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EFFECT OF BENZOIC ACID AND MYCORRHIZA ON *MENTHA VIRIDS* PLANTS GROWN UNDER DIFFERENT IRRIGATION LEVELS

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ABSTRACT: The response of spearmint plants growth, volatile oil production and volatile oil constituents to benzoic acid (BZA) treatments and inoculation by mycorrhizal fungi under different irrigation levels was studied. Three levels of soil moisture 30%, 60% and 100% field capacity (FC) were used. BZA was applied at three rates 0, 150 and 300 ppm, while Arbuscular Mycorrhizal fungi (AMF) were used at 1g/1kg soil. Results showed that benzoic acid (300ppm) or mycorrhizal fungi were capable to alleviate the deteriorative effect of drought stress. The plants which irrigated with 100% FC and treated by AMF or BZA at 300 ppm recorded an improvement in growth characters in term of plant height, fresh and dry weights and volatile oil yield. Meanwhile, the highest volatile oil percentage during the two seasons were recorded at 60% FC with mycorrhizal fungi followed by benzoic acid at 300 ppm treatment. It was noticed that, the vegetative growth, oil production and oil yield increased when the plants were treated by mycorrhizal fungi or BZA at 300 ppm and irrigated at 60% FC compared to those plants irrigated by 100% FC and untreated. Proline content was increased in plants under drought stress (30% FC) and untreated. The lowest values of proline content were found in the plants irrigated by 100% FC and treated by either benzoic acid or mycorrhizal fungi. Also, data showed that, dehydrogenase enzyme activity was the best under the highest level of field capacity (100% FC) with inoculation by mycorrhizal fungi. On the other hand; catalase enzyme activity was superior under the lowest level of FC (30%). AMF colonization infection on roots of spearmint plants increased with high level of FC (100%). AMF spores recorded significant increases under different levels of field capacity after harvesting period particular with mycorrhizal treatment followed by BZA (300ppm) treatment. It is of interest to mention that treating spearmint plants with benzoic acid and mycorrhiza induce mitigation effect on the harmful effect of stress condition.

Key words: Spearmint, benzoic acid, dehydrogenase, catalase enzyme, proline, mycorrhizal fungi, field capacity, drought stress.

INTRODUCTION

Spearmint (*Mentha virids* L.) belongs to Family Lamiaceae (Labiateae), its leaves and essential oil are used as carminative, antimicrobial, anti-inflammatory, antioxidant, popular flavoring and beverage (Poiata *et al*., 2006 and Bichra *et al*., 2013).

Drought stress is one of the most important abiotic stresses factors limiting growth and productivity of crop plants more than any other stress (Ghannoum, 2009).

Enhancing plant resistance to drought stress would be the most economical strategy to sustain agricultural productivity in areas prone to water scarcity (Xiong *et al.,* 2006). The plants respond to drought stress through phenological responses, morphological adaptations, physiological changes, and biochemical adaptations. Drought affects the water status, growth, development, yield, membrane integrity, and osmotic adjustment (Prabe *et al.*, 2009). The plants ability to sustain integral physiological process such as photosynthesis and gas exchange during drought stress, especially in the phases regarded sensitive to the crop, is a potential indication for maintaining productivity under water limiting conditions (Silva *et al.,* 2007). As the key process of primary metabolism, photosynthesis plays a key role in plant performance under drought stress (Pinheiro and Chaves, 2011). The balance between light capture and energy use are of great relevance to studies concerning the responsiveness of the photosynthetic apparatus to drought stress (Chaves *et al.,* 2009).

Benzoic acid is naturally synthesized by plants and classified in the group of carboxylic acids. These organic compounds are exudate toward the rhizosphere to facilitate the assimilation of mineral nutrients, also associated with the elevation of soil weathering and mineral lixiviation rate (Van Hees *et al.*, 2000). Benzoic acid and its compounds are derivatives of plants that are very soluble in water and organic polar solutions. Their effect in removing toxicity heavy metals, sodium chloride and harmful effect from drought stress were reported by Munne-Bosch and Penuelas (2003). Benzoic acid is potentially known to provide abiotic stress tolerance **(**Senaratna *et al.*, 2003), but benzoic acid induced drought tolerance has not been widely studied until now. Therefore, the present study was undertaken to investigate the possible role of BZA in improvement of drought tolerance.

In nature, plants interact with several microorganisms such as bacteria and fungi that improve their performance when facing various environmental stresses. One of these associations is referring to as mycorrhizal. Mycorrhiza form close symbiosis between fungi and plant roots. Mycorrhizl associations lead to crop improvement like growth rate, biomass, and mineral uptake under saline or drought conditions (Auge, 2004; Eveilin *et al.*, 2009; Subramanian and Charest, 1998 and 1999). Mycorrhizal fungi are decreasing the harmful effects resulting from environmental stress. Mycorrhizal fungi are unique between microorganisms occupying rhizosphere. These fungi form symbiotic colonies with most plants and increase inorganic nutrients in plant. Mycorrhizal fungi can improve plant tolerant due to environmental stress by stimulating growth regulators, increasing photosynthesis and improving regulation of osmotic pressure (Rabie and Almadani, 2005).

The purpose of this work was to study the role of benzoic acid and inoculation with mycorrhizal fungi for increasing mint plant production under different levels of irrigation.

MATERIALS AND METHODS

This study was carried out at the farm of Medicinal and Aromatic Plants Department at Dokky, Giza, Egypt during two successive seasons (2015 and 2016).

1. Experimental producers:

Spearmint seedlings at 15 cm height were obtained from El-Kanater El-Khairia, Experimental Farm of Medicinal and Aromatic Plants Research Department and transplanted on 20th February, 2015 and 2016 in No. 30 plastic pots each pot contained 10 kg soil.

Chemical fertilizers (NPK) were added at the recommended level, all phosphorus amounts were added during soil preparation and NK were applied in five doses, the 1st one was added during soil preparation, and the four doses were equally divided for the two cuts in the two seasons. Physical and chemical analyses of the soil are shown in Table (1).

2. Treatments:

- Irrigation was done at 30, 60 and 100% of field capacity (FC).
- Spearmint plants were sprayed four times by benzoic acid at 0, 150 and 300 ppm, the first and the second doses were spraying after 30 and 45 days from planting, the third one was after four days from the first cut, while the fourth spray was done after 15 days from the third spray in the two seasons.
- Inoculation with mycorrhiza (AMF) was conducted during transplanting at the rate of (1 g AMF/kg soil) in each pot.

The plants were harvested (twice), the first harvest cut was on 20th May, and the second one was on 20th July in both seasons.

Arbuscular mycorrhizae inoculation: Mixed spores of AM-fungi; *Glomus, Gigaspora* and *Acaulospora* were prepared after propagation and mixed with sterilized vermoculite (20% moisture) as a carrier were also estimated after 2^{ed} cut every season while, spore number of AM fungi were detected at harvest stage as described (Massoud *et al.*, 2013).

3. Experimental layout:

A split plot design of 12 treatments and three replicates and each replicate consisted of 9 pots (1 plant/pot) was used in this experiment. Different irrigation treatments represent the main plot, while the sub plots were the different treatments of benzoic acid (BZA) and mycorrhiza (AMF).

The statistical analysis was conducted according to Snedecor and Cochran (1980) means of the treatments were compared using LSD at 5%.

4. Data recorded:

The following data were recorded: Plant height (cm), Herb fresh and dry weight (g/plant), Essential oil percentage in dry herb according to the British Pharmacopeia (1963). Essential oil yield (ml/plant), GLC analysis of essential oil according to the methods described by Hoftman (1967) and Bunzen *et al.* (1969). The volatile oil was analyzed using DsChrom 6200 Gas Chromatograph equipped with a flame ionization detector for separation of volatile oil constituents**.** The analysis conditions were as follows: The chromatograph apparatus was fitted with capillary column Bpx_5% polystillphenylene_siloxane 30 mm x 0.25 mm ID x 0.25 µm film. Temperature program: Initial tem. Increase with a rate of 1 ^{o}C/min from 70 to 80 ^{o}C, rate of 5 ^{o}C/min from 80 to 120 ^{o}C and rate of 10 ^{o}C /min from 120 to 190 ^{o}C. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30ml/min and 330 ml/min for air. Detector and injector temperatures were 300 ^{o}C and 250 ^{o}C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of volatile oil.

Proline content in dry leaves was determined according to Bates *et al*. (1973).

Dehydrogenase enzyme activities (µg TPF/ g soil) were estimated according to the

Table 1. Physical and chemical analysis of the experimental soil.

Physical analysis			
Clay (%)	**Silt (%)**	**Sand (%)**	**Texture grade**
18.5	26.3	55.2	Sandy loam

Chemical analysis											
Total nutrients Content (ppm)			**pH**	**EC (dSm^{-1})**	**Soluble cation (meq/l)**				**Soluble anions (meq/l)**		
N	**P**	**K**			**Na^+**	**K^+**	**Ca^{++}**	**Mg^{++}**	**HCO_3^-**	**Cl^-**	**SO_4^{--}**
24.30	208.00	340.70	7.5	4.45	12.7	6.76	18.76	14.40	2.00	17.14	33.48

methods of Carbonell *et al.* (2000). Catalase enzyme activities (CAT) activity was estimated by monitoring the disappearance of H_2O_2 at 240 nm (ε = 40 mM−1 cm−1) according to the method of Aebi (1984). The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0), 33mM H_2O_2 and enzyme extract.

RESULTS AND DISCUSSION

Effect of different irrigation levels, benzoic acid and mycorrhizal fungi on vegetative growth:

1. Plant height (cm):

Data presented in Table (2) showed the effect of irrigation, benzoic acid and inoculation by mycorrhiza on plant height of spearmint plants. It could be noticed that, increasing the rates of irrigation from 30% up to 100% resulted in significant increases in plant height in both cuts in the two seasons. Actually, the tallest plants were recorded in case of 100% field capacity (FC) in the two cuts during both seasons. The recorded values were 39.34 and 35.36 cm/plant in 1st and 2nd cut for the 1st season, in the second one the values were 39.74 and 40.04 cm for the first and the second cuts, respectively. The shortest plants were observed at 30% FC during two cuts in the two seasons.

Benzoic acid treatment improved the plant growth as compared with the control under different irrigation levels. Values of plant height in the first season recorded 33.74 and 30.97 cm, in the second season the values were 31.94 and 32.83 cm for the first and the second cuts, respectively.

The tallest plants were recorded in case of those plants inoculated by mycorrhizal fungi, the values were 36.25 and 33.36 cm in the first season for the two cuts, respectively. Also, in the second season, the tallest plants attained 38.94 cm in the first cut, and 35.42 cm in the second one as shown in Table (2).

The interaction between irrigation levels (30, 60 and 100% FC) and benzoic acid at 0, 150, 300 ppm or mycorrhiza showed significant effect on spearmint plant growth (plant height). The beneficial effect of benzoic acid or mycorrhiza was found to be synergistically with increasing irrigation level from 30 to 100% FC. The tallest plants (45.03 and 39.23 cm) were recorded in the first season when these plants irrigated at 100% FC with inoculation by mycorhizal followed by the plants treated with 100% FC and 300 ppm benzoic acid (40.43 and 37.07 cm) through first and second cut, respectively. The same trend was observed in the second season. However, it was noticed that micorrhizal fungi or benzoic acid interacted with irrigation at 60% FC gave significant effect on plant height in comparison with the untreated plants at 100% FC.

On the other hand the shortest plants were observed when irrigation with 30% FC without AMF inoculation was used as well as 0 ppm of benzoic acid the plant height values were 20.57and 17.70 cm in the first season, and 22.57and 19.37 cm in the second one**.**

2. Fresh and dry weights g/plant:

Data in Table (3) indicated that increasing the rates of irrigation from 30%, 60% and 100% FC gave significant increase in fresh and dry weight in both cuts at the two seasons. The highest fresh and dry weight/plant were recorded when spearmint plants were irrigated at 100% FC, while the lowest values were recorded when spearmint plants irrigated at 30% FC. These results are in agreement with Abdalla (2011). Moisture deficiency induces various physiological and metabolic responses like stomatal closure and decline in growth rate and photosynthesis. Flexas and Medrano (2002); Baher *et al.* (2002) showed that greater soil water stress decreased plant height and total fresh and dry weight of *Satureja hortensis***,** Colom and Vazzana (2002) also showed that the number of stem per plant and plant dry weight was negatively related to water stress in *Eragrostis curvula.*

Table 2. Effect of irrigation levels, benzoic acid and mycorrhizal fungi on plant height of *Mentha viridis* plants at 2015 and 2016 seasons.

Irrigation / Treatments	Plant height (cm)															
	1st season (2015)								2nd season (2016)							
	1st cut				2nd cut				1st cut				2nd cut			
Treatments	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
Control	35.70	32.04	20.57	29.44	33.13	26.67	17.70	25.83	35.23	33.23	22.57	30.34	36.13	31.53	19.37	29.01
$BZA_{(1)}$	36.20	32.27	22.60	30.36	32.00	28.33	19.63	26.65	34.57	34.43	23.27	30.41	37.40	32.57	19.77	29.91
$BZA_{(2)}$	40.43	34.57	26.23	33.74	37.07	32.37	23.47	30.97	43.47	36.13	25.23	31.94	41.10	35.57	21.83	32.83
AMF	45.03	35.33	28.40	36.25	39.23	34.33	26.53	33.36	45.70	38.35	32.17	38.94	45.33	37.30	23.63	35.42
Mean	39.34	33.30	24.45		35.36	30.45	21.83		39.74	35.53	25.84		40.04	34.24	21.15	
LSD at 5% irrigation	0.221				0.160				0.146				0.669			
LSD at 5% treatments	0.155				0.168				0.099				0.610			
LSD at 5% interaction	0.278				0.279				0.179				0.979			

F_1=100% (Field capacity)
F_2= 60% (Field capacity)
F_3= 30% (Field capacity)

$BZA_{(1)}$ = benzoic acid 150 ppm
$BZA_{(2)}$ = benzoic acid 300 ppm
AMF= mycorrhizal fungi

Table 3. Effect of irrigation levels, benzoic acid and mycorrhizal fungi on herb fresh and dry weights of *Mentha viridis* plants at 2015 and 2016 seasons.

Irrigation / Treatments	1st season (2015)								2nd season (2016)							
	1st cut				2nd cut				1st cut				2nd cut			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
Herb fresh weight/plant (g)																
Control	29.27	22.40	8.67	20.11	27.37	18.13	6.17	17.22	31.13	24.47	10.50	22.03	29.67	21.17	8.50	19.78
$BZA_{(1)}$	31.17	23.90	10.33	21.80	27.90	19.33	7.33	18.19	33.23	25.10	12.47	23.60	30.93	22.30	11.33	21.52
$BZA_{(2)}$	32.37	26.53	14.13	24.34	30.43	22.43	11.03	21.30	35.43	28.33	15.23	26.33	32.83	24.60	12.27	22.33
AMF	37.63	28.50	17.53	27.89	35.30	25.23	15.30	25.28	39.33	33.43	14.97	29.24	36.43	28.47	16.57	27.16
Mean	32.61	25.33	12.67		30.25	21.28	9.96		34.78	27.83	13.29		32.47	24.14	12.17	
LSD at 5% irrigation	0.168				0.174				0.110				0.115			
LSD at 5% treatments	0.110				0.135				0.109				0.090			
LSD at 5% interaction	0.201				0.237				0.184				0.158			
Herb dry weight/plant (g)																
Control	9.37	7.17	2.61	6.38	9.39	6.11	3.08	6.19	10.50	8.07	3.01	7.19	9.66	6.35	2.66	6.22
$BZA_{(1)}$	9.97	7.46	2.89	6.77	9.67	6.70	2.42	6.26	11.19	8.37	3.74	7.77	10.72	7.14	3.47	7.11
$BZA_{(2)}$	12.58	8.49	4.24	8.44	10.79	7.48	3.16	7.14	12.71	9.83	4.83	9.12	11.49	8.36	3.84	7.90
AMF	13.24	9.59	4.91	9.25	12.36	7.83	4.49	8.23	13.77	11.03	5.67	10.16	12.76	9.34	5.47	9.19
Mean	11.29	8.18	3.66		10.55	7.03	3.29		12.04	9.33	4.31		11.16	7.80	3.86	
LSD at 5% irrigation	0.068				0.364				0.243				0.172			
LSD at 5% treatments	0.113				0.313				0.194				0.129			
LSD at 5% interaction	0.180				0.537				0.337				0.228			

F_1=100% (Field capacity)
F_2= 60% (Field capacity)
F_3= 30% (Field capacity)

$BZA_{(1)}$ = benzoic acid 150 ppm
$BZA_{(2)}$ = benzoic acid 300 ppm
AMF= mycorrhizal fungi

The addition of benzoic acid at 150 or 300 ppm had a significant effect on fresh and dry weight of spearmint plants in both seasons. In the first season, plants treated by BZA at 300 ppm showed an increase of fresh weight (24.34 and 21.30 g/plant) compared to the control (20.11 and 17.22 g/plant), in the same way dry weight of the same treatment was (8.44 and 7.14 g/plant) compared to the control (6.38 and 6.19 g/plant) in the first and second cuts, respectively. The same trend was observed in the second season for the two cuts. Data cleared that the fresh weights were 26.33 and 22.33 g/plant compared to 22.03 and 19.78 g/plant in the control and 9.12 and 7.90 g/plant for dry weight compared to the control 7.19 and 6.22 g/plant in the 1st and the 2nd cuts, respectively. Salicylic and benzoic acid are associated with cell wall alterations that increase their hardness, higher synthesis of antioxidants, and elevation of mitochondrial activity (Levine *et al.,* 1994 and Ding *et al.,* 2007). The role of benzoic acid as precursor of salicylic acid (SA) biosynthesis (Juli Chong *et al.*, 2001).

As for the effect of mycorrhizal fungi, it was noticed that inoculation by mycorrhiza achieved significantly highest increases in the fresh and dry weights in the two seasons. In the first season, data were 27.89 and 25.28 g/plant fresh weight compared to untreated plants 20.11 and 17.22 g/plant, in the first and second cuts, respectively. Also, in the second season data were 29.24 and 27.16 g/plant fresh weight compared to untreated plants 22.03 and 19.78 g/plant fresh weight in the two cuts. Regarding dry matter of spearmint plants as affected by AMF also, the same trend was recorded, plant dry weight data were 9.25 and 8.23 g/plant compared to untreated plants (6.38 and 6.19 g/plant) in the 1st and 2nd cuts at the first season, and 10.16 and 9.19 g/plant compared to untreated plants (7.19 and 6.22 g/plant) in the two cuts at the second season, respectively. Mycorrhizal fungi considerably improve the uptake of mineral nutrients to their host plant, whereas the plant supports the fungus with assimilation products (Harley and smith, 1983; Smith and Read, 1997 and Aggarwal *et al.,* 2011). Mycorrhizal fungi associations lead to crop improvement like growth rate, biomass, and mineral uptake under saline or drought conditions (Auge, 2004; Evelin *et al.,* 2009; Subramanian and Charest, 1998 & 1999).

Concerning the effect of the interaction between the irrigation and benzoic acid or mycorrhizal fungi on fresh and dry weight g/plant, it was clear from data in Table (3) that there were significant effect on growth of spearmint plants in term of biomass (fresh and dry weight) . The fresh weight recorded an increase when the plants were irrigated at 100% FC and sprayed by 300 ppm benzoic acid, the recorded data in this concern were 32.37, 30.43, 35.43 and 32.83 g/plant for the 1st and 2nd cuts in the 1st and 2nd seasons, respectively. As for the interaction effect between irrigation levels and AMF inoculation on spearmint plant growth a significant effect was showed in this concern, the heaviest fresh and dry weight were recorded at 100% FC with inoculation by mycorrhizal fungi in both seasons. The recorded data were 37.63, 35.30, 39.33 and 36.43 g/plant in the 1st and 2nd cuts for the 1st and 2nd season, respectively. Also, data shown the same trend observed on dry weight/plant where. mycorrhizal fungi tend to give highest dry weights with irrigation at 100% FC compared to the untreated plants.

Effect of different irrigation levels, benzoic acid and mycorrhizal fungi on essential oil:

1. Volatile oil percentage:

Data in Table (4) showed that irrigation had a significant effect on volatile oil of spearmint plants. The highest value of volatile oil percentage were recorded in plants irrigated with 60% FC 1.38, 1.36, 1.34 and 1.36% compared to 1.10, 1.25, 1.20 and 1.22% in plants irrigated with 100% FC and 1.04, 1.07, 1.08 and 1.13% in plants irrigated with 30% FC for the two cuts in the two seasons, respectively. These results are in harmony with Penka (1978); Afify *et al.*

Table 4. Effect of irrigation levels, benzoic acid and mycorrhizal fungi on volatile oil percentage of *Mentha viridis* plants at 2015 and 2016 seasons.

Treatments / Irrigation	Volatile oil percentage (dry herb)															
	1st season (2015)								2nd season (2016)							
	1st cut				2nd cut				1st cut				2nd cut			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
Control	1.13	1.27	0.92	1.11	1.17	1.24	0.94	1.12	1.10	1.26	0.91	1.09	1.08	1.13	1.10	1.10
$BZA_{(1)}$	0.95	1.32	1.04	1.10	1.22	1.21	1.03	1.15	1.18	1.30	1.03	1.17	1.21	1.33	1.08	1.21
$BZA_{(2)}$	1.14	1.45	1.09	1.23	1.25	1.43	1.14	1.27	1.25	1.38	1.16	1.26	1.28	1.47	1.15	1.30
AMF	1.18	1.48	1.12	1.26	1.36	1.55	1.17	1.36	1.28	1.40	1.20	1.29	1.31	1.52	1.19	1.34
Mean	1.10	1.38	1.04		1.25	1.36	1.07		1.20	1.34	1.08		1.22	1.36	1.13	
LSD at 5% irrigation	0.007				0.015				0.003				0.020			
LSD at 5% treatments	0.010				0.010				0.009				0.017			
LSD at 5% interaction	0.017				0.018				0.014				0.029			

F_1=100% (Field capacity)
F_2= 60% (Field capacity)
F_3= 30% (Field capacity)

$BZA_{(1)}$ = benzoic acid 150 ppm
$BZA_{(2)}$ = benzoic acid 300 ppm
AMF= mycorrhizal fungi

(1993); Abou Dahab *et al.* (2010) and Farahani *et al.* (2009) they explained that under drought stress more metabolites are produced in the plants and some substances prevent oxidization in the cells, so under drought stress the essential oil production in most of medicinal and aromatic plants was increased.

The addition of benzoic acid or mycorrhizal fungi to spearmint plants had a beneficial role on volatile oil percentage. The highest values of essential oil percentage of spearmint plants were recorded in case of those plants inoculated with mycorrhizal fungi, values were 1.26, 1.36, 1.29 and 1.34% compared to 1.11, 1.12, 1.09 and 1.10% in untreated plants in both cuts in the first and the second seasons, respectively. Mycorrhizal fungi significantly increased the volatile oil percentage of *Mentha viridis* plants as reported by Ali *et al*. (2014) and Saleh (2009) on anise plants. Also, when spearmint plants treated by benzoic acid at 300 ppm, data were 1.23, 1.27, 1.26 and 1.30% in the two cuts during the 1st and the 2nd seasons, respectively. However, it was noticed that mycorrhizal fungi was found to be more effective than benzoic acid in this respect.

The interactions between irrigation and benzoic acid or mycorrhizal inoculation spraying were more effective on volatile oil production of spearmint plants than the control. The highest volatile oil content was recorded in spearmint plants irrigated with 60% FC interacted with mycorrhizal fungi producing 1.48, 1.55, 1.40 and 1.52% compared to the same treatment under 100% FC it was achieved 1.18, 1.36, 1.28 and 1.31% in the two cuts during two seasons, followed by these plants treated with benzoic acid 300 ppm and irrigated with 60% FC 1.45, 1,43, 1.38 and 1.47% compared to 1.14, 1.25, 1.25 and 1.28% at irrigated with 100% FC interacted with benzoic acid 300 ppm in the two in the two seasons, respectively.

In general, it could be concluded that, irrigation, benzoic acid and mycorrhizal fungi had a significant role in biosynthesis of volatile oil in spearmint plant, so the highest oil content was found in those plants irrigated with 60% FC and inoculated with mycorrhizal fungi or with 300 ppm benzoic acid.

2. Volatile oil yield ml/plant:

Data recorded in Table (5) showed that the oil yield ml/plant was considerably affected by irrigation levels, benzoic acid (BZA) and mycorrhizal fungi inoculation.

Data revealed that, spearmint plants were found to be significantly affected by irrigation levels, *i.e.* the highest volatile oil yield was observed in the plants irrigated at 100% FC in the two cuts giving 0.133 and 0.126 ml/plant at the first season and 0.146 and 0.138 ml/plant in the two cuts in the second one, respectively. The lowest volatile oil yield was observed in the plants irrigated at 30% FC 0.034, 0.39, 0.048 and 0.044 ml/plant. These results showed that spearmint plant was stressed when irrigated with the low irrigation level (30%) so, plants produced low yield of essential oil as the growth was found to be sharply inhibited under low irrigation level. These results are in accordance with Ali *et al*. (2014).

As for mycorrhizal fungi, it was more significant in increasing oil yield ml/plant than the other treatments, values were 0.114 and 0.118 ml/plant in the two cuts at the first season and 0.133 and 0.125 in the two cuts in the second one. In the second rank, benzoic acid treatment significantly increased the volatile oil yield ml/plant at 300 ppm than the control.

The interaction between irrigation and mycorrhiza inoculated or benzoic acid was more effective on volatile oil yield ml/plant than the control. The superior values in this concern were recorded in spearmint plants irrigated at 100% FC with inoculation by mycorrhizal fungi, values were 0.168 and 0.157 ml/plant in the two cuts at the first season and 0.177 and 0.167 ml/plant in the two cuts at the second one, and followed by plants irrigated at 100% (FC) with benzoic

Table 5. Effect of irrigation levels, benzoic acid and mycorrhizal fungi on essential oil yield (ml)/plant of *Mentha viridis* plants at 2015 and 2016 seasons.

Treatments \ Irrigation	Essential oil yield (ml)/plant															
	1st season (2015)								2nd season (2016)							
	1st cut				2nd cut				1st cut				2nd cut			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
Control	0.111	0.076	0.020	0.069	0.106	0.091	0.024	0.074	0.116	0.101	0.027	0.081	0.107	0.072	0.029	0.069
$BZA_{(1)}$	0.118	0.081	0.025	0.075	0.095	0.108	0.030	0.080	0.132	0.109	0.039	0.093	0.129	0.095	0.038	0.087
$BZA_{(2)}$	0.134	0.107	0.036	0.092	0.146	0.123	0.047	0.105	0.159	0.136	0.056	0.117	0.147	0.123	0.044	0.105
AMF	0.168	0.121	0.053	0.114	0.157	0.142	0.055	0.118	0.177	0.155	0.068	0.133	0.167	0.141	0.065	0.125
Mean	0.133	0.096	0.034		0.126	0.116	0.039		0.146	0.125	0.048		0.138	0.108	0.044	
LSD at 5% irrigation	0.004				0.002				0.003				0.003			
LSD at 5% treatments	0.003				0.002				0.003				0.002			
LSD at 5% interaction	0.006				0.003				0.005				0.003			

F_1=100% (Field capacity)
F_2= 60% (Field capacity)
F_3= 30% (Field capacity)

$BZA_{(1)}$ = benzoic acid 150 ppm
$BZA_{(2)}$ = benzoic acid 300 ppm
AMF= mycorrhizal fungi

acid at 300 ppm values were 0.134 and 0.146 ml/plant in the two cuts in the first season and 0.159 and 0.147 ml/plant in the two cuts in the second one.

In general it could be concluded that, spearmint plants irrigated at 100% FC with mycorrhiza inoculation or benzoic acid spraying were found to be more effective on volatile oil production also, it was observed that, spearmint plants which were inoculated with mycorrhiza and irrigated at 60% gave high volatile oil yield ml/plant 0.121, 0.142, 0.155 and 0.141 ml/plant compared to these plants irrigated at 100% (FC) without treatments, in the same way those plants treated with 300ppm BZA and irrigated at 60% gave high volatile oil yield ml/plant compared to those plants irrigated at 100% (FC) without treatments except in the first cut in the first season.

3. GLC analysis of essential oil:

Data in Table (6) and Figs. (1 to 3) showed that fractionated components of the essential oil of spearmint plant under 100% and 60% FC with mycorrhizal fungi and 60% FC with benzoic acid at 300 ppm in the second cut of the second season. Carvon and Limonene were the main components of spearmint oil. It was observed that the best increase of Carvon content at 60% FC with mycorrhizal fungi treatments, it was achieved 72.41%, followed by 70.35% at 60% FC with benzoic acid at 300 ppm treatment, but Limonene recorded an increase under 100% FC with mycorrhizal fungi inoculation 19.3% and followed by 19.0% with 60% FC + benzoic acid (300 ppm) treatment. It was noticed that, while Carvon increased Limonene decreased.

Effect of benzoic acid and mycorrhiza treatments on biological activities of spearmint plants under different irrigation levels:

1. Proline content:

Data in Table (7) showed that proline content in spearmint plant significantly affected by irrigation rates. The highest content of proline was found in plants under drought stress 30% FC followed by 60% FC, but the lowest concentration of proline was recorded at 100% FC. Plants can partly protect themselves against mild drought stress by accumulating osmolytes. Proline is one of the most common compatible osmolytes in stressed plants.

Table 6. Effect of irrigation with benzoic acid (BZA) and mycorrhizal fungi on volatile oil component % of *Mentha viridis* plants in the 2nd season (2nd cut).

Volatile oil Components	Treatments		
	F_1 AMF	F_2 $BZA_{(2)}$	F_2 AMF
α-Pinene	1.03	0.97	0.89
Myrcene	0.80	1.86	1.74
β-Pinene	0.90	0.96	0.96
D-Limonene	19.3	19.00	17.16
ρ-Cymene	4.33	3.14	3.24
1,8- Cineole	0.23	0.57	0.74
γ –Terpinone	0.74	0.25	0.29
Carvone	70.22	70.35	72.41
α-Terpinone	1.93	1.32	1.41
Eugenol	0.35	0.58	0.46

F_1=100% (Field capacity) **$BZA_{(2)}$ = benzoic acid 300 ppm**
F_2= 60% (Field capacity) **AMF= mycorrhizal fungi**

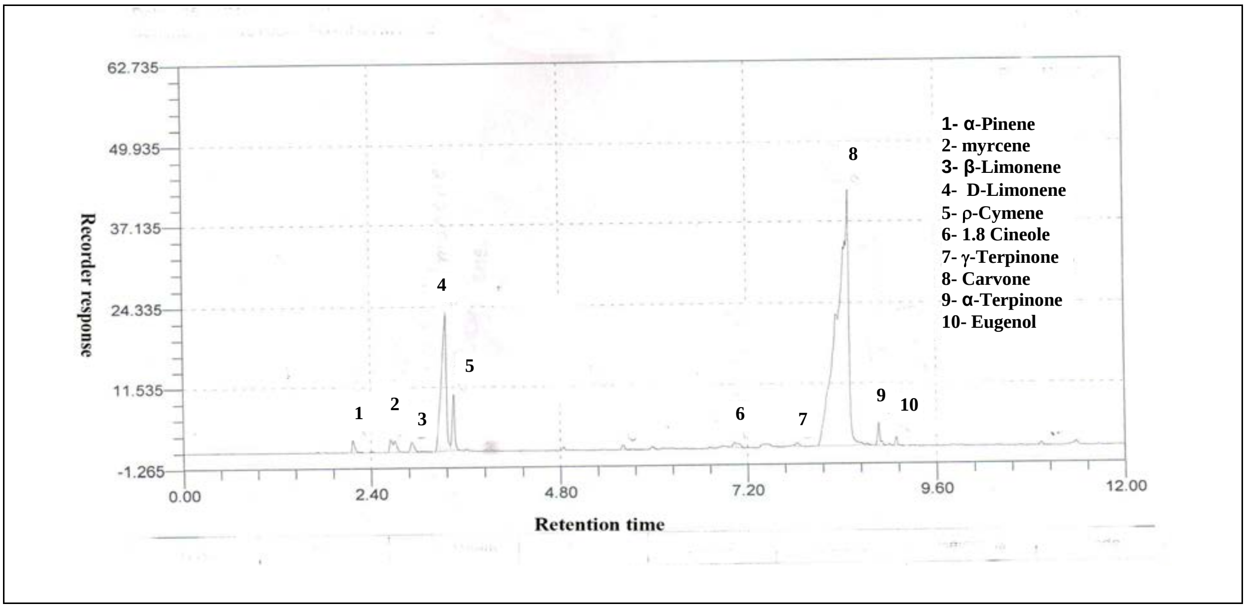

Fig. 1. (GLC) chromatogram of spearmint oil (F_2 AMF).

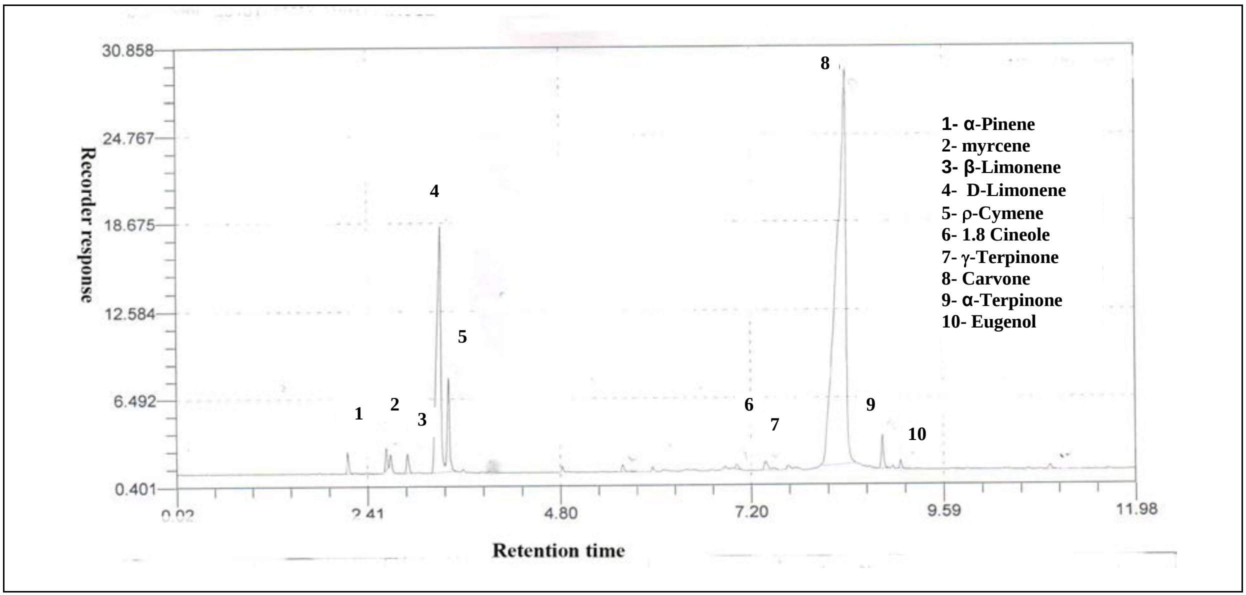

Fig. 2. (GLC) chromatogram of spearmint oil (F_2 $BZA_{(2)}$).

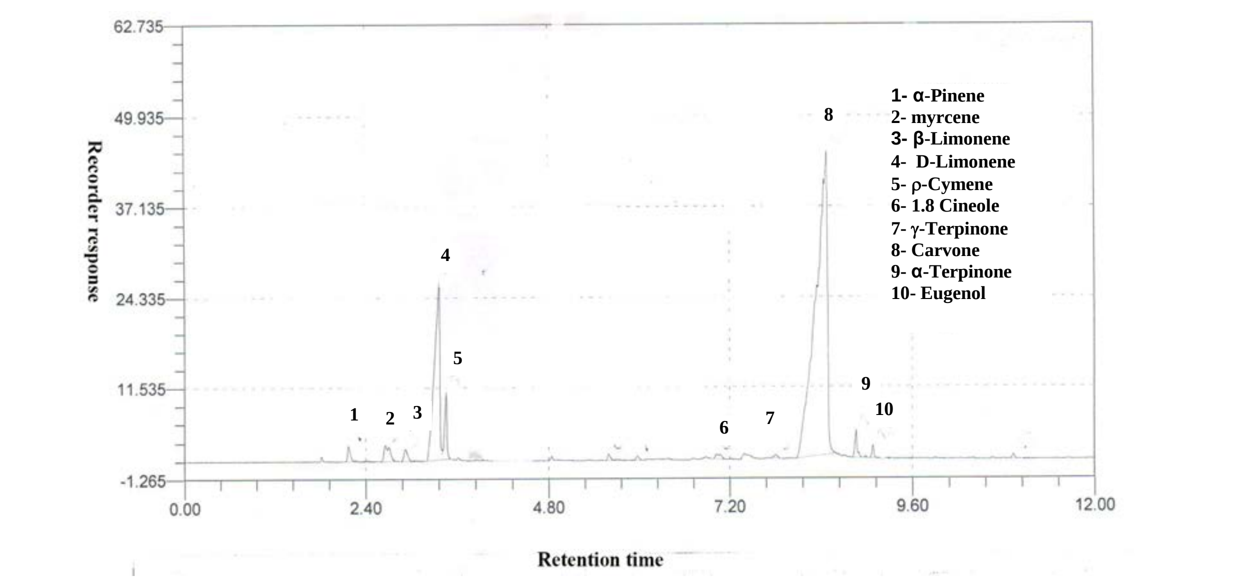

Fig. 3. (GLC) chromatogram of spearmint oil (F_1 AMF).

Table 7. Effect of irrigation levels, benzoic acid and mycorrhizal fungi on proline content μg/g dry leaves of *Mentha viridis* plants in the second Season (2016).

Treatments \ Irrigation	Proline content μg/g of (dry leaves)							
	1st cut				2nd cut			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
Control	433.7	820.5	1649.2	967.8	420.4	650.1	1559.7	860.7
$BZA_{(1)}$	419.6	750.7	1519.0	896.4	415.2	630.2	1319.0	788.3
$BZA_{(2)}$	389.6	670.0	969.2	676.3	369.8	590.5	860.0	606.8
AMF	369.2	550.5	758.9	559.5	350.1	520.6	670.5	713.7
Mean	403.0	697.9	1224.1		388.9	597.9	1102.3	
LSD at 5% irrigation	2.071				0.190			
LSD at 5% treatments	1.868				0.275			
LSD at 5% interaction	3.176				0.443			

F_1=100% (Field capacity) **$BZA_{(1)}$ = benzoic acid 150 ppm**
F_2= 60% (Field capacity) **$BZA_{(2)}$ = benzoic acid 300 ppm**
F_3= 30% (Field capacity) **AMF= mycorrhizal fungi**

For example, the proline content increased under drought stress in pea (Sanchez *et al.*, 1998 and Alexieva *et al.*, 2001). Proline does not interfere with normal biochemical reaction but allows the plants to survive under stress (Stewart, 1981). Also, Proline accumulation may also be part of the stress signal influencing adaptive response (Maggio *et al.*, 2002). The interaction between irrigation and inoculation by mycorrhizal or treating with benzoic acid recorded a significant effect on the value of proline content in spearmint plants the highest value of proline content was observed in plants irrigated under stress 30% FC without any treatments, the recorded data were 1649.2 μg/g of dry herb in the first cut and 1559.7 μg/g of dry herb in the second cut. But the lowest proline contents were recorded in plants which irrigated with 100% FC and inoculated by mycorrhizal 369.2 and 350.1 μg/g of dry herb in the first and second cuts, respectively, and followed by plants which irrigated at 100% FC with BZA at 300 ppm data were 389.6 and 369.8 µg/g of dry herb in the first cut and the second one, respectively.

It was worthy to mention that, all irrigation levels in control plants recorded the high values of proline, also results indicated that treating spearmint plants with mycorrhiza and benzoic acid showed a mitigation effect on stress condition, as the contents of proline were decreased.

2. Dehydrogenase and catalase enzymes activities:

Dehydrogenases (DHA) are intracellular enzymes that are active in living cells and are an important indicator of microbial activity of the soil Trasar-Cepeda *et al.* (1999). As shown in Table (8), DHA activities in rhizosphere region of *Mentha viridis* plants were achieved the highest significant value with treatment mycorrhiza 189.5 µ g TPF / g soil in the first season also, DHA was higher value than other treatments under 100% FC 150.7 µ g TPF/g soil also, DHA activities were the best with mycorrhizal treatment. Meanwhile, the lowest value of DHA activities were under 30% FC so, interaction treatments between mycorrhiza and irrigation under 100% FC was the best treatment in DHA activities recorded 201µ g TPF / g soil. In the second season, the same treatment was more efficient where; mycorrhiza was stilling the best treatment under 100% FC achieved 220 µ g TPF / g soil.

Table 8. Effect of irrigation levels, benzoic acid and mycorrhizal fungi on dehydrogenase and catalase enzymes activities.

Treatments \ Irrigation	1st season				2nd season			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
Dehydrogenase enzyme (µ g TPF /g dry rhizosphere)								
Control	140	129	21.37	96.79	165	144	36.2	115.1
$BZA_{(1)}$	66.2	175	189.2	143.5	71.83	195	204	156.9
$BZA_{(2)}$	195.7	35.3	30.1	87.03	214	52.6	42.9	103.2
AMF	201	195	172.5	189.5	220	210	191.2	207.1
Mean	150.7	133.6	103.3		167.7	150.4	118.65	
LSD at 5% irrigation	0.867				0.574			
LSD at 5% treatments	0.364				0.463			
LSD at 5% interaction	0.789				0.804			
Catalase enzyme (µ mol min^{-1} /mg)								
Control	120	105	99	108	165.3	146.7	103.9	138.6
$BZA_{(1)}$	113	120	135	122.7	173.7	186.7	201.7	187.4
$BZA_{(2)}$	158	169	186	171	211.7	216.5	228.8	218.4
AMF	195	200	205	200	224.7	231.6	247.8	234.4
Mean	146.5	148.5	156.25		193.85	195.41	196.3	
LSD at 5% irrigation	6.801				1.636			
LSD at 5% treatments	5.942				1.261			
LSD at 5% interaction	10.160				3.508			

F_1=100% (Field capacity) **$BZA_{(1)}$ = benzoic acid 150 ppm**
F_2= 60% (Field capacity) **$BZA_{(2)}$ = benzoic acid 300 ppm**
F_3= 30% (Field capacity) **AMF= mycorrhizal fungi**

Dehydrogenase is an oxidoreductase, which is only present in viable cells, this enzyme has been considered as a sensitive indicator of soil quality and it has been proposed as a valid biomarker to indicate changes in total microbial load due to changes in soil management (Roldan *et al.*, 2004).

Increasing the tolerance drought stress in *Mentha viridis* plants is associated with the antioxidant enzyme activities (catalase enzyme). While DHA was increasing with 100% FC in contrast, catalase enzyme was at high level under the lowest level of FC. Catalase enzyme play key role in protecting plants from oxidative stress by increasing their activities under drought stress where, results revealed that *Mentha viridis* plants showed an increase in drought tolerance under low level of irrigation (30% FC) it was 156.25 µ mol min^{-1} /mg. Also, data cleared the mycorrhizal treatment recorded the best value of drought tolerance for *Mentha viridis* plants it was 200 µ mol min^{-1} /mg. So, interaction between treatments which contents of mycorrhiza under lowest level of FC was the greatest in significant increases in catalase enzyme activities 205 µ mol min^{-1} /mg during the first season. The same behavior for values of catalase enzyme was noticed in the second season, but they were more than first season where, mycorrhiza treatment under the lowest level of water (30% FC) achieved 247.8 µ mol min^{-1} /mg. This results are agreement with Mardukhi *et al.*, (2011) where, they reported the Arbuscular mycorrhizal association with plant roots usually increases the growth of plants especially the presence of N_2 – fixing bacteria, which enhance water and nutrient uptake specially phosphorus. Therefore, Mycorrhizal fungi have a potential to be considered as a major component of sustainable agro-ecosystem. Antioxidant enzymes activity increases in plant cells as a response to environmental stresses. Environmental stresses can result in the production of Reactive Oxygen Species (ROS), including O, H_2O_2 and OH these ROS adversely affect crops yield and quality Baby and Jini (2011). To prevent damage to cellular components by ROS, plants have developed a complex antioxidant system. The primary components of his system include carotenoids, ascorbate, glutathione and tocopherols, in addition to enzymes such as superoxide dismutase, catalase, glutathione peroxidase, peroxidases and the enzymes involved in ascorbate-glutathion cycle such as ascorbate peroxidase and glutathione reductase (GR) (Rahimizadeh *et al.,* 2007).

3. Mycorrhizal colonization and mycorrhizal dependency percentages:

Data in Table (9) cleared that the mycorrhizal colonization on root of *Mentha viridis* plants with arbuscular mycorrhizal fungi treatment achieved better colonization during two seasons they obtained 97 and 101.7% respectively. Also, the highest level of water filed capacity (100% FC) was the best treatment during two seasons 77.8 and 81.43% respectively. Arbuscular mycorrhizal fungi were dominating at all levels of FC particular in second season this mean mycorrhiza found under the lowest level of FC for soil. Mycorrhizal dependency counted from equation:

$$\mathbf{MD} = \frac{\text{Dry wt. of AM plants - Dry wt. of non AM plants}}{\text{Dry wt. of AM plants}} \times 100$$

The highest percentage of mycorrhizal dependency (MD) exhibited with benzoic acid (300 ppm) treatment where, it achieved the highest percentage of MD either with the first season (40.9%) or second season (48.73%) also; the same treatment exhibited under 100% FC the highest percentage of MD 45.9 and 49.8% during two seasons respectively.

Mycorrhizal infection (100%) obtained through two seasons after seedling stage, this reflected on increasing the number of spores (g) in soil with mycorrhiza treatment (806.4 spores) after harvesting period. Field capacity 100% treatment recorded 345 g of numbers of spores after harvesting period.

Table 9. Changes of mycorrhizal colonization and mycorrhizal dependency percentages as affected by different treatments under different irrigation levels.

Treatments \ Irrigation	1st season				2nd season			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
AMF colonization (%)								
Control	59	40	25	41.33	62.67	44.67	29.67	45.67
$BZA_{(1)}$	71	66	63	66.67	75.67	68.67	65.67	70
$BZA_{(2)}$	82	75	62.3	73.1	85.67	78.67	66.67	77
AMF	99	97	95	97	101.7	101.7	101.7	101.7
Mean	77.8	69.5	61.3		81.43	73.43	65.93	
LSD at 5% irrigation	0.378				0.945			
LSD at 5% treatments	0.330				1.167			
LSD at 5% interaction	0.564				1.902			
Mycorrhizal dependency (%)								
Control	33.1	22.4	14	23.17	39.5	27.8	18.1	28.47
$BZA_{(1)}$	39.8	37	35.3	37.37	47.9	43.4	41.4	44.23
$BZA_{(2)}$	45.9	41.5	35.3	40.9	54.3	49.8	42.1	48.73
AMF	0	0	0	0	0	0	0	0
Mean	29.7	25.225	21.15		35.4	30.3	25.4	
LSD at 5% irrigation	0.294				1.529			
LSD at 5% treatments	0.380				0.049			
LSD at 5% interaction	0.617				0.077			

F_1=100% (Field capacity) **$BZA_{(1)}$ = benzoic acid 150 ppm**
F_2= 60% (Field capacity) **$BZA_{(2)}$ = benzoic acid 300 ppm**
F_3= 30% (Field capacity) **AMF= mycorrhizal fungi**

Also, mycorrhiza treatment under high level of FC was superior in increases numbers of spores (870 spores) after harvesting period as shown in Table (10). Birhane *et al.* (2012) reported that under drought conditions, arbuscular mycorrhizal fungi alter water relationships of plants and improve their resistance to drought. There were significantly higher levels of AMF colonization under irregular precipitation regime than under continuous precipitation also, significantly higher for mycorrhizal seedlings. Mycorrhizal seedlings under irregular watering had the highest biomass. Also, Schmitz and Harrison (2014) said under the levels of water and nutrients, plants will enter into symbiosis with arbuscular mycorrhizal fungi for the enhancement of mineral nutrient acquisition from the surrounding soil. AMF lives in close, intracellular association with plant roots where they transfer phosphate and nitrogen to the plant in exchange for carbon and protect plants against of environmental stress. They are obligate fungi, relying on their host as their only carbon source. This results harmony with Emiru Birhane *et al.*, (2015) they stated AMF symbiosis enhanced the acquisition of water and nutrients and increased gas exchange resulting in increased *Acacia* and *Boswellia* seedling biomass. The rapidly growing *Acacia* species (acquisitive strategy) showed larger mycorrhizal benefit at higher water availability. The slow-growing *Boswellia* (conservative strategy),

Table 10. A change of spore's number for mycorrhizal after harvest.

Treatments \ Irrigation	Spore number after harvest/g soil			
	2nd season			
	F_1	F_2	F_3	Mean
Control	100	95	37	77.33
$BZA_{(1)}$	160	150	140	150
$BZA_{(2)}$	250	220	200	223.3
AMF	870	800	749.3	806.4
Mean	345	316.3	281.6	
LSD at 5% irrigation	7.557			
LSD at 5% treatments	6.603			
LSD at 5% interaction	11.289			

F_1=100% (Field capacity) **$BZA_{(1)}$ = benzoic acid 150 ppm**
F_2= 60% (Field capacity) **$BZA_{(2)}$ = benzoic acid 300 ppm**
F_3= 30% (Field capacity) **AMF= mycorrhizal fungi**

in contrast, showed larger mycorrhizal benefit at lower water availability.

CONCLUSION

Spearmint plants showed significant response in growth and yield as well as essential oil production by irrigation, benzoic acid and mycorrhizal fungi. Spearmint plants irrigated at 100% FC and inoculated with mycorrhizal fungi or treated by 300ppm benzoic acid gave the highest response of vegetative growth, essential oil yield and main constituents. The highest essential oil percentage was observed at 60% FC and mycorrhizal fungi or treated with benzoic acid at 300ppm. Spearmint plants irrigated at 60% FC with mycorrhizal fungi or treated by 300ppm BZA gave high vegetative growth, essential oil production compared to control plants under 100% FC. The highest proline content was found in control plants under stress 30% FC, while the lowest content of proline was detected in those plants irrigated under 100% FC and inoculated by mycorrhiza. Relationship of biological activities, catalase enzymes increase with the lowest level of FC (30%) on the other hand, dehydrogenase enzymes activities were with the highest level of FC (100%). It is of interest to mention that treating spearmint plants with benzoic acid and mycorrhiza induce mitigation effect on the harmful effect of stress condition.

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تاثير حمض البنزويك و فطر الميكورايزا على نباتات النعناع البلدي النامية تحت مستويات ري مختلفة

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أجري هذا البحث لدراسة مدى إستجابة نباتات النعناع البلدي من حيث النمو الخضري وإنتاج الزيت الطيار للمعاملة بحمض البنزويك و التلقيح بفطر الميكوريزا تحت مستويات ري مختلفة.

استخدم ثلاثة مستويات ري مختلفة ١٠٠%، ٦٠% و٣٠ % من السعة الحقلية. وتمت اضافة حمض البنزويك بمعدلات صفر، ١٥٠، ٣٠٠ جزء فى المليون و التلقيح بفطر الميكوريزا بمعدل ١جم/١ كجم تربة.

وأدت النتائج إلى أن المعاملة بحمض البنزويك بمعدل ٣٠٠ جزء فى المليون أو الميكوريزا إلى تخفيف تدهور نباتات النعناع البلدي الناتجة عن الإجهاد المائي. وكانت أهم النتائج زيادة النمو الخضري من حيث إرتفاع النبات و الوزن الطازج والجاف و محصول الزيت الطيار عند الري بمعدل ١٠٠% من السعة الحقلية و المعاملة بالميكوريزا أو بالمعاملة بحمض البنزويك بمعدل ٣٠٠ جزء فى المليون. بينما أعلى نسبة الزيت الطيار كانت عند الري بمعدل ٦٠% من السعة الحقلية مع المعاملة بالميكوريزا ويليها المعاملة بحمض البنزويك بمعدل ٣٠٠% جزئ فى المليون. ومن الملاحظ أن النمو الخضري و نسبة ومحصول الزيت الطيار يزيد عند المعاملة بالميكروريزا أو البنزويك أسد بمعدل ٣٠٠ جزء فى المليون والري بمعدل ٦٠% من السعة الحقلية بالمقارنة بالنباتات التي رويت بمعدل ١٠٠% من السعة الحقلية والغير معاملة. البرولين المتكون في نباتات النعناع البلدي إزداد تحت ظروف الإجهاد المائي ٣٠% من السعة الحقلية مع عدم معاملة النباتات بأي من الميكوريزا أو حمض البنزويك وكانت أقل نسبة برولين متكونة في النباتات التي رويت ١٠٠% من السعة الحقلية والتي عوملت بفطر الميكوريزا أو حمض البنزويك. اما بالنسبة للنشاط البيولوجى متمثل فى انزيم الدي هيدروجينيز فحققت المعاملة بالميكوريزا تحت مستوى سعة حقلية ١٠٠ % اعلى معدل فى الانزيم وعلى النقيض حقق انزيم الكتاليز فى النبات اعلى معدل له عند اقل مستوى ري ٣٠% من السعة الحقلية. مستعمرة العدوى بفطر الميكوريزا على جذور نباتات النعناع البلدي زاد مع المستوى العالي من السعة الحقلية (١٠٠%) و جراثيم فطر الميكوريزا سجل زيادة معنوية تحت المستويات المختلفة من السعة الحقلية بعد فترة الحصاد وخصوصاً مع المعاملة بفطر الميكوريزا يليها المعاملة بحمض البنزويك بمعدل ٣٠٠ جزء في المليون.

وجدير بالذكر أن معاملة نباتات النعناع البلدي بحمض البنزويك و الميكوريزا يحدث له تأثير في تخفيف الأثر الضار لحالات الإجهاد.