting rhizobacteria (PGPR) on physiological traits of lemon balm under drought stress condition was investigated in year 2012 at Karaj Research Institute of Forest and Rangelands. Experiment was conducted as split-split plot in a randomized complete block design with three replications. irrigation (normal: irrigated to field capacity; drought stress: watered to 60% of field capacity) was considered as main plot, vermicompost fertilizer (consuming 0, 5 and 10 t ha^{-1}) as split plot and biofertilizers (*Pseudomonas fluorescent, Azotobacter chrococum+Azospirillm brasilense, Azotobacter+ Azospirillum + Pseudomonas* and no fertilizer) as split-split plot. Results showed that water stress significantly increased essential oil content, while decreased essential oil yield, total chlorophyll, cell membrane stability, relative water content and proline. Only relative water content and total chlorophyll were positively affected by biofertilizer application. Essential oil content, essential oil yield, total chlorophyll, cell membrane stability, relative water content and proline were significantly increased by vermicompost application, especially, relative water content increased with an increase in amount of vermicompost application, but there were strong interactions with irrigation. These interactions are important in practical use of vermicompost and biofertilizers.

Keywords: Vericompost, PGPR, Drought Stress, Lemon Balm

Introduction

Lemon balm is a herbal medicine plant with the height of 30 to 80 cm, its leaves are oval and heart-shaped, serrated, covered with fluff, and the leaf surface is uneven and has multiple nodules including secretory fluff, and its fruit is tetraalkene and brown [1]. Environmental stresses (e.g. inadequate mineral nutrients supply and water deficiency) are increasing due to intensive use of natural resources and increasing world population [2]. Water stress is one of the most severe limitations of crop growth especially in arid and semiarid regions, which usually results in high yield losses [3].

Water stress not only limits plant growth and survival, but also induces various physiological and metabolic responses like stomatal closure, decline in growth rate, solute and antioxidant accumulation and expression of stress specific genes [4]. Solinas and Deiana [5], reported that plants secondary products can be altered by environmental factors and water stress is a major factor affecting the synthesis of natural products. Changes in essential oils extracted from aromatic plants and their composition were observed with water stress [6]. Water stress resulted in significant reduction of

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fresh and dry matter, nutrient content, and essential oil yield of Japanese mint plants [7]. The secondary metabolite production is believed to be stimulated by stressful environment. However, there is too little experimental data to support this notion. Misra and Srivastava [7] investigated the effect of irrigation on mint plant and found that adequate irrigation would increase the growth and essential oil of mint.

A biofertilizer is a substance which contains living microorganisms which, when applied to seed, plant surfaces or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant [8]. The well known genera of PGPR are *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, and *Pseudomonas*, but some of these genera include endophytic species as well [9]. Bacterial mechanisms of plant growth promotion include biological nitrogen fixation (BNF), synthesis of phytohormones, environmental stress relief, synergism with other bacteria-plant interactions, inhibition of plant ethylene synthesis, as well as increasing availability of nutrients like phosphorus, iron and minor elements, and growth enhancement by volatile compounds [9]. The use of fertilizers containing *Azotobacter*, *Azospirillum*, *Bacillus* and 50 percent complete fertilizer, increased the vegetative growth and essential oil yield of fennel [10] . Azzaz *et al*. [11] assessed possibility of using organic fertilizers, instead of chemical fertilizers, in fennel and concluded that the growth, yield and quantity of essential oil in fennel increased under treatment of organic fertilizer.

Vermicompost is a very important biofertilizer produced through the artificial cultivation of worms, ie Vermiculture. Vermicompost is enriched with all beneficial soil bacteria and also contain many of the essential plant nutrients like N, P, K and micronutrients [12]. Therefore, applying it in sustainable agriculture led to high and rapid growth of plants including medicinal plants as well as increasing population growth and activity of beneficial soil microorganisms [13]. The aim of this study was to investigate the effect of drought stress, vermicompost fertilizer and bio-fertilizers on physiological traits of lemon balm.

Material and Methods

The field experiments were carried out in year 2012 at Karaj Research Institute of Forest and Rangelands (latitude 35.48, longitude 51.00). The climate is semi-arid with an annual average rainfall of about 245 mm. Chemical and physical properties of experimental soil are listed in Table 1. Experiment was conducted as split- split plot in a randomized complete block design with three replications. Irrigation (normal and drought stress) was considered as main plot, vermicompost fertilizer (consuming 0, 5 and 10 t ha^{-1}) as split plot and biofertilizers (*Pseudomonas fluorescent*, *Azotobacter chrococum*+*Azospirillm brasilense*, *Azotobacter*+*Azospirillum*+*Pseudomonas* and no fertilizer) as split-split plot. In the normal watered treatment, plants were irrigated to field capacity every two days. Plants in the drought-stressed treatment were watered to 60% of field capacity as same times.

Plant Material

Each subplot was 8 square meters, distance of each stack was 50 cm, the distance between plants was 40 cm on each line and there were 10 plants on each line. Distance within each block was 34 m and distance between plots within a block was 2 lines or a meter. When 6 leaves grew on lemon balm and its height was 8-10 cm, it was removed from medium and roots were washed completely and placed in growth promoting bacteria inoculum for 24 hours (Populations of bacteria in each inoculum was 1×108 cell Ml-1). 100 grams of air dried shoot were cut in small pieces, and the essential oil was obtained by steam distillation in 1500 mL water for 2 h by Clevenger apparatus.

Then 2ml of the filtered extracts were transferred to tubes having caps. To each of these tubes were added 2 ml of the Ninhydrin Reagent and two milliliters of glacial acetic acid.

Table 1 Chemical and physical properties of experimental soil

Soil depth (cm)	silt %	Sand %	Clay %	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Mg (ppm)	Ca (mg/lit)	K (ppm)	P (ppm)	N %	EC ds/m	pH
0-30	38.78	25.51	35.71	3.18	12.88	0.42	0.37	68	9.87	580	8.16	0.09	1.02	7.48

After the tubes were capped, they were placed at 100- degree- centigrade water for one hour. The tubes were then cooled, four milliliters of toluene were added to each of them, and they were shaken for 15 to 20 seconds in a vortex mixer. The red supernatant that contained the proline dissolved in toluene was removed and put in the spectrophotometer simultaneously with the standard sample and its absorption level was read at 520 nanometers. The standard curve was used to determine the proline concentration in milligrams per gram of fresh leaf tissue [14].

Measuring the Total Chlorophyll

Firstly, 0.25 grams of fresh leaves were chopped and pulverized in a porcelain mortar with 5 ml distilled water to form a uniform mass. Then, the mixture was poured into a volumetric flask 25ml and raised to the 25 ml volume with distilled water. The 0.5 ml of the resulting solution was removed and mixed with 4.5 ml acetone 0.80 and then centrifuged (300 rpm for 10 min). The supernatant was removed (there was a little sediment at the bottom of the centrifuge tube) and poured in clean tubes and obtained the absorption at wavelengths 490, 638, 663, 645, and 647 nm by spectrophotometer and calculated the concentration of pigments using the following relations. (A, b, and c are read numbers at different wavelengths).

1. Chla (mg/l)=(12.25*a663)-(2.79*a647)
2. Chlb (mg/l)=(21.5*a647)-(5.1*a663)
3. Chla+b (mg/l) = (7.15*a663)+(18.71*a647)

2.4. Measuring relative water content and Electrical conductivity

For measuring the relative water content, 10 expanded leaves from the same height of plant were selected and their wet weight was measured. Then, these segments were placed in distilled water in low intensity light for 24 hours to determine the turgor weight. After measuring turgor weight, the leaves were dried in 75 ^{o}C for 48 hours and their dry weight was calculated. (FW, DW, and TW are fresh weight, dry weight, and turgor weight, respectively). For measuring the Electrical conductivity, 7 fully mature leaves were selected from each treatment and after weighing, were placed in 10 ml mannitol solution with osmotic pressure of 2 - load at 20 °C for 24 hours. Electrical conductivity of the solution was measured using Micro Electrical Conductivity Meter Devices (Model GP 383).

Measuring Essential Oil Yield and Content

100 grams of flowering branch was selected from each treatment and dried adjacent to the open air and shade, each sample was separately chopped in mill and poured in the 2-liter flask and after adding 1.5 l distilled water, shook the flask to mix water and plant. Essential oil extraction by Clevenger apparatus was continued for 2 hours.

Data was analyzed with SAS software and the mean comparison was compared with Duncan multiple range test at 5% level.

Results and Discussion

Essential Oil Yield

Analysis of variance showed that the main effect of irrigation, vermicompost and biofertilizers on essential oil yield were significant at the level of 1, 1 and 5%, respectively (Table 2). But, there were no significant effect recorded for two and three way interactions (Table 2). The results of the mean comparison of irrigation effect on essential oil yield showed that normal and drought irrigation with the means of 7.64 and 6.6 kilogram per hectare had the highest and lowest essential oil yield, respectively (Table 3). Fatima *et al*. [6] studied the effect of water stress on *Cymbopogon martini* (Roxb.) W.Watson and found that water stress significantly increased essential oil content, while decreased essential oil yield.

The mean comparison results of vermicompost effect on the oil yield showed that consuming 5 and 10 t ha, respectively, with the means of 7.55 and 6.96 kg.ha^{-1} had the highest and control treatment with a mean of 6.84 kg per hectare had the lowest oil yield. Consuming 5 and 10 tons per hectare showed 10.38 and 1. 75% increases in comparison with control treatment (Table 4). A study conducted on the effect of vermicompost fertilizer on basil medicinal plant showed that consuming 5 tons vermicompost improved significantly the essential oil content and the quality in basil [15].

The mean comparison results of bio-fertilizer effect on the oil yield showed that Pseudomonas biofertilizer and control treatment with the means of 7.7 and 6.39 (kg ha^{-1}) had the highest and the lowest oil yield, respectively. Pseudomonas biofertilizer showed 20.5% increases in comparison with control treatment (Table 5). In a greenhouse experiment conducted on comparing organic and mineral fertilizers and combined application of *Azospirillum*, *Azotobacter* and phosphate solubilizing bacteria on marjoram1 no significant differences observed in oil yield and biomass between treatments [16].

The mean comparison results of interaction effect of drought×vermicompost showed that normal irrigation treatment×consuming 5 tons vermicompost fertilizer and drought×consuming 5 tons per hectare vermicompost with the means of 8.25 and 5.43 kg per hectare had the highest and lowest oil yield, respectively. Normal irrigation× consuming 5 tons vermicompost fertilizer had 51.93% increases in comparison with drought× consuming 5 tons per hectare vermicompost (Table). The highest essential oil yield and vegetative yield was related to the integrated treatment of 75% nitrogen, phosphorus, and potassium chemical fertilizers with biological fertilizers reported in a research on different levels of nitrogen, phosphorus, and potassium chemical fertilizers and bio- fertilizers on basil [16].

The mean comparison results of interaction effect of vermicompost× biological fertilizers showed that the treatment of consuming 10 tons per hectare Pseudomonas+Azotobacter+Azospirillum and consuming 10 tons per hectare× Pseudomonas with the means of 8.41 and 8.28 kilogram per hectare had the highest and the control treatment with the mean of 5.46 kilogram per hectare had the lowest oil yield. Consuming 10 tons per hectare× Pseudomonas+Azotobacter+Azospirillum and consuming 10 tons per hectare× Pseudomonas showed, respectively, 54.09 and 51 .64 %8 increases in comparison with control treatment (Table 6).

The mean comparison results of interaction effect of drought×vermicompost×biological fertilizers on oil yield showed that normal irrigation treatment× consuming 10 tons per hectare×Pseudomonas and normal irrigation×consuming 10 tons per hectare×Pseudomonas inoculation+Azotobacter+ Azospirillum with the means of 8.96 and 8.93 kg ha^{-1} 232 had the highest and drought treatment× lack of consuming vermicompost×lack of consuming bio-fertilizer with the mean of 4.43 kg ha^{-1} 234 had the lowest oil yield (figure 1). Karla [17], in a research conducted on different fertilizer treatments on the oil percentage of peppermint observed that the essential oil in vermicompost, cow manure, *Azotobacter* and *Azospirillum* treatments would be equal to control treatment (using chemical fertilizers).

Essential Oil Content

Results of variance analysis showed that the effect of irrigation, vermicompost and bio- fertilizer on oil percentage was significant at the level of 1% (Table 2). The mean comparison results of irrigation effect on essential oil content showed that drought and normal irrigation with the means of 0.24 % and 0.2% had the highest and the lowest amount (Table 3). The mean comparison results of vermicompost effect on essential oil showed that consuming 5 and 10 tons per hectare and the control treatment with the means of 0.23 % and 0.2 % had the highest and the lowest oil percentage, respectively. In a research, effect of different fertilizer treatments on the oil percentage of mentha (*Mentha pipertia* L.) was examined and the results showed that vermicompost treatment, cow manure and *Azospirillumsp* and *Azotobacters* would be equal to control treatment (using chemical fertilizer) [17]. The mean comparison results of biofertilizers effect on the mentioned traits showed that *Pseudomonas fluorescence* bacteria and the control treatment with the means of 0.24 and 0.2 % had the highest and lowest oil percentage; Respectively. *Pseudomonas fluorescence* bacteria had 20% increases in comparison with the control treatment (Table 5). Research conducted on consuming bio- fertilizers on fennel showed that consuming different levels of bio fertilizers improved quality and quantity of essential oil in this medicinal plant in comparison with chemical fertilizers treatment [18].

Total Chlorophyll

Results of variance analysis showed that the effect of irrigation, vermicompost, bio fertilizer and interaction effect of drought × vermicompost on total chlorophyll was significant at the level of 1%.

Furthermore, the interaction effect of drought × vermicompost × bio-fertilizer on total chlorophyll was significant at the level of 5% (Table 2).

The mean comparison results of irrigation effect on the mentioned trait showed that in normal irrigation and drought with the means of 17.3 and 14.28 mg per fresh weight had the highest and lowest total chlorophyll, respectively. Normal irrigation had 16. 73% increases in comparison with drought (able 3). The mean comparison results of vermicompost fertilizer effect showed that consuming 5 and 10 t ha with the means of 16.5 and 16.43 mg per fresh weight had the highest and lack of consuming vermicompost with the mean of 15.26 mg ml had the lowest total chlorophyll.

In each column, means with same letters are not significantly different from each other at the 5% level by Duncan's multiple range tests. Consuming 5 and 10 tons per hectare had 8.12 and 7.66%

increases in comparison with control treatment (Table 4). Thus, consuming appropriate amounts of vermicompost increased the total chlorophyll and raised the rate of photosynthesis in plants through improving soil microbial activities and producing growth regulators, as well as accessing to a greater amount of food for the plant [19]. The mean comparison results of bio-fertilizers effect on total chlorophyll showed that Pseudomonas fluorescent and control with the means of 16.7 and 15 mg per fresh weight had the highest and lowest total chlorophyll, respectively. It was also observed that the Pseudomonas bacteria had 10.59% increases in comparison with control (Table 5).

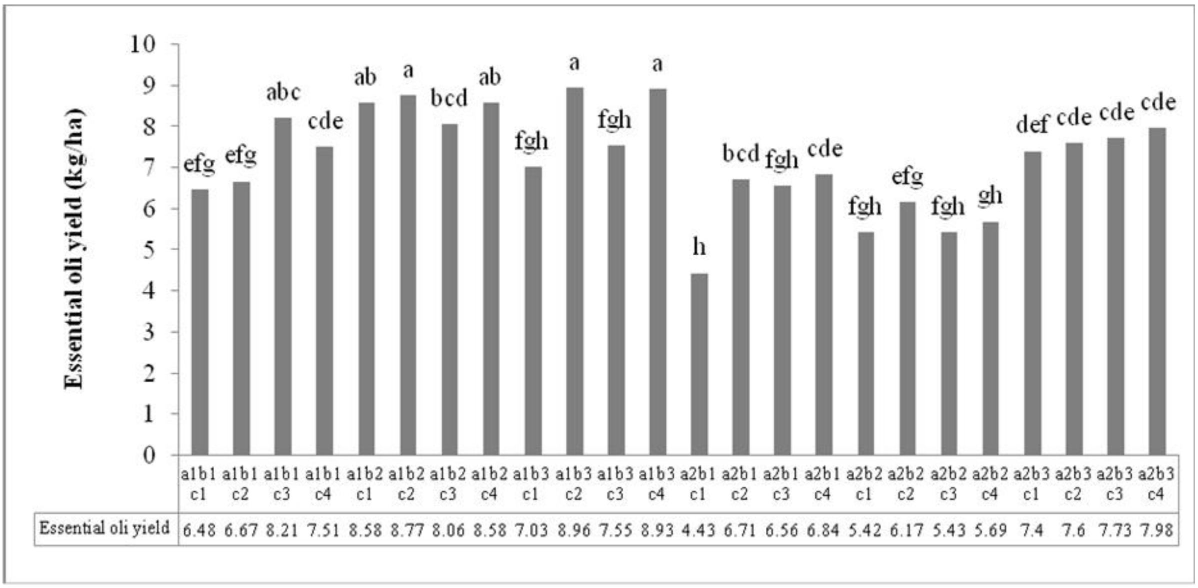

Fig. 1 Three- way interaction of irrigation×vermicompost×biofertilizer on essential oil yield of lemon balm plants. a1: normal irrigation, a2 drought stress, b1: without vermicompost, b2 and b3: application of 5 and 10 tones vermicompost per ha, respectively; c1: without biofertilizer; c2: *Pseudomonas*; c3: *Azotobacter* + *Azospirillum*; c4: *Azotobacter* +*Azospirillum*+ *Pseudomonas.* Means with same letters are not significantly different from each other at the 5% level by Duncan's multiple range tests.

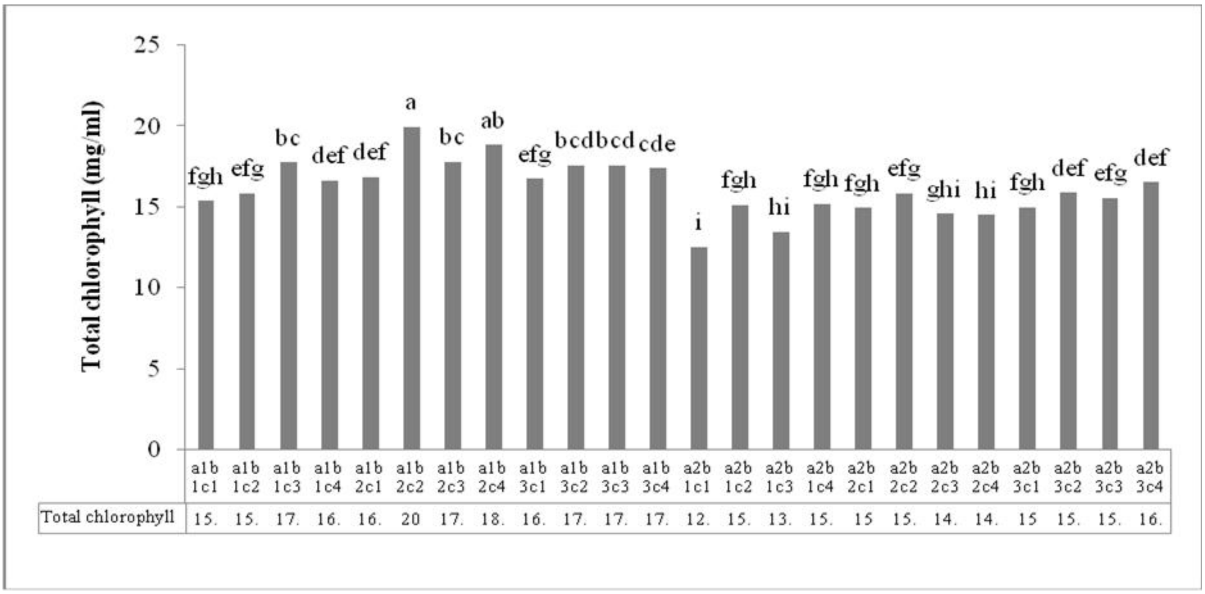

Fig. 2 Three- way interaction of irrigation×vermicompost×biofertilizer on total chlorophyll content of lemon balm plants. a1: normal irrigation, a2 drought stress, b1: without vermicompost, b2 and b3: application of 5 and 10 tones vermicompost per ha, respectively; c1: without biofertilizer; c2: *Pseudomonas*; c3: *Azotobacter* + *Azospirillum*; c4: *Azotobacter*+ *Azospirillum* +*Pseudomonas.* Means with same letters are not significantly different from each other at the 5% level by Duncan's multiple range tests.

Table 2 ANOVA of essential oil yield (EY), Essential oil concentration (EC), total chlorophyll (TC), cell membrane stability (CMS), relative water content (RWC) and proline

Source of variation	df	Proline (ug/g)	RWC (%)	CMS (uS/s)	TC (mg/ml)	EC (%)	EY (kg/ha)
Rep	2	0.29	34.2	26.8	0.02	0.0004^{ns}	1.09^{ns}
Irrigation (A)	1	1173.62**	1067.91**	70408**	110.18**	0.02**	19.46**
A error	2	4303.76	1194.31	183518.02	133.95	0.009	19.45
Vermicompost (B)	2	5.1ns	278.98**	14435.99*	11.6**	0.008**	3.47*
A×B	2	14.99*	97.25**	67956.65**	8.93**	0.0004^{ns}	15**
Error AB	8	7.27	289.95	47703.09	6.03	0.002	22.28
Biofertilizer (C)	3	12.6*	35.49*	3265.05^{ns}	8.33**	0.005**	5.78**
A × C	3	4.57^{ns}	8.14^{ns}	3786.65^{ns}	1.47^{ns}	0.0008^{ns}	0.25^{ns}
B × C	6	0.58^{ns}	9.22^{ns}	1804.51^{ns}	2.15^{ns}	0.0002^{ns}	3.65*
A×B×C	6	0.75^{ns}	9.21^{ns}	1806.04^{ns}	3.4*	0.0006^{ns}	3.87**
error	-	4.26	10.64	3845.77	1.49	0.0009	1.13
cv	-	15.76	4.36	14.46	7.6	13.34	14.97

^{ns}, ** and *; not significant and significant at 1 and 5%, respectively

Table 3 Mean comparison effect of irrigation on essential oil yield (EY), Essential oil concentration (EC), total chlorophyll (TC), cell membrane stability (CMS), relative water content (RWC) and proline of Lemon Balm

Treatments	Physiological Traits					
	Proline (ug/g)	RWC (%)	CMS (uS/cm)	TC (mg/ml)	EC (%)	EY (kg/ha)
Normal irrigation	17.14a	78.57a	460.04a	17.3a	0.20b	7.64a
Drought Stress	9.06b	70.87b	397.5b	1^{4}.82b	0.24a	$6^{.6}0^{b}$

In each column, means with same letters are not significantly different from each other at the 5% level by Duncan's multiple range tests.

Table 4 Mean comparison effect of vermicompost on essential oil yield (EY), Essential oil concentration (EC), total chlorophyll (TC), cell membrane stability (CMS), relative water content (RWC) and proline of Lemon Balm

Treatments	Physiological Traits					
	Proline (ug/g)	RWC (%)	CMS (uS/cm)	TC (mg/ml)	EC (%)	EY (kg/ha)
5 ton/ha	13.49a	77.73a	456.84a	16.5a	0.23a	7.55a
10 ton/ha	13.23a	75.41b	417.97b	16.43a	0.23a	6.96ab
Control	12.59a	71.02c	411.5b	15.26b	0.20b	6.84b

In each column, means with same letters are not significantly different from each other at the 5% level by Duncan's multiple range test

Table 5 Mean comparison effect of bio-fertilizer on essential oil yield (EY), Essential oil concentration (EC), total chlorophyll (TC), cell membrane stability (CMS), relative water content (RWC) and proline of Lemon Balm

Treatments	Physiological Traits					
	Proline (ug/g)	RWC (%)	CMS (uS/cm)	TC (mg/ml)	EC (%)	EY (kg/ha)
Pseudomonas	11.93b	75.9a	444. 6a	16.7a	0.24a	7.7a
Azotobacter + Azospirillum	13.36ab	75.36a	430.04a	16. 3a	0.22b	7.4a
Azotobacter+Azospirillum+Pseudomonas	13.91 a	74.93a	411.69a	16.14a	0.22b	6.97ab
Control	13.2ab	72.7b	411.69a	15.lb	0.20b	6.39b

In each column, means with same letters are not significantly different from each other at the 5% level by Duncan's multiple range tests

The mean comparison results of irrigation interaction effect vermicompost showed that normal irrigation×consuming 5 tons per ha vermicompost and drought treatment lack of consuming vermicompost fertilizer with the means of 18.38 and 14 mg ml had the highest and the lowest total chlorophyll, respectively (Table 6). Vermicompost is the source of organic substance, improving soil water 278 holding capacity, increasing nutrients, increasing the activity of hormone-like plants, and consequently increases the plants morphological and physiological traits [17].

Table 6 Two- way interaction of irrigation×vermicompost and vermicompost×biofertilizer on essential oil yield (EY), Essential oil concentration (EC), total chlorophyll (TC), cell membrane stability (CMS), relative water content (RWC) and proline of lemon balm plants

Treatments	Physiological Traits					
	Proline (ug/g)	RWC (%)	CMS (uS/cm)	TC (mg/ml)	EC (%)	EY (kg/ha)
albl	8.83c	76.53b	381.99cd	16.42bc	0.18c	7.22b
alb2	9.80c	79.84a	374.87d	18.38a	0.22b	8.25a
alb3	8.56c	79.50a	435 64bc	17.0b	0 21bc	7.44ab
a2bl	16.40b	65.5Id	453.21b	14.10d	0.22b	6.7b
a2b2	16.65b	70.99c	358.81a	14.62d	0.25a	5.43c
a2b3	18.42a	76.12b	381.36cd	15.76c	0.25a	7.65ab
blcl	11.49b	67. 64e	389.38b	13.98c	0.18e	5 46d
blc2	12.69ab	72.36cd	428.64ab	15.63b	0.19de	7.34abc
blc3	13.41ab	72.68cd	431.13ab	15.94b	0.20cde	7.38abc
blc4	12.77ab	71.41de	422.72ab	15.92b	0.22cde	7.67ab
b2cl	11.91ab	74.45bcd	424.35ab	17.88a	0.20cde	7.00abc
b2c2	14.01ab	77.35ab	487.63a	16.98ab	0.24abc	7.47ab
b2c3	13.90ab	75.57bcd	453.24ab	16.2lb	0.23bcd	6.75bcd
b2c4	13.09ab	74.29bcd	462.15ab	15.98b	0.26a	6.14cd
b3cl	12.40ab	76.03abc	421.33ab	15.41b	0.22cde	6.72bcd
b3c2	13.39ab	77.99ab	417.54ab	16.73ab	0.23bcd	8.28a
b3c3	14.42a	77.83ab	401.87b	16.58ab	0.22bcd	6.79bcd
b3c4	13.74ab	79.09a	405.24ab	16.98ab	0.25ab	8.41a

a1: normal irrigation, a2 drought stress, b1: without vermicompost, b2 and b3: application of 5 and 10 tones vermicompost per ha, respectively; c1: without biofertilizer; c2: *Pseudomonas*; c3: *Azotobacter* + *Azospirillum*; c4: *Azotobacter* + *Azospirillum*+*Pseudomonas.*

The mean comparison results of irrigation interaction effect ×vermicompost×bio-fertilizer on total chlorophyll showed that normal irrigation treatment ×consuming 5 tons per ha vermicompost × Pseudomonas fluorescent bacteria and drought × lack of consuming vermicompost fertilizer× lack of consuming bio- fertilizer with the means of 20 and 12.6 mg ml had the highest and the lowest total chlorophyll, respectively (Fig. 2).

Zaidi and khan [20] increased yield in the experiments conducted on the effects of nitrogen fixation (*Bradyrhizobium sp.*), Phosphate Solubilizing bacteria (*Bacillus subtilis*) and mycorrhizal fungi (*Glumus fasciculatum*) on growth, chlorophyll content, grain yield, node, grain protein, nitrogen and phosphorus uptake of mung bean [*Vigna radiate* (L.) R. Wilczek], which grew on soil under phosphorus deficiency.

Electrical Conductivity Measurement for Cell Membrane Stability

Cell membrane response changes against various environmental factors and conditions such as heat, drought and freezing and affects the plant growth due to its role on controlling exchanges of water and solutes to maintain cell turgor. Plants resistance against environmental stresses increased through cytoplasmic membrane stability by enhancing the ability of plants to maintain turgor [21]. Analysis of variance showed that the irrigation effect and the interaction effect of irrigation×vermicompost fertilizer on electrical conductivity were significant at the level of 1% and vermicompost effect was significant at the level of 5% (Table 2). The mean comparison results of irrigation effects on electrical conductivity showed that drought stress and normal irrigation with the means of 460.04 and 497.5 µ S/cm had the highest and the lowest stability (Table 3). Heuer [22] proved that there was a logical relationship between physiological reactions such as high relative humidity, high cytoplasmic membrane stability and water potential and resistance mechanisms to drought. The mean comparison results of vermicompost effects on electrical conductivity showed that control treatment and consuming 5 tons per hectare vermicompost with the means of 456.84 and 411.5 µ Siemens/cm had the highest and the lowest electrical conductivity, respectively. Control had 11.01% increases in comparison with consuming 5 tons per hectare vermicompost (Table 4).

The mean comparison results of irrigation interaction effect vermicompost fertilizer on electrical conductivity showed that the drought consuming 5 tons ha vermicompost and normal irrigation Non vermicompost fertilizer with the means of 458.81 and 374.87 µ Siemens/cm had the highest and lowest electrical conductivity, respectively (table 6).

Relative Water Content (RWC)

Results of variance analysis showed that the effect of irrigation, vermicompost fertilizer and interaction effect of irrigation vermicompost on relative water content were significant at the 1% level and the effect of bio- fertilizer was significant at the 5% level (Table 2). The mean comparison results of the irrigation effect on the mentioned trait showed that normal irrigation and drought with the means of 78.57 % and 70.87% had the highest and the lowest relative water content, respectively. Drought decreased by 10.86% in comparison with normal irrigation (Table 2).

The mean comparison results of vermicompost fertilizer effect on relative water content showed that consuming 5 tons vermicompost and control treatment with the means of 77.73and 71 .2 had the highest and lowest relative water content, respectively. Consuming 5 tons per hectare vermicompost had 9.17 % increases in comparison with the control treatment (Table 4). Investigating the effect of vermicompost fertilizer on tomato growth traits showed that vermicompost fertilizer improved growth traits such as relative water content [23].

The mean comparison results of biologic fertilizer effect on the mentioned trait showed that *Pseudomonas* fluorescent bacteria and the control treatment with the means of 75.9% and 72.7% had the highest and the lowest relative water content, respectively. *Pseudomonas fluorescent* bacteria had 4.4% increases in comparison with control treatment (table 5). Bio-fertilizer plays a significant role in the process of photosynthesis and producing green levels and increasing relative water content by increasing nitrogen uptake and increasing its efficiency, followed by growth and flowering [24]. The mean comparison results of interaction effect of irrigation vermicompost fertilizer on relative water content showed that the normal irrigation treatment consuming 5 tons per hectare vermicompost and normal irrigation consuming 10 tons per hectare with the means of 79.84% and 79.5%, respectively, had the highest and drought irrigation in absence of vermicompost fertilizer with a mean of 65.51% had the lowest relative water content. Normal irrigation treatment 5 tons per hectare vermicompost and normal irrigation× consuming 10 tons per hectare showed, respectively, 21.87% and 21.35% increases in comparison with control treatment.

Proline

Results of variance analysis showed that the effect of irrigation on proline contents was significant at the level of 1% and the effect of biological fertilizer and interaction effect of irrigation vermicompost was significant at the level of 5% (Table 2). The mean comparison results of the irrigation effect on proline showed that drought and normal irrigation tension with the means of 17/14 and 9.06 micrograms per gram dry weight had the highest and the lowest proline weight, respectively. Drought had 89.18 % increases in comparison with normal irrigation tension (table 3).

Although, proline can be one of the potential regulators, it cannot be a very good indicator for water scarcity because it is produced in plant tissues due to different stresses [25]. The mean comparison results of biological fertilizer effects on proline showed that *Azotobacter* + *Azospirillum* and *Pseudomonas* bacteria with the means of 13.91 and 11.93 micrograms per gram dry weight had the highest and the lowest proline, respectively. *Pseudomonas fleourcent* bacteria had 16.59% decreases Azotobacter+Azospirillum (Table 5). The biological fertilizer would enhance plant growth as it was observed in a study conducted on the effect of biological fertilizers treatments on fennel medicinal plant [26]. The mean comparison results of irrigation interaction effect×vermicompost on proline showed that drought×consuming 10 tons vermicompost and normal irrigation×5 tons vericom7post, normal irrigation×10 tons vermicompost, and normal irrigation×the lack of consuming vermicompost treatments with the means of 18.42 and 9.8, 8.56, and 8.38 mg per g dry weight had the highest and the lowest proline, respectively (Table 6). Proline content rose with increasing severity of drought tension. Proline molecules consist of hydrophilic and hydrophobic sections. Soluble proline can affect the solubility of various proteins and prevent from albumin abnormality. Interaction relationship was established between proline and hydrophobic proteins surface and increased levels of hydrophilic protein molecules enhanced their stability and prevented from changing, and the plants increased probably their proline due to the above mentioned reasons [27].

Conclusions

The results of the mean comparison of irrigation effect on essential oil yield showed that normal irrigation and drought with the means of 7 .64 and 6.6 kilogram per hectare had the highest and the lowest oil yield, respectively.

The mean comparison results of biological fertilizer effects on proline showed that *Azotobacter+Azospirillum* brasilense and *Pseudomonas* fleourcent bacteria with the means of 13.91 and 11.93 micrograms per gram dry weight had the highest and the lowest proline, respectively. *Pseudomonas fleourcent* bacteria had 16.59% decreases Azotobacter+Azospirillum.

The mean comparison results of biologic fertilizer effect on the mentioned trait showed that *Pseudomonas* fluorescent bacteria and the control treatment with the means of 75.9% and 72.7% had the highest and the lowest relative water content, respectively. *Pseudomonas* fluorescent bacteria had 4.4% increases in comparison with control treatment.

The mean comparison results of irrigation effect on essential oil percentage showed that drought and normal irrigation with the means of 0.24% and 0.2% had the highest and the lowest percentage.

References

1. Douglas, M. 1993. Lemon balm-*Melissa officinalis*, Crop and food Research. (http://crop.cri.nz/psp/ broadshe/lemon. htm).
2. Said-Al Ahl HA, Abdou MA, Omer EA. Effect of potassium fertilizer on lemon balm (*Melissa officinalis* L.) grown under water stress conditions. Planta Medica. 2009;75:PJ155.
3. García AC, Santos LA, Izquierdo FG, Rumjanek VM, Castro RN, dos Santos FS, de Souza LG, Berbara RL. Potentialities of vermicompost humic acids to alleviate water stress in rice plants (*Oryza sativa* L.). J Geochem Explor. 2014;136:48-54.
4. Hughes SG, Bryant JA, Smirnoff NI. Molecular biology: application to studies of stress tolerance. Plants under stress. Biochemistry, physiology and ecology and their application to plant improvement. Cambridge University Press, Cambridge. 1989:131-155.
5. Salinas V, Deiana S. Effect of water and nutritional condition on the *Rosmarinus officinalis* L. phenolic fraction and essential oil yield. Riv Ital EPPOS. 1996;19:189-198.
6. Fatima S, Abad Farooqi AH, Ansari SR, Sharma S. Effect of water stress on growth and essential oil metabolism in *Cymbopogon martinii* (palmarosa) cultivars. J Essent Oil Res. 1999;11:491-496.
7. Misra A, Srivastava NK. Influence of water stress on Japanese mint. J Herbs, Spices & Med Plants. 2000;7:51-58.
8. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255:571-586.
9. Fuentes-Ramirez LE, Caballero-Mellado J. Bacterial biofertilizers. In PGPR: Biocontrol and biofertilization 2005 (pp. 143-172). Springer Netherlands.
10. Mahfouz SA, Sharaf-Eldin MA. Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.). Int Agron. 2007;21:361.
11. Azzaz NA, Hassan EA, Hamad EH. The chemical constituent and vegetative and yielding characteristics of fennel plants treated with organic and bio-fertilizer instead of mineral fertilizer. Australian J Basic Appl Sci. 2009;3:579-587.
12. Sinha J, Biswas CK, Ghosh A, Saha A. Efficacy of Vermicompost against fertilizers on Cicer and Pisum and on population diversity of N. J Environ Biol. 2010;31:287-292.
13. Prabha ML, Jayraaj IA, Jayaraj R, Rao DS. Effect of vermicompost and compost on growth parameters of selected vegetable and medicinal plants. Asian J Microbiol Biotechnol Environ Sci. 2007;9:321.
14. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant soil. 1973;39:205-207.
15. Anwar M, Patra DD, Chand S, Khanuja SPS. Effect of organic manures and inorganic fertilizer on growth, herb and oil yield, Nutrient Accumulation, and oil quality of French basil. Communicat Soil plant Aanal. 2005;36:1737-1746.
16. Gewaily EM, Fatma I, El-Zamik T, El-Hadidy T, Abd El-Fattah HI, Salem SH. Efficiency of bio-fertilizers, organic and inorganic amendments application on growth and essential oil of marjoram (*Majorana hortensis* L.) plants grown in sandy and calcareous soils. Zagazig J Agric Res. 2006;33:205-230.
17. Karla A. Organic cultivation of medicinal and aromatic plants. A hope for sustainability and quality enhancement. Journal of Organic Production of

Medicinal, Aromatic and Dye-Yielding plants (MADPs). FAO. 2003.

18. El Ghadban EA, Shalan MN, Abdel Latif TA. Influence of biofertilizers on growth, volatile oil yieldand constituents of fennel (*Foeniculum vulgare* Mill.). Egyptian J Agric Res. 2006;84:977-992.
19. Jat RS, Ahlawat IP. Effect of vermicompost, biofertilizer and phosphorus on growth, yield and nutrient uptake by gram (*Cicer arietinum*) and their residual effect on fodder maize (*Zea mays*). Indian J Agric Sci. 2004;74:359-361.
20. Zaidi A, Khan MS. Co-inoculation effects of phosphate solubilizing microorganisms and *Glomus fasciculatum* on green gram-Bradyrhizobium symbiosis. Turkish J Agric Forest. 2006;30:223-230.
21. Ingram J, Bartels D. The molecular basis of dehydration tolerance in plants. Ann Rev Plant Biol. 1996;47:377-403.
22. Heuer B. Osmoregulatory role of proline in water-and salt-stressed plants. Handbook of Plant and Crop Stress. 1994:363-381.
23. Scott MA. use of worm-digested animal waste as a supplement to peat in loamless composts for hardy nursery stock. Earthworms in waste and environmental management/edited by Clive A. Edwards and Edward F. Neuhauser. 1988.
24. Han HS, Lee KD. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Res J Agric Biol Sci. 2005;1:210-215.
25. Natali S, Bignami C, Fusari A. Water consumption, photosynthesis, transpiration and leaf water potential in *Olea europaea* L., cv. Frantoio, at different levels of available water. Agric Med. 1991;121:205-212.
26. Giri B, Kapoor R, Mukerji KG. VA Mycorrhizal techniques/VAM technology in establishment of plants under salinity stress conditions. In Techniques in Mycorrhizal Studies 2002 (pp. 313-327). Springer Netherlands.
27. Kuznetsov VV, Shevyakova NI. Proline under stress: biological role, metabolism, and regulation. Russian J Plant Physiol. 1999;46:274-287.