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The Response of Drought Stressed Lemon Balm (*Melissa officinalis* L.) to Vermicompost and PGPR

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ABSTRACT. In this research, effect of vermicompost and PGPR of Physiological traits on lemon balm under experimental drought stress was investigated as the split- split plot in a randomized complete block design with three replications. Treatments included irrigation (normal and drought irrigation), vermicompost fertilizer (consuming 0, 5 and 10 t/ha) and PGPR (Pseudomonas fluorescent, Azotobacter + Azospirillum, Azotobacter + Azospirillum + Pseudomonas and no fertilizer). Traits including oil yield, oil percentage, total chlorophyll content, electrical conductivity, relative water content and proline were evaluated. Analysis of variance showed that the effect of irrigation on all traits was significant at the statistical probability level of 1 %. Vermicompost fertilizer effect on oil percentage, total chlorophyll and relative water content was significant at the level of 1% and its effect on essential oil yield and Electrical conductivity was significant at the level of 5%. Effect of bio-fertilizers on oil percentage, oil yield and total chlorophyll was significant at the level of 1% and its effect on proline and relative water content was significant at the level of 5%. The mean comparison results of irrigation effect showed that normal irrigation had the highest oil yield, total chlorophyll, relative water content and proline.

Keywords: Vermicompost, PGPR, Drought Stress, Lemon Balm.

INTRODUCTION

Drought stress is especially important in countries where crop agriculture is essentially rain-fed (Ludlow and Muchow, 1990). Drought stress causes an increase in solute concentration in the environment, leading to an osmotic flow of water out of plant cells. This in turn causes the solute concentration inside plant cells to increase, thus lowering water potential and disrupting membranes along with essential processes like photosynthesis. Lemon balm is a perennial 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 tetraakene and brown (Douglas, 1993). Drought is the most common abiotic environmental stress limited the production at approximately 25 % of the world agricultural land. Yield losses include more than two- thirds of the total damage of abiotic stresses due to drought, salinity and other factors (Bohnert and Bressan, 2001). Drought is a widespread and unpredictable phenomenon that can severely decrease grain and forage yield and yield stability in many areas (Yadav *et al.*, 2002). Bio-fertilizer is a densely populated preservative of one or more types of useful terricolous microorganism, their metabolic phenomenon are used to provide the nutrients needed by plants, control soil-borne diseases and maintain the stability of soil structure (Vessey, 2003). Vermicompost is a rich source of high and low consuming elements, vitamins, enzymes and hormones promoting plant growth. 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 (Prabha *et al.*, 2007). Misra and Srivastava (2005) investigated the effect of irrigation on mint plant and found that adequate irrigation would increase the growth and essential oil of mint. Azospirillum and Azotobacter increased the quality and yield stability, particularly in the production of medicinal plants (Sharma, 2003).

The results of a greenhouse experiment on marjoram (*Majorana hortensis*) in Egypt suggested that bio-fertilizers containing Azotobacter and phosphate solubilizing bacteria had a significant effect on growth, oil rate and also the inhibitory effect of essential oils on the growth of gram-positive bacteria, gram-negative bacteria and fungi (Fatima *et al.,* 1999). The research conducted on the effect of vermicompost on the quantity and quality fertilizer active substance showed that consuming vermicompost led to significant improvement 62 in the quantity and quality of essential oil s in basil medicinal plant (Anwar *et al.,* 2005). Effect of different levels of biological and chemical fertilizers on Pogostemon cablin indicated that the integrated treatment of Azospirillum, Azotobacter, mycorrhiza, 75 % nitrogen and phosphorous and 100% potassium led to the highest growth, essential oil yield and fresh biomass (Manjunatha *et al.,* 2002). Effect of biofertilizers on the two medicinal plants of chamomile and calendula were examined in an experiment in Cuba. The results showed that applying these fertilizers increased oil yield and improved the medicinal qualities in calendula, while it increased flower yield in chamomile, had no effect in the essential oils quality (Sanches *et al.,* 2005). In this study, the effect of drought, vermicompost fertilizer and bio-fertilizers on physiological traits of lemon balm was investigated.

MATERIALS AND METHODS

A. Treatments

In this research, effect of vermicompost and bio-fertilizers on lemon balm under experimental drought stress was investigated as the split- split plot in a randomized complete block design with three replications. Treatments included irrigation (normal and drought irrigation), vermicompost fertilizer (consuming 0, 5 and 10 t/ha) and biological fertilizers (Pseudomonas fluorescent, Azotobacter + Azospirillum, Azotobacter + Azospirillum + Pseudomonas and no fertilizer). Traits including oil yield, oil percentage, total chlorophyll content, membrane stability, relative water content and proline were evaluated.

B. 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 $1 \times 10^{\text{cell/ Ml}}$).

Table 1: Chemical and physical characteristics for soil of experimental field.

Soil depth (cm)	Silt %	Sand %	Clay %	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Mg (ppm)	Ca Meg/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

From the branches exposed to the open air and dried in shade, 100 g was selected and after crushing the samples using steam distillation method by Clevenger apparatus, the plant oil was extracted. Characteristics of the site soil were shown in Table 1.

C. Assessing proline concentration

Two tenths of a gram of a seedling was weighed and pulverized in a china mortar containing three milliliters of three percent sulfosalicylic acid. The homogenate obtained was centrifuged for 150 minutes in a centrifuge machine at 18000 revolutions per minute and two milliliters of the filtered extracts were transferred to tubes having caps. To each of these tubes were added two milliliters of the Ninhydrin Reagent and two milliliters of glacial acetic acid. After the tubes were capped, they were placed at 100°C 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 (Bates *et al.*, 1973).

D. Measuring the total chlorophyll

Firstly, 0.25 g 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. Chb (mg/l) = (21.5*a647) - (5.1*a663)
3. Chla + b (mg/l) = (7.15*a663) + (18.71*a647)

E. 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 129 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) (Heath and Packer, 1968).

F. Measuring Percentage and Oil Yield

100 g 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

A. Oil yield

Analysis of variance showed that the effect of irrigation on essential oil yield and the effect of vermicompost were significant at the level of 1% and 5%, respectively. Effect of bio- fertilizers on the mentioned trait was significant at the level of 1%. The mutual effect of irrigation× vermicompost and irrigation × vermicompost × biological fertilizers on oil yield and the mutual effect of vermicompost × biological fertilizers were significant at the level of 1% and 5%, respectively (Table 2). 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 (Table 3). Fatima *et al.* (1999) showed in a research that water stress in studied varieties of *C. winterianus* increased significantly oil content and decreased significantly oil yield.

Table 2. Analysis of variance effect of irrigation, Bio-Fertilizers and vermicompost on Physiological Traits of Lemon Balm

	df	Proline	RWC	Cytoplasmic Membrane	Total chlorophyll	Percentage Oil	Oil Yield
Replication	2	0.29	34.2	26.8	0.02	0.0004ns	1.09ns
Drought Stress	1	1173.62**	1067.91**	70408**	110.18**	0.02**	19.46**
Error a	2	4303.76	1194.31	183518.02	133.95	0.009	19.45
Vericompost	2	5.1ns	278.98**	14435.99*	11.6**	0.008**	3.47*
× Drought Stress Vericompost	2	14.99*	97.25**	67956.65**	8.93**	0.0004ns	15**
Error ab	8	7.27	289.95	47703.09	6.03	0.002	22.28
Bio-Fertilizer	3	12.6*	35.49*	3265.05ns	8.33**	0.005**	5.78**
Bio-× Drought Stress Fertilizer	3	4.57ns	8.14ns	3786.65ns	1.47ns	0.0008ns	0.25ns
Vericompost × Bio-Fertilizer	6	0.58ns	9.22ns	1804.51ns	2.15ns	0.0002ns	3.65*
Drought Stress× Vericompost × Bio-Fertilizer	6	0.75ns	9.21ns	1806.04ns	3.4*	0.0006ns	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 ** showed significant and non significant on levels 0.05 and 0.01 respectively.

Table 3. Mean comparison effect of irrigation on Physiological Traits of Lemon Balm

	Proline (Mg per dry weight)	RWC (Relative water content) (%)	Electrical conductivity ($\mu S.cm^{-1}$)	Total chlorophyll (Mg per fresh weight gram)	Percentage Oil (%)	Oil Yield ($kg.ha^{-1}$)
Normal irrigation	17/14a	78/57a	460/04a	17/3a	0/2b	7/64a
Drought Stress	9/06b	70/87b	397/5b	14/82b	0/24a	6/6b

Different letters on the top of columns indicate statistically significant differences.

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 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).

Furthermore, 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 (Anwar *et al.*, 2005).

The mean comparison results of bio-fertilizer effect on the oil yield showed that Pseudomonas bio-fertilizer and control treatment with the means of 7.7 and 6.39 (kg/ha) had the highest and the lowest oil yield, respectively.

Table 4. Mean comparison effect of vermicompost on Physiological Traits of Lemon Balm

	Proline (Mg per dry weight)	RWC (Relative water content) (%)	Electrical conductivity ($\mu S.cm^{-1}$)	Total chlorophyll (Mg per fresh weight gram)	Percentage Oil (%)	Oil Yield ($kg.ha^{-1}$)
5 ton.ha^{-1}	13/49a	77/73a	456/84a	16/5a	0/23a	7/55a
10 ton.ha^{-1}	13/23a	75/41b	417/97b	16/43a	0/23a	6/96 ab
Treatment	12/59a	71/02c	411/5b	15/26b	0/2b	6/84b

Different letters on the top of columns indicate statistically significant differences.

Table 5. Mean comparison effect of bio-fertilizer on Physiological Traits of Lemon Balm

	Proline (Mg per dry weight)	RWC (Relative water content) (%)	Electrical conductivity ($\mu S.cm^{-1}$)	Total chlorophyll (Mg per fresh weight gram)	Percentage Oil (%)	Oil Yield ($kg.ha^{-1}$)
Pseudomonas	11/93b	75/9a	444/6a	16/7a	0/24a	7/7a
Azotobacter+ Azospirillum + Pseudomonas	13/36ab	75/36a	430/04a	16/3a	0/22b	7/4a
Azotobacter+ Azospirillum	13/91a	74/93a	411/69a	16/14a	0/22b	6/97ab
Treatment	13/2ab	72/7b	411/69a	15/1b	0/2b	6/39b

Different letters on the top of columns indicate statistically significant differences.

Table 6: Mean comparison effect of irrigation × vermicompost and vermicompost × biofertilizer on physiological traits of Lemon Balm.

	Proine (Mg per dry weight)	RWC (Relative water content) (%)	Electrical conductivity ($\mu S.cm^{-1}$)	Total chlorophyll (Mg per fresh weight gram)	Percentage Oil (%)	Oil Yield ($kg.ha^{-1}$)
a1b1	8/83c	76/55b	381/99cd	16/42bc	0/18c	7/22b
a1b2	9/80c	79/84a	374/87d	18/38a	0/22b	8/25a
a1b3	8/56c	79/5a	435/64bc	17/0b	0/21bc	7/44ab
a2b1	16/4b	65/51d	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
b1c1	11/49b	67/64e	389/38b	13/98c	0/18e	5/46d
b1c2	12/69ab	72/36cd	428/64ab	15/63b	0/19de	7/34abc
b1c3	13/41ab	72/68cd	431/13ab	15/94b	0/20cde	7/38abc
b1c4	12/77ab	71/41de	422/72ab	15/92b	0/22cde	7/67ab
b2c1	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	13/89ab	75/57bcd	453/24ab	16/21b	0/23bcd	6/75bcd
b2c4	13/09ab	74/29bcd	462/15ab	15/98b	0/26a	6/14cd
b3c1	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

Different letters on the top of columns indicate statistically significant differences. irrigation, a_2= Drought Stress, b_1= not used vermicompost(treatment), b_2= vermicompost(5 ton.ha^{-1}), b_3= vermicompost(10 ton.ha^{-1}), c_1= not bio- fertilizer, c_2= Pseudomonas, c_3= Azotobacter + Azospirillum, c_4= Azotobacter+ Azospirillum + Pseudomonas)

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 (Gewailyet *et al.,* 2006) The mean comparison results of mutual 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.

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 (Ajimoddin *et al.*, 2005). The mean comparison results of mutual 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% increases in comparison with control treatment (Table 6). The mean comparison results of mutual 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 had the highest and drought treatment × lack of consuming vermicompost × lack of consuming bio-fertilizer with the mean of 4.43 kg/ha had the lowest oil yield (Fig. 1). Kalra (2003), 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 brasilense* treatments would be equal to control treatment (using chemical fertilizers).

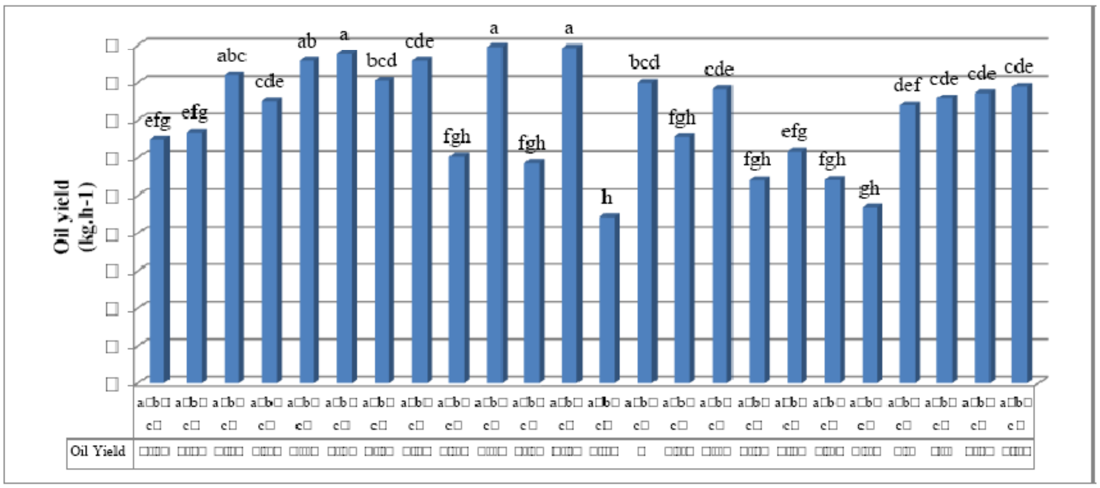

Fig 1. Comparison of the mutual mean irrigation, vermicompost and bio-fertilizer of oil yield. (a_1= normal irrigation, a_2= Drought Stress, b_1= not used vermicompost(treatment), b_2= vermicompost(5 ton.ha^{-1}), b_3= vermicompost(10 ton.ha^{-1}), c_1= not bio- fertilizer, c_2= Pseudomonas, c_3= Azotobacter + Azospirillum, c_4= Azotobacter+ Azospirillum + Pseudomonas)

B. Essential oil percentage

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 irrigatio1n effect on essential oil percentage showed that drought and normal irrigation with the means 42 of 0.24% and 0.2% had the highest and the lowest percentage (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 piperita*) was examined and the results showed that vermicompost treatment, cow manure and Azospirillum sp and Azotobacter sp would be equal to control treatment (using chemical fertilizer) (Kalra, 2003). The mean comparison results of biofertilizers effect on the mentioned trait 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 (EL-Ghadban *et al.*, 2006).

C. Total chlorophyll

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

Furthermore, the mutual 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 (Table 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. 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 (Jat and Ahlawal, 2004). 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). The mean comparison results of irrigation mutual 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 (Bachman and Metzger 2008). The mean comparison results of irrigation mutual 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 Saghir Khan (2005) increased yield in the experiments conducted on the effects of Nitrogen Fixation (*Brady rhizobium* sp.), Phosphate Solubilizing bacteria (*Bacillus subtilis*) and mycorrhiza fungi (*Glumus fasciculatum*) on growth, chlorophyll content, grain yield, node, grain protein, nitrogen and phosphorus uptake of mung bean (*Vigna radiate* L.), which grew on soil under phosphorus deficiency.

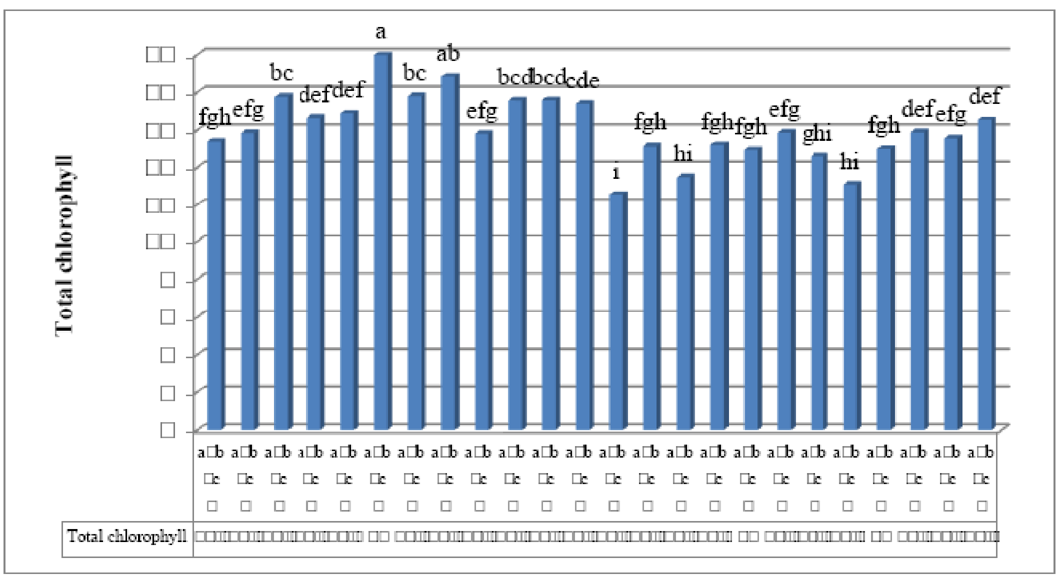

Fig 2. Comparison of the mutual mean irrigation, vermicompost and bio-fertilizer of Total chlorophyll irrigation, a_2= Drought Stress, b_1= not used vermicompost(treatment), b_2= vermicompost(5 ton.ha^{-1}), b_3= vermicompost(10 ton.ha^{-1}), c_1= not bio- fertilizer, c_2= Pseudomonas, c_3= Azotobacter + Azospirillum, c_4= Azotobacter+ Azospirillum + Pseudomonas)

D. Electrical conductivity measurement for Cytoplasmic Membrane Stability

Cytoplasmic 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 (Ingram and Bartels, 1996). Analysis of variance showed that the irrigation effect and the mutual 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 ? Siemens/cm had the highest and the lowest stability. (Table 3). Heuer (1994) 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 411.5µSiemens/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 mutual 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 374.87µSiemens/cm had the highest and lowest electrical conductivity, respectively (Table 6).

E. Relative Water Content (RWC)

Results of variance analysis showed that the effect of irrigation, vermicompost fertilizer and mutual 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 77.73 and 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 (Scott, 1988).

The mean comparison results of biologic fertilizer effect on the mentioned trait showed that P. 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. *P. 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 (Han and Lee, 2006). The mean comparison results of mutual 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 tension Lack 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.

F. Prolin

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 mutual 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 (Natalie *et. al.* 1991). The mean comparison results of biological fertilizer effects on proline showed that Azotobacter Bacteria + *Azospirillum brasilense* and P. fluorescent bacteria with the means of 13.91 and 11.93 micrograms per gram dry weight had the highest and the lowest proline, respectively. P. fluorescent bacteria had 16.59% decreases Azotobacter Bacteria + *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 (Kapoor *et al.,* 2002).

The mean comparison results of irrigation mutual effect × vermicompost on proline showed that drought × consuming 10 tons vermicompost and normal irrigation × 5 tons vericompost, 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 prolin, 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. Mutual 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 prolin due to the above mentioned reasons (Kuznestov and Shevykova, 1999).

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 Bacteria + Azospirillum brasilense and P. fluorescent bacteria with the means of 13.91 and 11.93 micrograms per gram dry weight had the highest and the lowest proline, respectively. P. fluorescent bacteria had 16.59% decreases Azotobacter Bacteria + Azospirillum.

The mean comparison results of biologic fertilizer effect on the mentioned trait showed that P. 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. P. 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

Ajimoddin, I., Vasundhara, M., Radhakrishna, D., Biradar, S.L. & Rao, G.G.E. 2005. Integrated nutrient management studies in sweet basil (*Ocimum basilicum* L.). *Indian Perfume*. **49**: 95-101.

Anwar, M., Patra, D.D., Chand, S. & Khanuja, S.P.S. 2005. Effect of organic manures and inorganic fertilizer on growth, herb and oil yield, nutrient accumulation, and oil quality of French basil. *Communications in Soil Science and Plant Analysis*. **36**: 1737-1746.

Bachman, G.R. & Metzger, J.D. 2008. Growth of bedding plants in commercial potting substrate amended with vermicompost. *Bioresource Technology*. **99**: 3155-3161.

Bates, I.S., Waldren, R.P. & Teare, I.D. 1973. Rapid determination of free proline for water- stress studies. *Plant and Soil*. **39**: 205-207.

Bohnert, H.J. & Bressan, R.A. 2001. Abiotic stresses, plant reaction and new approaches towards understanding stress tolerance. *Crop Science*. **6**: 81-100.

Douglas, M. 1993. Lemon balm-Melissa officinalis, Crop and Food Research. Available on: http://crop.cri.nz.

El-Ghadban, E.A.E., Shalan, M.N. & Abdel-Latif, T.A.T. 2006. Influence of biofertilizers on growth, volatile oil yield and constituents of fennel (Foeniculum vulgare Mill.). *Egyptian Journal Agricultural Research*. **84**: 977-992.

Fatima, S., Farooqi, A.H.A., Ansari, S.R. & Sharma, S. 1999. Effect of water stress on growth and essential oil metabolism in Cymbopogon martini (Palmarosa) cultivars. *Journal of Essential Oil Research*. **11**: 491-496.

Fatma, E.M., El-Zamik, I., Tomader, T., El-Hadidy, H.I., Abd El-Fattah, L. & Seham Salem, H. 2006. Efficiency of biofertilizers, organic and in organic amendments application on growth and essential oil of marjoram (*Majorana hortensis* L.) plants grown in sandy and calcareous. Agric. Microbiology Dept., Faculty of Agric., Zagazig University and Soil Fertility and Microbiology Dept., Desert Research Center, Cairo, Egypt.

Gewaily, E.M., El-Zamik, F.I., El-Hadidy, T.T., Abd El-Fattah, H.I. & Salem, S.H. 2006. Efficiency of biofertilizers, organic and inorganic amendment application of growth and essential oil of Marjoram (*Majorana hortensis* L.) plants grown in sandy and calcareous soils. *Zagazig Journal of Agricultural Research*. **33**: 205-396.

Han, H.S. & Lee, K.D. 2006. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. *Research Journal of Agriculture and Biological Sciences*. ***1***: 210-215.

Heath, R.L. & Packer, L. 1968. Photoperoxidatin in isolated chloroplast. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*. **125**: 189-198.

Heuer, B. 1994. Osmorgulatory Role of Proline in Water and Salt Stressed Plants: Handbook of Plant and Crop Stress. Pessarkli M, ed. Marcel Dekker, USA, p. 363-381.

Ingram, J. & Bartels, D. 1996. The molecular basis of dehydration tolerance in plants. Annual Reviews in Plant *Physiology and Plant Molecular Biology*. **47**: 377-403.

Jat, R.S. & Ahlawat, I.P.S. 2004. 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 Journal of Agricultural Sciences.* **74**: 359-361.

Kalra, A. 2003. 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.

Kapoor, R., Mukerji, K.G. & Giri, B. 2002. VA Mycorrhizal Techniques VAM Technology in Establishment of Plants under Salinity Stress Condition. (Mukerji, K.G., Manoracheir, C. and Singh, I.) Techniques in Mycorrhizal Studies. Kluwer, Dordrecht, p. 313-327.

Kuznestov, V.I. & Shevykova, N.I. 1999. Prolinennder stress: Biological role, metabolism and regulation. Russian *Journal of Plant Physiology.* **46**: 274-287.

Ludlow, M.M. & Muchow, R.C. 1990. A critical evaluation of the traits for improving crop yield in water limited environments. *Advances in Agronomy.* **43**: 107-153.

Manjunatha, R., Farooqi, A.A., Vasundhara, M. & Srinivasappa, K.N. 2002. Effect of biofertilizers on growth, yield and essential oil content in patchouli (*Pogostemon cablin* Pellet.). *Indian Perfumer*. **46**: 97-104.

Misra, A. & Srivastava, N.K. 2000. Influence of water stress on Japanese mint. *Journal of Herbs, Spices and Medicinal Plants*. **7**: 51-58.

Natali, S., Bignami, C. & Fusari, A. 1991. Water consumption, photosynthesis, transpiration and leaf water potential in Olea europeae L. cv. "Frantoio" at different levels of available water. *Agri. Medi.* **121**: 205-212.

Prabha, M.L., Jayraaj, I.A., Jayraaj, R. & Rao, D.S. 2007. Effective of vermicompost on growth parameters of selected vegetable and medicinal plants. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences.* **9**: 321-326.

Sanches Govin, E., Rodrigues Gonzales, H. & Carballo Guerra, C. 2005. Ifluencia de los abonosorganicos ybiofertilizantes en la calidad de las especiesmedicinales calendula *officinalis* L. *Matricariarecutita* L. *Revista Cubana de Plantas Medicinales.* **10**: 1.

Scott, M.A. 1988. The use of worm-digested animal wastes as a supplement to peat in leas composts for hardy nursery stocks (Edwards, C.A. and Neuhayser, E.) Earthworm in Waste and Environmental Management. SPB Academic Press, Netherlands, p. 1221-229.

Sharma, A.K. 2003. Biofertilizers for Sustainable Agriculture. Agrobios, India.

Vesse, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil.* **255**: 571-586.

Yadav, R.S., Hash, C.T., Bidinger, F.R., Cavan, G.P. & Howarth, C.J. 2002. Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought- stress conditions. *Theoretical and Applied Genetics.* **104**: 67-83.

Zaidi, A. & Saghir Khan, M. 2005. Co-inoculation effects of phosphate solubilizing microorganisms and Glomus fasciculatum on green gram-Brady rhizobium symbiosis. *Turkish Journal of Agriculture and Forestry.* **30**: 223-230.